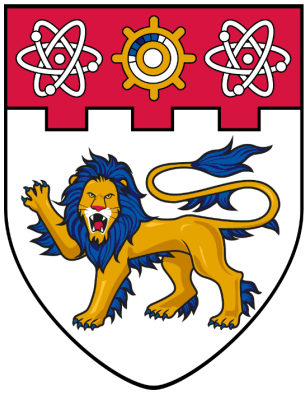

NANYANG TECHNOLOGICAL UNIVERSITY
SINGAPORE

RELATIONSHIP OF ATOPIC DERMATITIS WITH OBESITY AND ITS RELATED INFLAMMATORY METABOLIC DISTURBANCES IN AN ADULT GENERAL POPULATION COHORT

YEW YIK WENG

LEE KONG CHIAN SCHOOL OF MEDICINE

2020

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Lee Kong Chian School of Medicine

A thesis submitted to the Nanyang Technological University in partial fulfilment of the requirement for the degree of Doctor of Philosophy

2020

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution

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Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

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Date Professor John C. Chambers

Authorship Attribution Statement

This thesis contains materials from one paper published in the following peer-reviewed journal where I was first and/or corresponding author.

Chapter 8 is published as Yew YW, Loh M, Thng STG, Chambers JC. Investigating causal relationships between Body Mass Index and risk of atopic dermatitis: a Mendelian randomization analysis. Sci Rep. 2020 Sep 17;10(1):15279. doi: 10.1038/s41598-020-72301-2. The original research paper is attached at the end of the thesis.

The contributions of the co-authors are as follows:

- I drafted the manuscript, performed the data extraction, analyzed and interpreted the data.
- Asst Prof Marie Loh provided valuable scientific and technical inputs, assisted in data analysis, data interpretation and manuscript preparation.
- Prof Steven Thng provided the broad project directions, assisted in data interpretation and manuscript preparation.
- Prof John C. Chambers vetted the manuscript, provided the broad project directions and valuable scientific inputs, supervised the planning and execution of the experiments and data analysis.

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Abbreviations

AD	Atopic dermatitis
B-H	Benjamini-Hochberg
BMI	Body mass index
CCL	Chemokine ligand
CD	Cluster of differentiation
CNS	Central nervous system
CT	Computed tomography
CTCL	Cutaneous T cell lymphoma
DNA	Deoxyribonucleic acid
DXA	Dual energy x-ray absorptiometry
EA	East Asia
FDR	False discovery rate
FLG	Filaggrin (gene)
GWAS	Genome wide association studies
HELIOS	Health for Life in Singapore
HRNR	Hornerin (gene)
IgE	Immunoglobulin E
IKK	inhibitor of κ kinase
IL	Interleukin
ILC	innate lymphoid cells
IV	Instrument variable
JNK	c-jun N-terminal kinase
KW	Kruskal Wallis

LDA	Linear discriminant analysis
LEfSe	Linear discriminant analysis Effect Size
LOD	Limit of detection
MATT	Mattrin (gene)
MR	Mendelian randomization
MRI	Magnetic resonance imaging
NMF	Natural moisturising factors
NOD2	Nucleotide-binding oligomerization domain-containing protein 2
NPX	Normalized protein expression
OR	Odds ratio
PCA	Principal component analysis
PCoA	Principal coordinates analysis
PCR	Polymerase chain reaction
PEA	Proximity extension assay
PKR	Protein kinase R
PLS-DA	Partial least squares discriminant analysis
RH	Relative humidity
SC	Stratum corneum
SEA	South East Asia
SES	Social economic status
SNP	Single nucleotide polymorphisms
SPINK5	Serine protease inhibitor Kazal-type 5 (gene)
STAT	Signal Transducer and Activator of Transcription.
TARC	Thymus- and activation regulated chemokine
TCA	Tricarboxylic acid

TEWL	Trans epidermal water loss
Th	T helper
TLR2	Toll-like receptor 2
TNF	Tumour necrosis factor
TSLP	Thymic stromal lymphopoietin
UKWP	UK Working Party
VIP	Variable importance in projection
WC	Waist circumference
WHR	Waist hip ratio

Summary

Atopic dermatitis (AD) is a chronic inflammatory skin disease with significant patient and population burden. It has been observed that obesity is associated with a higher risk of AD. However, the underlying mechanisms are not clear.

The purpose of this thesis is to assess the relationship of AD and obesity in a general adult population cohort and explore possible underlying mechanistic links. Specifically, there are five different aims to determine epidemiological associations, skin physiology differences, skin microbiome diversity, serum inflammatory biomarkers profile and genomic associations between AD and obesity. These are specifically to address the various hypotheses based on our current understanding of AD's pathophysiological mechanisms.

Participants of the Health for Life in Singapore (HELIOS) study cohort, aged 30 to 85 years old from the general population, were included in the analysis. Participants were screened for AD using the modified UK Working Party criteria and obesity classified according to anthropometric, bio-impedance and dual x-ray absorptiometry measures. Skin physiology parameters such as trans epidermal water loss (TEWL), skin surface moisture and pH were also measured for all participants. In addition, skin microbiome profiling and serum inflammatory biomarkers proteomics analysis were performed in 300 selected Chinese participants (150 AD participants age and gender matched in a 1:1 ratio with healthy controls). Genetic associations and the concept of Mendelian randomisation (MR) were also used to explore causal relationship between obesity, and AD using genetic data published in public domain. Finally, analysis was performed to integrate results from the skin microbiome and serum proteomic studies to evaluate interactions between the various omics platforms.

A total of 5560 participants were recruited from initiation till January 2020. About 8.8% of participants had AD while 40.2% were overweight or obese. Participants with higher visceral fat mass were more likely to have AD after adjusting for age, gender and ethnicity (Odds Ratio (OR): 1.52; p=0.028). Skin physiology, skin microbiome and serum proteomic profile related to AD were found to be significant with increasing body mass index (BMI). Obese participants (BMI $\geqslant$ 30 kgm^{-2} had a significantly higher trans-epidermal water loss (TEWL) values (β=0.059 $p < 0.001$) while having a lower skin surface hydration (β= - 0.047 $p = 0.003$). Serum proteomic analysis revealed that levels of 98 specific serum biomarkers were significantly different with increasing BMI. Known biomarkers of obesity such as serum leptin and IL6 and several AD related chemokines such as CCL17, CCL20, CCL3 and CCL4 were among these significantly different serum biomarkers. The IL18 family of cytokines, previously hypothesized to play a role in initiating AD like dermatitis, were also found to be significantly elevated with increasing BMI. While the diversity of the skin microbiome was similar between the obese and the lean, there were increased proportions of *Corynebacterium* species, *Staphylococcus hominis* and *Malassezia globosa* and reduced proportions of *Propionibacterium (Cutibacterium) acnes* in participants with increasing BMI. Finally, with MR analysis, it has been observed that genetically determined increase in obesity is associated with increased risk of AD (OR of AD 1.08; p = 0.015).

This study provided new evidence to improve our understanding of how obesity is associated with the risk of AD. Obesity, particularly abdominal/visceral obesity was associated with an increased risk of AD. It was established by the MR analyses that the direction of this relationship likely reflects an effect of obesity on the risk of developing AD. This was further reinforced by the observations that obese participants exhibited features of skin barrier dysfunction. Serum proteomic and skin microbiome

analyses also revealed unique profile among obese participants. These findings provided basis for future studies to evaluate possible underlying mechanistic pathways of this relationship.

1. Introduction

Atopic dermatitis (AD) is a very common chronic relapsing inflammatory skin disorder characterized by intense itch, dryness and inflammation. While prevalence of AD among children is similar to that of most developed countries, adult atopic dermatitis appears to be more common in Singapore, comprising 14% of all cases of AD seen at the National Skin Centre [1]. The local prevalence of AD among school children was about 20% in 2002 and 2017, with recent data estimating the prevalence of AD among young adults aged 18 to 19 years old at about 10% [2-4]. The exact local prevalence of AD among adults has not been widely reported.

Atopic dermatitis is associated with high morbidity, affecting the quality of life of patients as well as their caregivers [5]. Persistent itch, recurrent bacterial skin infection and highly conspicuous skin lesions severely impact the patient's self-esteem, daily activities, sports participation and psycho-social well-being.

Our understanding of AD's aetiology and pathogenesis is incomplete. It is however recognized that AD is a multifactorial disorder with interaction between genetic and environmental factors, affecting the skin's epidermal barrier function and immune profile [6]. It has increasingly been hypothesized to be more of a systemic disorder, based upon observations that several non-allergic comorbidities are associated with AD, especially among adults [7]. There has therefore been considerable interest among researchers, clinicians and patients alike on whether other inflammatory co-morbidities play any role in the aetiology of AD or its severity. At the same time, there have been reports of association of AD with metabolic syndrome conditions, especially obesity and cardiovascular conditions. With increasing rates of obesity and prevalence of AD, there have been postulations that the positive correlations between two chronic diseases might

be related to common patho-physiological mechanisms [8, 9]. Increased waist circumference and raised body fat percentage were found to be significantly associated with adult Korean women having AD [10]. Indeed, overweight or obesity has been reported to be associated with an increased prevalence of AD in the regions of North America and Asia [11].

It is not known whether this association is a result of similar inflammatory mechanisms observed in other inflammatory skin diseases such as psoriasis or lichen planus, or if these associated metabolic diseases are in fact downstream complications of the disease or its treatments. Obesity has previously been reported to have an association with dysregulated skin epidermal barrier function [12]. There is also a low-grade inflammation that exists chronically in obese individuals which can impact on cytokine pathways related to AD [13, 14]. Skin microbiota might also be different in obesity [15]. With advancement in techniques and technologies, we are now in a better position to assess the mechanistic links between AD and obesity (as well as other co-morbidities). Understanding the relationship between AD and obesity would be valuable in understanding disease pathophysiology and generating insights on possible future novel AD interventions/treatments.

There are altogether 12 chapters of this thesis, including the current chapter that forms the introduction and structure of the thesis. This current chapter summarizes the background and value of the entire study. Chapter 2 is a literature review chapter with information on the epidemiology, disease definition and diagnostic criteria of atopic dermatitis. It presents evidence on the clinical presentation as well as observed disease associations with AD. Chapter 2 provides background information on obesity, along with its pathophysiology mechanisms and severity classification, and presents the aims of this thesis. Chapter 3 is a methodology chapter that is separated into several main sections.

It elaborates on the study design, study population as well as how AD, obesity and association factors are classified. Sample size calculation for the studies in the thesis is also included in this chapter. Separate methods section detailing the specific analysis and technologies used in each study, as well as corresponding results are presented from Chapter 4 to 8 according to the respective specific studies. Chapter 9 is an integrated analysis chapter that combines the results of the various profiling platforms. Chapter 10 is a discussion chapter that summarizes the results of the five studies presented in the earlier chapters, the strengths and limitations of this thesis and provides recommendations for future research in the area. Finally, Chapter 11 provides a closing and final summary of the thesis. Chapter 12 is the bibliography section listing all the references used in the entire thesis.

2. Background

2.1. Epidemiology of atopic dermatitis (AD)

2.1.1. Disease prevalence of AD

Atopic dermatitis (AD) is a chronic inflammatory skin condition with a significant disease burden globally and locally. It typically presents early in infancy, but can remain persistent in adulthood with various disease severity. The lifetime prevalence of AD has been reported to be about 15 to 30% in children and 2 to 10% in adults [16]. It was previously estimated that about 25% of patients with early onset AD will progress to persistent AD in adulthood [17]. However, a prospective cohort study in Denmark which followed up 1,501 children for 15 years showed that 50% had persistent AD in adulthood, highlighting the significant disease burden of AD among adults [18].

In the current literature, the existing epidemiological data is largely centred on children. The 1-year prevalence among children can vary from 1% to 20%, with the highest prevalence observed in the Northern Europe region [8]. A recent multi-centre international study of young children aged 6-7 year olds and 13-14 year olds found a decrease in prevalence rates of AD in developed countries in Europe while the rates of AD have increased in some parts of Asia [19]. It was noted to be high in Indonesia, Thailand and Taiwan, but less so in Hong Kong, Korea and China [19]. This suggests a plateau effect in developed countries, due to possible changing environmental or allergen exposure factors. A plausible explanation for this trend may be due to increased awareness and better patient understanding of disease, leading to active treatment of mild disease with topical therapy. However, there has been an overall rising global prevalence of AD in recent years.

In a multi-centre study looking at international trends of AD, it was found that the prevalence rate has increased in Asia and Southeast Asia [20]. In particular, the prevalence rate of AD in Singapore has increased from 7.4% in 1994 to 9.2% in 2001 for 13-14 year olds, and has remained relatively stable since. A study in Japan, another developed Asian nation, found the point prevalence of AD to be 11% in the 16 to 18-year group, which was almost five times higher than 20 years ago [21]. Moreover, AD is also known to be more prevalent in urban and developed countries, and it is therefore expected that Singapore similarly has a higher disease burden [22].

Prevalence of atopic dermatitis was noted to be as high as 20.8% in an earlier study by Tay et al. [4]. This may suggest that the disease load in the adults may be equally high, given the increasing prevalence rates in Asia. However, AD prevalence data among adults is limited by the small number of studies conducted in Singapore.

A recent local, community-based, cross-sectional survey of Singaporeans between the ages of 1 and 80 years was conducted in 2017 [2]. It was a well-represented community study conducted among randomly selected housing blocks across Singapore. Participants were assessed for AD in their homes by trained medical students with the UK Working Party Criteria [2]. Overall, the prevalence of AD was 13.1%, with 20.6% of AD among children (20.6%) and 11.1% among adults. In another local national study of young military males aged 18 to 19 years old, the period prevalence of AD among all male servicemen was 9.76% [3].

However, it should be noted that any reported AD prevalence would be influenced by the disease definition and AD diagnostic criteria applied.

2.1.2. Disease burden of AD

Beyond being the most prevalent inflammatory skin disease, AD often seriously affects the quality of life in patients. Skin disease is the 4th leading cause of non-fatal disease burden worldwide according to the WHO Global Burden of Disease project and in Singapore, this is mainly attributed to AD [23]. In 2017. Skin and subcutaneous diseases rank 8th amongst leading cases of years lived with disability (YLDs), with Singapore ranking among the top countries in terms of YLDs attributed to skin diseases [23]. Besides its impact on quality of life, AD also imposes substantial direct and indirect costs with a large proportion arising from out-of-pocket expenses (37%) [24]. It has been estimated that AD costs over USD$5 billion annually in the United States of America [25]. In Singapore, the average annual cost per child with AD was estimated at USD$7,943 in 2017 [24]. Besides excess health care costs, it also imposes costs in the form of lost work productivity and lost work days and morbidities in terms of itch and loss of sleep [25].

2.2. Diagnostic criteria of AD

The term “atopic dermatitis” was first coined by Wise and Sulzberger in 1933 [26]. Some regions of the world prefer the word eczema. “Atopic eczema” and “atopic dermatitis” are still being used interchangeably in some parts of the world [27]. Dermatitis refers to some form of inflammation of the skin while atopy refers to a tendency to produce IgE antibodies when sensitized and exposed to allergen [28]. However, it remains debatable whether IgE is a reliable biomarker to determine atopy since there are situations when oligosensitization may be more important than overall levels of IgE [27].

Based on this definition of the condition and understanding, various diagnostic criteria were proposed. The Hanifin and Rajka criteria was the first AD diagnostic criteria, proposed in 1980 [29]. The major and minor criteria used provided some uniformity in

diagnosing AD in studies of hospital settings. It was developed mainly for clinical research purposes and consists of four major and 23 minor criteria (Figure 2.1). Despite being commonly used in clinical research setting, it has not been extensively validated. It has been reported that the sensitivity of Hanifin and Rajka diagnostic criteria was about 87.9% to 96.0% and its specificity about 77.6% to 93.8% [30, 31]. However, this diagnostic criteria is often deemed too complex and time consuming to be used in population cohort studies and in general clinical practice [32]. In the subsequent years, several diagnostic criteria were proposed. These included the Kang and Tian criteria and Japanese Dermatological Association criteria developed for use in their respective ethnic population [33, 34]. They were modified after the Hanifin and Rajka criteria or its simplified versions. There were either no validation done on these criteria or any validation done had inherent bias.

The UK Working Party Criteria was proposed in 1997 in view that the Hanifin and Rajka criteria could not be easily applied in clinical or epidemiological settings [31]. Since then, it has been widely used for clinical and research purposes. The criteria is made up of one mandatory itch criteria and five other minor criteria. Diagnosis of AD requires the fulfilment by the itch criteria and three out of the five criteria (Figure 2.2). Description of the individual components are being discussed in the following section.

3 or more basic features:

1. Pruritus
2. Typical morphology and distribution:
    a. Flexural lichenification or linearity in adults
    b. Facial and extensor involvement in infants and children
3. Chronic or chronically-relapsing dermatitis
4. Personal or family history of atopy (asthma, allergic rhinitis, atopic dermatitis)

Plus 3 or more minor features:

- Xerosis
- Ichthyosis/palmar hyperlinearity/keratosis pilaris
- Nipple eczema
- Cheilitis
- Recurrent conjunctivitis
- Dennie-Morgan infraorbital fold
- Keratoconus
- Anterior subcapsular cataracts
- Orbital darkening
- Facial pallor/facial erythema
- Pityriasis alba
- Anterior neck folds
- White dermatographism/ delayed blanch

- Immediate (type 1) skin test reactivity
- Elevated serum IgE
- Early age of onset
- Tendency toward cutaneous infections (especially *S. aureus* & *HSV*)/ impaired cell-mediated immunity
- Tendency toward non-specific hand or foot dermatitis
- Itch when sweating
- Intolerance to wool or lipid solvents
- Perifollicular accentuation
- Food intolerance
- Course influenced by environmental/emotional factors

Figure 2.1. Hanifin and Rajka Diagnostic Criteria

Mandatory: An itchy skin condition (parental report of scratching or rubbing in a child)	
Plus 3 or more of the following:	
1.	History of involvement of the skin creases such as folds of elbow, behind the knees, fronts of ankles or around the neck (including cheeks in children under 10 years old).
2.	A personal history of asthma or hay fever (or history of AD in a first-degree relative in children under 4 years old).
3.	History of general dry skin in the last year
4.	Visible flexural eczema (or eczema involving the cheeks/forehead and outer limbs in children under 4 years old)
5.	Onset under the age of 2 (not used if child is under 4 years old)

Figure 2.2. Components of the UK Working Party Diagnostic Criteria

Pruritus (Itch)

Itch is consistently present and therefore an important central feature to diagnose atopic dermatitis. In almost all available diagnostic criteria, itch remains an essential major or mandatory criterion in diagnosing AD. It has been the general consensus that patients with AD usually itches. However, this criterion does have the potential of missing out rare, mild asymptomatic cases.

History of Flexural Involvement

This was ascertained with the question if the skin condition affected skin creases over the limbs, neck and around the eyes. Flexural involvement is a common region of involvement in AD patients. This is deemed a classical area of involvement.

History of atopy

This minor criterion assessed for the presence of personal history of atopy which is important in diagnosing atopic diseases. In the original validation study, omitting this criterion resulted in a drop in sensitivity from 84.6% to 71.8% while the specificity increased from 96.0% to 97.3% [31].

History of a general dry skin

General dry skin or xerosis is an important feature in AD. This is probably related to the underlying skin epidermal barrier dysfunction of AD [35]. The *FLG* gene is one of the most important genetic determinants of AD and its mutation is related to a relative deficiency of natural moisturising factors (NMF) leading to xerosis in AD [36, 37]. This is why xerosis is often a lifelong feature in AD.

Early onset disease

AD is often deemed as a disease of early onset and therefore early age of onset was included as one of the minor criteria in the UK Working Party criteria. It is reported that about half the cases present before the age of 1 year and 85% have the disease by age of 5 years [38]. However, it should be noted that these observations were based on cohort studies of younger children and there might be bias in this reporting [39]. It has been reported that about 25% of adult with AD presented with the disease in adulthood [40]. This proportion can be as high as 54% in the United States and 60% in Italy [41, 42].

However, reporting of adult onset AD can be controversial as it can be affected by recall bias [39].

Visible flexural dermatitis

This relates to the observation that flexural involvement is commonly found in AD patients. The presence of visible flexural dermatitis, however, reflects current, active disease. This physical sign as one of the five minor criteria needs to be ascertained by an informed observer [43]. This person is usually trained by a dermatologist with standardized materials and instructions in a population setting [44].

UK Working Party diagnostic criteria of AD is among the most commonly used criteria in research studies, especially in community based epidemiological research [31]. Despite its validation in both the community- and hospital-based setting internationally, there are some potential limitations and challenges in its use [45, 46].

The diagnostic criteria was originally proposed and designed with epidemiological studies among paediatric population in mind and therefore has been mainly validated several times in children population but seldom in adult population [31, 47]. Clinical presentations and phenotypes of AD may be different in the adult population and in certain racial/ethnic groups. The original authors who proposed the criteria had acknowledged that clinical experience suggested that Afro-Caribbean children have a tendency to present with follicular lichenification, extensor involvement, later onset and a lower frequency of atopy [31]. The different clinical phenotypes among different age and ethnic/racial groups are being discussed in the subsequent sections. These could pose important limitations when the criteria is used in such adult populations. For example, a recent study from China applied the UK Working Party Criteria in a cohort of over 6000 Chinese children and adolescents and reported overall fair agreement (kappa = 0.40) between the UKWP

diagnostic criteria and clinical diagnosis [48]. It has been observed that sensitivity of the UK Working Party criteria fluctuated in various international population studies [47]. A study in Iran showed a low sensitivity of 10% while two other Japanese studies and an African study also reported slightly lower sensitivity [49-52]. This observed lower sensitivity has been attributed to the differences in phenotypes, environmental factors, cultural and language settings that these studies were conducted in [47].

Several validation studies have also been done using different cut off points to evaluate the sensitivities and specificities [43, 49, 53, 54]. Results of the original validation study is summarized in Table 2.1 [43]. Flexural dermatitis had the greatest positive predictive value of 54% among all the individual minor criteria. It also had a high specificity and negative predictive value. The study also examined the various arrangements and cut offs for the various criteria. Questions about atopy may result in a poorer performance of the overall diagnostic criteria.

	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
Visible flexural dermatitis	63	95	54	96
UKWP criteria (Itch plus 3 or more (3 out of 5))	70	93	47	97
Itch plus 2 or more (Questionnaire based (2 out of 4))	76	86	34	98
Itch plus 2 or more (2 out of 5)	80	86	34	98

Table 2.1. Sensitivity, specificity and positive predictive values of different arrangements of UK Working Party diagnostic criteria (UKWP criteria) against dermatologist's clinical diagnosis.

Although visible flexural dermatitis is the "best performing" criterion feature, the need for an informed observer to administer the minor criterion of visible flexural dermatitis could

possibly pose a challenge for its use in large scale population cohort studies. Some studies have examined the use of a modified UKWP diagnostic criteria based entirely on self-reported questions [43, 55]. A more recent study in a Danish general population examined the differences in using three different disease definition in an association study with cardiovascular comorbidity [56]. One definition was based on self-reported physician diagnosis of AD. The other two definitions were modified versions of questionnaire-based UK Working Party criteria and required at least 2 (UKWP 2/4) or 3 (UKWP 3/4) of 4 minor of the modified UK Working Party criteria respectively. UKWP 2/4 as a definition has previously been used in several large-scale Danish population cohort studies [53, 54]. It has been suggested that this gives a slightly lower specificity, but a higher sensitivity compared to using the UKWP 3/4 version. In this recent study, different risks of cardio-metabolic diseases were noted among adults with AD using different definitions, with the authors concluding that there was poor agreement using the different disease definitions [56].

Subsequent to Hanifin and Rajka criteria and UK Working Party criteria, various simplified diagnostic criteria were developed specifically to suit the needs of respective countries' context, particularly in Asian countries [47]. These include the criteria of the American Academy of Dermatology as well as national criteria in Thailand, India and China [57-60]. However, the UK Working Party criteria remains the most widely validated criteria [47].

2.3. Clinical presentation of AD

Atopic dermatitis is a heterogeneous skin condition. As discussed in the previous section, there is lack of a gold standard disease definition, likely attributable to the diverse clinical phenotypic presentation in the different age groups and ethnic groups in different geographical regions.

2.3.1. Disease by age group

Clinical phenotypes of AD are known to vary depending on the different phases in life [61]. They are broadly defined as the infantile, childhood, adolescent/adult and elderly type [62]. Infantile AD typically affects the cheeks and is generally the more acute lesions with edema and papulo-vesiculation. The scalp, neck and extensor parts of the limbs are also involved, but sparing the diaper area. There is prominent scalp involvement during this stage. This stage lasts from 3 months to about 2 years of age. Childhood AD starts from age 2 to 12. Chronic lichenification is noted to be more prominent and main sites are located over the flexurals (antecubital fossa and popliteal fossa). These are the classical sites that we are familiar with. Nummular plaques are relatively common at this stage. The next stage from adolescent to adults ranged from above 12 years of age to about 60 years of age. The classical typical areas remain, but there is a higher likelihood of hand and periorbital eczema as well as a more erythrodermic variant. Eczema in the elderly occurs in patients older than 60 years of age. This form of eczema often lacks clear characterizations and with the elderly, there could be many differential diagnoses such as cutaneous drug eruptions, allergic contact dermatitis and cutaneous T cell lymphoma. The lesions are now more extensor, often sparing the flexural regions and can be more scaly, lichenified and erythrodermic [63].

2.3.2. Disease by ethnicity/geographical region

It is relatively known that the disease prevalence and clinical phenotypic presentation are different among different ethnic groups and geographical region. This difference could be secondary to underlying genetic factors or environmental or cultural determinants [64]. Differences are mainly observed because of the differences in skin colour and distribution of the eczema rashes [64]. It has been reported that follicular, papular and lichenoid eczema are more in common in patients with skin of colour [65]. Notably, a widely quoted

prospective study of AD among the Nigerian black population revealed that papular lichenoid lesions can be as common as 54% in the study population [66]. These rashes were more commonly found in extensor joints (70.3%) compared to 51.8% involvement in the antecubital fossa region [66]. Indeed a 2012 review study comparing AD features between African Americans and whites reported that African Americans were more likely to have prurigo nodularis, lichenification and papular lesions over the extensors and trunk [67]. AD patients of darker skin colour also have a higher tendency of having post-inflammation pigmentary changes over areas of previous eczema affected skin [67]. Perception of erythema in AD patients with darker skin can also be challenging as they often appear violaceous or barely discernible [64].

However, racial differences may not be limited to just patients of European white, Asian or African descent. A systematic and meta-analysis reported different clinical characteristics of AD in eight different geographical regions in the world [68]. This study illustrated the differences in clinical characteristics of AD in the various regions. For example, AD patients from the South East Asia (SEA) region had more extensor involvement with lichenification and exudation.

These differences might be secondary to underlying immune phenotypes of the various ethnic populations as well as underlying differences in genetic and environmental factors such as climate and allergen exposure [68]. There can also be a varied behavioural response to itch in different populations [68]. Recent studies have reported that Asians exhibit an elevated cytokine production by type 1 and type 17 helper T cells, in addition to the classical type 2 helper T cell activation [69-71]. It is postulated that this is the reason why Asian AD phenotypes are more chronic, lichenified and psoriasiform, resembling the rashes of psoriasis. This is in contrast to African American AD patients having an up-regulation of T helper 22 response in addition to an elevated T helper 2 response [72].

Unravelling the pathophysiological mechanisms of AD might therefore shed light on the abovementioned differences observed among different population groups, as well as the risk factors and disease co-morbidities associated with AD.

2.4. Pathogenesis of AD

Given the heterogeneity of AD in its presentation, it is not surprising that the pathogenesis of the disease is a result of a complex interplay of multiple factors including genetics, skin barrier dysfunction, immune dysregulation, neuroimmune modulation (itch) and skin microbiota [73].

2.4.1. Genetic factors

Genetics has an important role in the development of AD. It can be inferred from the observation that having a family history of atopy including AD confers a very high risk of developing AD. Twin studies show that monozygotic twins have a concordance rate of about 80% compared to a concordance rate of 20% in dizygotic twins [74, 75]. Genomic association studies using genome-wide or targeted high-throughput approaches had identified about 34 genomic regions with AD risks associated genetic variants [76]. These genetic variants are often associated with skin barrier function or cutaneous inflammation. The downstream mechanisms are discussed in the subsequent sections.

FLG gene is the most known and well-studied gene associated with AD [77]. It encodes for the protein filaggrin, whose breakdown products are important in skin barrier function [77]. A single loss-of-function mutation within *FLG* exon 3 can be found in about 10% of European and Japanese individuals, while a previous local study reported a wider spectrum of filaggrin null mutations among Singaporean Chinese [78, 79]. As a result of this loss of function mutation, the profilaggrin molecule is truncated and cannot be converted into filaggrin monomers, leading to a decreased expression of filaggrin in the

skin. The resultant disease manifestation would be that of generalized xerosis, hyperlinear palms, keratosis pilaris and an increased tendency of having AD and a more severe disease [78, 79]. However, it should be noted that these mutations could not account for all cases of AD. Only about 20% of mild to moderate cases have the *FLG* mutations while more than half of those with the mutation do not develop any disease [80]. In some ethnic groups such as African Americans or African populations, the *FLG* mutations are rare in these AD patients [81, 82]. It has been reported previously that extrinsic AD has stronger association with *FLG* mutations compared to intrinsic AD [83]. Extrinsic AD is known to be associated with raised total IgE with specific sensitization [84]. Therefore, the presence of *FLG* mutations might be related to specific AD subtypes given the heterogenous nature of AD. Besides, *FLG* mutations, other genes contributing to the epidermal barrier function would include the *FLG2, hornerin (HRNR), MATT* and *SPINK5* [85-88].

Genes related to the innate and adaptive immune activation pathways also play an important role in the pathogenesis of AD. For example, genes related to AD are located among the Th2 cytokine cluster on chromosome 5q31.1 and are responsible for coding the Th2-related interleukins: IL-4, IL-13 [89, 90]. Polymorphisms of other Th2 related genes include IL-31 and receptors of IL-4 and IL-13 [91-93]. Some immune related genes are specifically important in certain ethnic groups, such as a particular mutation of the gene coding for STAT6 in Taiwanese population and that for TSLP in African American population [94, 95].

Given the advancement in technology for increasingly faster and cheaper sequencing, it is expected that newer discoveries of other gene loci associated with risk of AD will be made from both larger genome wide association analyses as well as whole genome/exome sequencing studies.

2.4.2. Barrier dysfunction

AD is often associated with significant epidermal barrier dysfunction. This could be secondary to either underlying genetic *FLG* mutation or as a consequence of ongoing skin inflammation. The latter could be a result of a reduced expression of important epidermal barrier proteins as a response to specific Th2 inflammatory cytokines such as IL4, IL-13 and IL-33 [96, 97]. Associated changes with reduced expression of these barrier proteins include increased pH, increased trans-epidermal water loss, increased irritability and permeability to small molecular weight haptens [98-100].

Epidermal barrier function relies on the intact function of the stratum corneum and tight junctions. Stratum corneum (SC) is the outermost layer of the epidermis and is often described as analogous to the "brick and mortar" structure [101]. The "bricks" are composed of the denucleated corneocytes while the "mortar" that gels the structure together refers to the intercellular lipids. Filaggrin as coded by the *FLG* gene is a protein responsible for binding the keratin intermediate filaments in the SC layer [102]. It is formed by enzymatic processing of its precursor, profilaggrin by proteases [103]. At the upper portion of SC, filaggrin is in turn further broken down by proteases into amino acids, urocanic acid and pyrrolidine carboxylic acid [104]. They provide the acidity of the skin and natural moisturizing factors in the epidermis [73].

Together with the tight junctions that regulate paracellular transport of water and solutes, both have important responsibilities in the barrier function of the skin [105]. Disruptions of both components are often seen in AD skin. There is often a reduced expression of epidermal differentiation-related structural proteins (e.g. filaggrin) and/or tight junction proteins as well as an imbalance of protease-protease inhibitors and epidermal lipids composition [76]. The epidermal disruption and change in pH would enhance the function

of serine proteases which in turn stimulate production of inflammatory cytokines and thymic stromal lymphopoietin (TSLP) to perpetuate the inflammatory process [106-108].

2.4.3. Immune dysregulation

Barrier disruption secondary to itch-scratch-cycle or barrier dysfunction triggers and promotes inflammation via the release of cytokines such as IL-1β, IL-25, IL33 and TSLP [109, 110]. These cytokines stimulate the innate lymphoid cells (ILCs) to produce Th2 chemokines such as CCL17 (thymus- and activation regulated chemokine (TARC)), CCL22 and CCL5 [111]. In addition, Th2 cytokines such as IL-4, IL-5, IL-13 which are known to be important in the pathogenesis of AD are produced by ILC2 [76]. The release of IL4 and IL-13 in turn drive the Th2 inflammation in AD by completing the vicious cycle of stimulating the keratinocytes to produce TSLP [112]. TSLP, besides activating Th2 cells, can also act on dendritic cells to migrate to lymph nodes and drive naive T cells into Th2 phenotypes [113, 114].

The main Th2 cytokines IL-4 and IL-13 can downregulate skin barrier function proteins such as filaggrin, loricrin and involucrin as well as activate the signal transducer and activator of transcription pathways (STAT3 and STAT6) to affect keratinocyte differentiation [115-117]. They can also induce B cell class switching and production of IgE [118].

Beyond involvement of the Th2 cytokines, Th1 and Th17 cytokines are also elevated in chronic AD and particularly in AD patients of Asian descent [69, 119]. Studies have also shown that severe AD patients have an increased number of circulating Th17 cells [120]. Such a characteristic inflammatory profile would have an impact on possible accompanying disease associations and subsequent treatment selection.

2.4.4. Neuroimmune modulation (itch)

Itch or pruritus is one of the most common manifestations of AD and remains an important mandatory component in diagnostic criteria of AD. Slow-conducting, non-myelinated C fibres and fast myelinated Aδ fibres transmit the sensation of itch via afferent fibres to the spinal cord with possible subsequent activation of the anterior cingulated and dorsolateral prefrontal cortex of the brain [121, 122]. It is also observed that there is an increase in nerve fibre density and a lower activation threshold of these nerves in AD patients [123, 124]. This is likely secondary to a disruption in the balance of nerve sprouting and nerve retraction factors.

This itch pathway can be activated by other pruritogens besides the well-known histamine in AD. The acute barrier disruption caused by scratching up-regulates inflammatory proteins which in turn drive Th2 inflammation via aforementioned mechanisms. These proteins can act as pruritogens and activate the itch pathways. For example, TSLP released by keratinocytes can directly activate the sensory nerves [125]. Type 2 cytokines can directly activate afferent itch nerves via IL-4 receptor subunit α and Janus Kinase 1 [126]. IL-31 similarly can activate itch pathways via its receptors on peripheral nerve fibres, dorsal root ganglions and keratinocytes [127]. All these would therefore contribute to the itch scratch cycle, perpetuating the AD inflammation and itch.

2.4.5. Skin microbiota

Skin of AD patients is often colonized with increased *Staphylococcus aureus* and predisposition to infection [128]. Among AD patients, S. aureus could be found in close to 70% of lesional skin and 39% of non lesional skin using culture-based methods [129]. The prevalence is affected by patient factors such as age and disease severity. The overall microbiota diversity is reduced with the corresponding increase in *S aureus* and

other members of the genus *Staphylococcus* during AD flares [129]. This was previously explained by a reduction in the levels of antimicrobial peptides influenced by Th2 mediated inflammation in the skin [130]. However, newer evidence has suggested that it might be secondary to the relative insufficiency or the dysfunction of these antimicrobial peptides [129, 131].

More evidence is needed to determine if these microbiota changes are merely consequences of the AD barrier dysfunction and Th2 inflammatory response, or if these changes can lead to and worsen AD [76]. *S. aureus* can cause further skin barrier dysfunction by releasing enzymes and toxins [132]. Via pattern recognition receptors in the skin like TLR2 and NOD2 receptors, S. aureus can also evoke a cutaneous proinflammatory state by T cells activation, recruitment, IgE mediation [133]. It can also trigger Th17 responses with an increased in IL-17 production [131].

2.5. Disease associations of AD

While our appreciation and understanding of AD as a chronic inflammatory, heterogenous skin disease might have improved in recent years, many pathogenic mechanisms of disease associations observed in epidemiologic studies remained unclear [134]. These observed associations from epidemiology studies provide important opportunities to understand the disease better. Its relationship with atopic, inflammatory and autoimmune conditions provides some insights on the background immune activation in AD [134]. Indeed, there is increasing evidence from numerous studies suggesting that AD is likely a systemic disorder.

It is widely accepted that AD is closely associated with other allergic diseases such as asthma and allergic rhino-conjunctivitis [135]. The latter diseases have been described to occur after the onset of AD and together they form the “atopic march”. The barrier

disruption in AD facilitates allergic sensitization by cutaneous allergen penetration with activation of inflammatory cascade. As a result, the Th2 and Th22 cytokines can lead to further barrier dysfunction. The subsequent migration of sensitization T cells and IgE into the nasal and airways contributes to subsequent allergic diseases in the atopic march [136, 137].

The lesional AD abnormalities such as epidermal hyperplasia, immune T cell infiltrates as well as increased levels of inflammatory cytokines are similarly found in patients with psoriasis [138]. Psoriasis is another chronic inflammatory skin disease, but with a better known relationship with other co-morbidities such as joints, cardiovascular systems and gastrointestinal [139]. However, in contrast to psoriasis, AD immune activation can also be observed in non-lesional skin and blood [140]. With these important similarities and differences with psoriasis, there are increasing interests to evaluate for similar associations of immune mediated skin diseases with AD.

AD has been observed to be associated with auto-inflammatory and auto-immunological diseases such as inflammatory bowel disease, rheumatoid arthritis, alopecia areata, a decreased risk for type 1 diabetes and lupus erythematosus in several epidemiology studies [141, 142]. These autoinflammatory and autoimmune diseases are known to have prominent Th1 and Th17 immune responses and suggest a possible link with the increased Th17 responses found in chronic AD or AD among Asians [118].

Besides this group of diseases, AD has been observed to be associated with cancer, neuropsychiatric disorders as well as cardiovascular risk factors. However, such reported relationships can be both conflicting and complex with many biases and unknown confounding factors.

2.5.1. Cancer and Neuropsychiatric disorders

Evidence surrounding the association of AD with malignancies has always been conflicting and controversial [134]. Evidence to date suggests a possible small significant increased risk of lymphoma among AD patients [143]. However, this was only observed in pooled cohort studies but not case control studies. This is especially so among the more severe cases. Several biases and confounding factors may affect the true association between AD and lymphoma. There is a possible risk of misclassification bias as cutaneous T cell lymphoma (CTCL) is a likely and important differential diagnosis of AD. Some cases of CTCL might be misclassified as AD cases in adults and therefore lead to an overestimation of the association [144]. More severe AD cases are also more likely to be on long term oral immunosuppressants such as cyclosporine and azathioprine which are known to confer risks of haematological malignancies [145]. This may be an important confounding factor in this relationship. Previously, to explain this observed association, it has been suggested that the long term chronic inflammation of AD might have contributed to the carcinogenesis observed [146]. It is however reassuring to note that more recent meta-analyses and reviews found no or inverse association between AD and other forms of haematological malignancies such as acute lymphoblastic leukemia and acute myelogenous leukemia [147]. Similarly, no or inverse associations are observed between AD and cancers such as bladder, pancreatic, brain and skin cancers [148]. It could possibly be related to the heightened immune surveillance with a resultant eradication of dysregulated cells by inflammation [149].

AD has consistently been shown to have an association with neuropsychiatric disorders such as depression, anxiety, suicidal ideation, attention deficit and autism spectrum disorder [134]. The likely underlying mechanism would be the huge disease impact that AD has on psychological health. The chronic itch and sleeplessness of the disease can

severely affect the quality of life and mental well-being of patients with AD [150]. Some have proposed an explanation that the pro-inflammatory state of AD with an increased level of inflammatory cytokines could penetrate the blood brain barrier and affect behavior and emotions via the central nervous system (CNS) [151]. Furthermore, as discussed in the prior sections, central mechanisms of itch sensation are known to exist and could also possibly affect neuropsychiatric behavior.

2.5.2. Cardiovascular risks/diseases

Emerging evidence suggests that adult AD patients have a higher risk of having coronary artery disease and congestive heart failure [152, 153]. A recent study has found that there is increased coronary artery calcium score in the computed tomography angiography of adult AD patients [153]. However, the evidence for risk of myocardial infarction and stroke among AD patients remained conflicted. While the earlier cohorts from US and Taiwan revealed an increased risk, subsequent cohort studies from Denmark and US suggested otherwise after adjusting for confounding factors [154-157]. It is therefore likely that AD mediates its risks of cardiovascular disease indirectly via the shared risk factors of metabolic risks and mental wellbeing. It has been observed that adult AD patients have significant risks of having a self-reported history of hypertension, diabetes and hypercholesterolemia before and after adjusting for confounding factors [7]. This could either be mediated by the chronic inflammatory state or contributed by poor health behaviors among AD patients. AD patients are known to have a higher risk of smoking, drinking alcohol and having reduced physical activity [154].

2.5.3. Association with Obesity

Obesity is the main co-morbidity of interest with AD in our study and will therefore be discussed in detail in this and the following section.

In a recent meta-analysis, it was observed that both adult and children AD patients have a higher risk of being overweight and obese in studies from North America and Asia, but not in Europe [11]. In the analysis, adults who were obese had 1.56 times the odds of having AD (95% CI: 1.24-1.95) and those who were overweight had 1.29 times the odds of AD (95% CI: 1.05-1.59). Two subsequent population-based case control studies have further found significant positive associations between obesity and AD patients in Germany [158, 159]. A national cross-sectional study in Korea of more than 5,000 adults observed a positive association between BMI equal or greater than 25kg/m^2 and AD [10]. In contrast, two questionnaire-based surveys of 30,000 adults and 8,617 participants (6 to 44 years old) from Sweden and Poland did not find a significant relationship between obesity and AD [160, 161]. Both were self-administered, questionnaire-based survey study.

Only two studies evaluated the relationship between AD and adiposity in terms of waist circumference (WC). In the aforementioned Korean study, those with abdominal obesity (WC ≥ 80cm) had a higher risk of AD [10]. This association was similarly observed in a cross-sectional study of children with moderate to severe AD in US. In this study, AD was associated with a higher percentile of WC (OR 3.92; 95% CI: 1.50-10.26) [162].

Obesity was also found to be associated with greater severity of AD in children and adolescents in three studies [163-165]. One US case control study did not find an association with disease severity [162]. A recent national local study evaluating more than 10,000 Singaporean military conscripts aged 18 to 20 years observed that those who were overweight had 1.23 times the odds of moderate to severe AD (95% CI: 1.12 - 1.35) while those who were obese had 1.44 times the odds of moderate to severe AD (95% CI: 1.28 to 1.63) [166].

While most studies show consistent results in the association between obesity and AD, review and analysis of current studies revealed how heterogeneous the reporting of outcomes and classification of obesity could be. Some studies have relied on self-reported diagnosis of AD while others diagnosed AD with specific diagnostic criteria. Classification of obesity was also different in various studies and regions of the world. This could potentially influence the results and rendered some studies not amendable to meta-analysis.

It is important to be aware of these associations in order to relieve the burden for AD patients. The mechanistic link between obesity and AD is still poorly understood. Understanding the burden of obesity, pathogenesis and its classification would be important to hypothesize the possible mechanistic link between the two chronic diseases.

2.6. Obesity

2.6.1. Epidemiology

Obesity is defined as a state of excessive accumulation of body fat with adverse effects on the health. There is a rising worldwide prevalence of overweight and obesity. It has been estimated in 2014 that 38% of men and 40% of women were overweight or obese and the worldwide prevalence has doubled since 1980 [167, 168].

In 2013, 34.3% of all Singapore adults in 2013 was overweight (BMI cut off ≥ 25 kg/m^2), with 40.2% of males and 28.6% of females classified overweight [169]. The prevalence of obesity was on a rising trend from 5.5% in 1992 to 8.6% in 2013. Those who are in the 30-39-year-old have the highest prevalence of obesity. Based on the Asian cut offs for BMI risk categories, 34.8% have a moderate risk BMI and 17.7% have a high-risk BMI [169].

2.6.2. Classification

It is known that there can be variable distribution of fat around the body, such as over the abdomen, subcutaneous or around/within the organs which may be different between population groups. Measurement of adiposity requires methods that can measure body fat accurately, taking into account such variability.

BMI (kg/m^2) is an index to assess total body fat and is recommended by the World Health Organization (WHO) for classifying adults into underweight, overweight and obesity.

Classification	BMI
Underweight	<18.5
Normal	18.5-24.9
Overweight	25.0-29.9
Obese class I	30.0-34.9
Obese class II	35.0-39.9
Obese class III	≥ 40.0

Table 2.2. Classification of Obesity

However, it should be noted that BMI as an index to stratify health risks may be less accurate in adults older than 65 years old. This is due to a loss of lean body mass with age. While the WHO Expert Consultation Group recommends the above cut offs as international classifications, individual countries need to decide on their cut offs based on different risks situation [169].

It has been reported that several population groups from China, Taiwan, Pakistan and Singapore exhibit a higher prevalence of Type 2 diabetes and other cardiovascular risk

factors even at normal BMI of < 25 kg/m^2. For Asians, BMI cut offs of 23.0 kg/m^2 and 27.5 kg/m^2 have been used to infer cardiovascular risks instead of 25.0 kg/m^2 and 30.0 kg/m^2 cut offs [169, 170].

Cardiovascular risks	Asian BMI cut-offs (kg/m^2)
	<18.5
Low	18.5-22.9
Moderate	23.0-27.4
High	27.5-32.4
Very High	32.5-37.4
	≥ 37.5

Table 2.3. Asian BMI cut-offs for cardiovascular risks

Specifically, for abdominal obesity, other markers of measurement have been proposed. These include waist circumference, waist-hip ratio and waist to height ratio.

Waist circumference reflects well both the degree of visceral adiposity as well as overall adiposity. Similarly, the cut offs recommended for Asians are lower (Table 2.4) [169].

Guideline	Waist circumference (cm)	
	Men	Women
WHO	102	88
Asia Pacific Consensus	90	80

Table 2.4. Waist circumference cut offs for men and women

Waist-hip ratio (WHR) is another measure of abdominal obesity. The cut-offs for obesity among men and women are 0.90 and 0.85 respectively [169].

2.6.3. Pathogenesis

Obesity is often simply caused by an imbalance of energy intake and energy expenditure, resulting in an over-accumulation of fat tissue in the body [13]. This accumulated obese adipose tissue then secretes inflammatory signals (cytokines) that cause a chronic state of inflammation. Within the adipose tissue, increased levels of TNF-α, IL-6, IL1β, CCL2 and adipokines are found but in modest levels compared to the usual immune response to infection and trauma [171, 172]. Kinases c-jun N-terminal kinase (JNK), inhibitor of κ kinase (IKK), protein kinase R (PKR) and Toll like receptors are activated and contribute to the increased cytokine levels [173-175]. It has been shown that macrophages are also recruited into adipose tissue and contribute to the inflammation [176]. This inflammatory state is chronically maintained but with a reduced metabolic rate [177]. Genetic studies have also revealed an association between obesity and inflammatory gene network [13].

2.7. Hypothesis behind the mechanistic link between AD and obesity

2.7.1. Inflammation in AD and obesity

The chronic inflammatory state of obesity mediated via raised levels of adipokines and cytokines such as leptin, adiponectin, TNF-α, and IL-6 could possibly be associated with AD [178]. Pro-inflammatory cytokines such as leptin, TNF, IL-6 and interferon γ can provide a positive feedback loop leading to sustained promotion of inflammation. For example, leptin can enhance T cell survival and result in the production of more proinflammatory cytokines [179]. Another adipokine, adiponectin, has anti-inflammatory effects but is found decreased in obesity. Adiponectin can inhibit the levels of IL-6 and TNF [180]. Low levels of adiponectin have been observed with an increased AD prevalence [181]. All these can lead to a chronic inflammatory state that can predispose individuals to hypersensitivity reactions in AD.

Recent evidence has also described the presence and role of IL-33 in human adipose tissue inflammation [182]. IL-33 is also known to promote the production of Th2 cytokines and mast cell degranulation in AD patients [183].

The association of obesity and AD could also be mediated via gender specific factors such as hormonal changes. It has been reported in two studies that the association between obesity and AD/asthma was observed only in females [184, 185]. It is known that peripheral conversion of androstenedione to oestrone and testosterone to oestradiol occurs in adipose tissue [186]. Oestradiol has been shown to shift Th1 to Th2 inflammation [187]. It can also increase IL-4 and IL13 expression with eosinophil recruitment and degranulation [187].

2.7.2. Skin physiology parameters changes in AD and obese skin

It has been shown in some studies that there are significant epidermal barrier changes in obese individuals, but results are conflicting [12, 188-190]. Two studies observed increased trans epidermal water loss (TEWL) in both obese children and adult participants [12, 188]. The adult study attributed the increased TEWL to increased sweating and hypothesized that the parameter merely mimics a disrupted skin barrier instead of a true abnormal epidermal change since sodium lauryl sulphate (SLS) susceptibility was not increased [12]. The children study attributed this to a change in the stratum corneum structure and hypothesized that adipokines in the obese state may activate keratinocytes and cause changes to the stratum corneum [188]. With the resultant dry skin, it can exacerbate or predispose someone to having AD.

However, a study in Italy demonstrated that the TEWL was significantly lower in obese subjects compared to controls instead [189]. The authors suggested that this could be due to obesity causing a drop in the water permeability of the cutaneous barrier [189].

Leptin, an adipokine, increases mitosis of the keratinocytes and stimulates in vitro fibroblast proliferation and collagen synthesis leading to a change in water permeability [191].

A recent study in a group of European adult females reported a “U” shape relationship between TEWL values and BMI categories [190]. Higher TEWL were reported in the normal weight and morbidly obese females compared to those who were overweight and obese. An inverted “U” shape relationship between skin surface hydration was correspondingly observed between TEWL values and BMI groups. The authors therefore proposed that increased weight can improve skin physiology within certain optimal limits [190].

2.7.3. Microbiome in AD and obesity

There have also been multiple studies reporting the relationship of gut microbiome and obesity as well as that of skin microbiome and AD [192, 193]. Increasingly, emerging evidence is suggesting that the “overlying” body surface microbiota have a role in impacting the underlying immunological profile or response of AD [192]. In turn, skin microbiome composition might be affected by gut microbiome flora, whose composition also has an impact on the development of both obesity and AD.

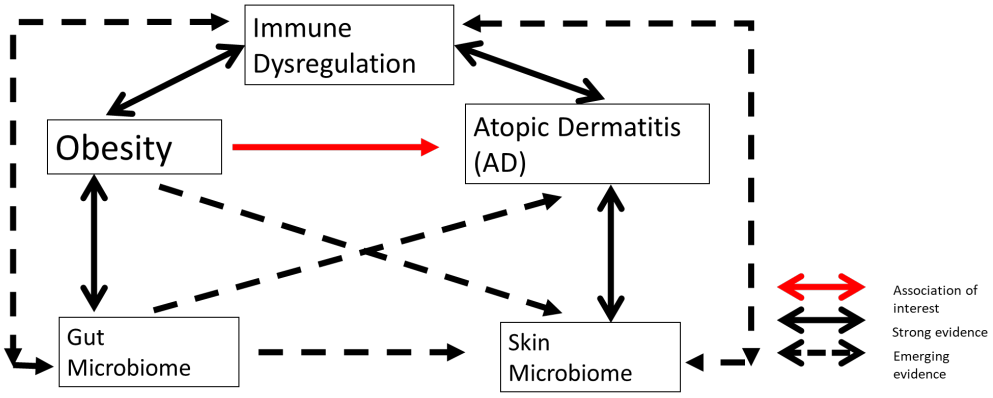


Figure 2.3. Hypothesis and overview of how obesity influence AD via immune dysregulation and microbiome profile.

The relationship of obesity and AD could potentially be mediated or confounded by complex interplay of skin or gut microbiome differences. Local community microbial composition over flexural skin areas is distinctly different in AD patients compared with controls even during periods of non-flare states [194]. There is an increase in relative abundance of *Streptococcus* and *Gemella* but decreased for *Dermacoccus* in AD-prone versus normal healthy skin. This illustrates the cross-interactions between the skin microbiome, the overlying cutaneous microenvironment and the underlying immune system, perpetuating a vicious feedback cycle that leads to AD flare [194].

There has also been emerging evidence to suggest that obesity could also potentially influence the skin microbiome diversity at various sites. This was reported in a study with gravid women with class III obesity where the skin microbiome diversity differed between the various skin sites on their abdomen [195]. There was also another recent skin microbiome study that reported a decreased in microbiome diversity and increased relative abundance of *Corynebacterium* species with increasing BMI [15]. Beyond these studies, we currently have limited understanding of the mechanisms by which obesity status impacts the diversity and composition of skin microbiome in humans.

Comparatively, multiple high--profile studies have shown that obese individuals have a distinctive microbial composition of the gut [196]. It has been observed that the characteristic “obesogenic microbiota” consists of a relative decrease in the abundance of *Bacteroidetes* coupled with a relative increase in the *Firmicutes* [197]. The relative abundance of various microbial species has been observed to be different in obesity. These include *Clostridium innocuum, Eubacterium dolichum*, and *Catenibacterium mitsuokai, Lactobacillus reuteri, Lactobacillus sakei, Actinobacteria*, and *Methanobrevibacter smithii* [198]. There have been several proposed mechanisms on how underlying gut microbiota might affect obesity. These include changes in energy

harvesting, changes in metabolic pathways, possible changes in brain and behaviour as well as the role of induced inflammatory responses. More evidence is however needed to understand whether the observed gut microbiome changes increase the risk of developing obesity, or are downstream sequelae of obesity [199]. It is not easy to discern the two possibilities as there is a complex interplay between diet, microbiota, immunity and obesity development.

There have been studies to suggest an association between prior gut microbiome dysbiosis and subsequent development of AD [200]. Longitudinal birth cohort studies have observed that those who developed atopic dermatitis later in life tend to have a preceding less diverse gut microbiome [201]. It has been hypothesized that colonization of gut flora during early years affects the subsequent priming and development of the immune system [202]. The microbiome and their metabolic products such as butyric acid can affect the differentiation of naïve T cells into various types of Th cells or T regulatory cells [203, 204]. This in turn have an impact on the relative Th1 or Th2 skewing resulting in chronic inflammation and autoimmune or allergic conditions [205]. However, this has not been observed from individuals with established atopic dermatitis [201]. Gut microbiome was analyzed with next-generation sequencing in both children and adults with AD and compared with healthy controls. There was no difference in overall gut microbiota diversity between the two groups. Increased abundance of *Faecalibacterium prausnitzii* and reduced short-chain fatty acids were however detected in patients with early onset AD [206]. In addition, some early basic science studies have observed that the gut microbiome composition could affect the skin microbiome distribution via metabolic products from the guy microbiota such as short chain fatty acids and propionic acid [207, 208]. This could offer explanations on how gut microbiome and skin microbiome might be intimately related.

From the above, we therefore hypothesize that obesity may partly influence AD and its severity by changing the underlying systemic immunophenotypic profile as well as altering the biodiversity and functional capacity of the gut and skin microbiomes.

2.7.4. Genetic links between AD and obesity

Although genetic links between AD and obesity have not been widely reported, there were previous reports of genetic links between asthma, atopy and obesity. β2-adrenergic (*ADRB2*), glucocorticoid (NR3C1) and fractalkine receptors genes have previously been reported in the relationship between asthma/atopy and obesity [209, 210]. The strong relationship between AD and atopy/asthma may suggest that there might be similar genetic links between AD and obesity. These genetic links might also be mediated through common factors influencing both AD and obesity, e.g. psychiatric stress or physical inactivity.

2.8. Using genetics to understand causation (Mendelian Randomization)

Observational epidemiological studies investigating disease and risk factors associations can be subjected to various biases and confounding. They are especially prone to the problem of reverse causation. Various methods have been proposed, including evaluating the dose and strength of association or other components in the Hill Bradford Criteria, as well as using various study methods to triangulate the true relationships [211]. Mendelian randomization is one of these methods that utilizes the law of random assortment and serves as a mean of reducing confounding in examining exposure-disease associations in epidemiology studies [212].

With the improvement in genomic sequencing technologies, there has been a significant explosion of many genome-wide association studies (GWAS) evaluating genotype-

phenotype associations. Large numbers of genetic variants in the form of single nucleotide polymorphism are being identified for various disease exposures. With a method known as two-sample Mendelian randomization analysis where exposure GWAS data are interpreted together with outcome GWAS studies, various disease associations could be interrogated to assess if there might be a causal relationship [212].

Using Mendelian randomization, genetic associations of an instrument with an exposure and outcome can be used to assess the effect of an exposure on an outcome. Three assumptions of Mendelian Randomization need to be fulfilled [213].

(IV1) the instrument is associated with the exposure;

(IV2) the instrument is independent of known and unknown confounders;

(IV3) the instrument is independent of the outcome conditional on the exposure and confounders

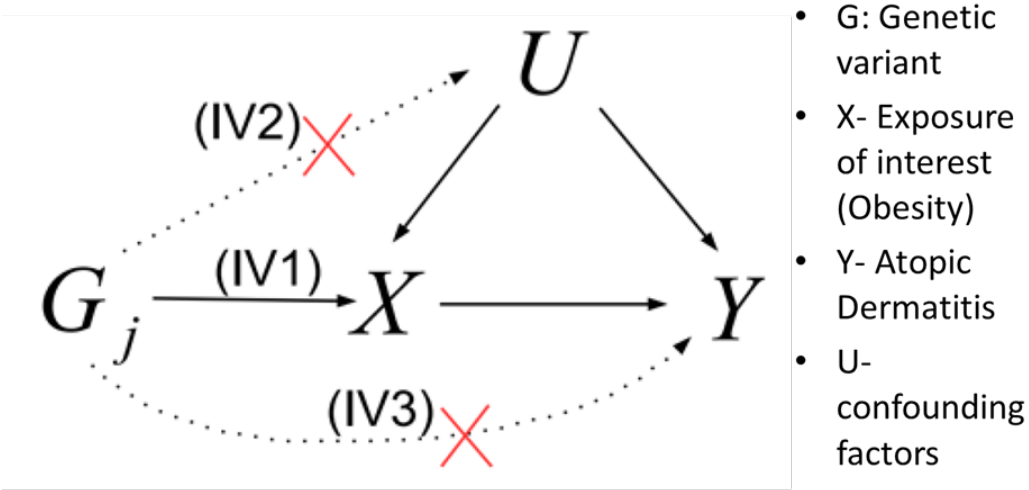


Figure 2.4. Genetic instrument in Mendelian randomization analysis

Assumption 1 (IV1) can be easily and directly tested. However, Assumption 2 and 3 (IV 2 and 3) cannot be readily tested and there may be several threats to the assumptions. Horizontal pleiotropy (genetic variant affecting multiple traits via several pathways),

linkage disequilibrium and population stratification (different subgroups having different genetic ancestries) can introduce genetic confounding in the context of assumption 2 and 3 [212].

Details on how to perform a two-sample Mendelian Randomization analysis are being discussed in the methods section.

2.9. Aims of this thesis

This thesis aims to evaluate the relationship of atopic dermatitis with obesity and its related inflammatory metabolic disturbances in a general adult population cohort and understand possible underlying mechanistic links behind this relationship.

Specific aims were:

SA1. To determine the prevalence of AD and its association with obesity and its related metabolic disturbances.

SA2. To determine the extent to which obesity contributes to the differences in skin physiology parameters in AD.

SA3. To determine the extent to which obesity affects the diversity of skin microbiome in AD.

SA4. To investigate the inflammatory biomarkers in AD using multiplexed proteomics platform and the extent obesity underpins the inflammatory condition in AD.

SA5. To use genetic associations and concept of Mendelian randomization to explore causal relationship between obesity and AD.

3. Materials and methods

3.1. Study design

The studies of this dissertation included participants of the ongoing Health for Life in Singapore (HELIOS) cohort study and analyzed data collected in the dermatology domain of the study.

The HELIOS study by LKC School of Medicine is a population cohort study with an eventual target sample size of 10,000 participants from 30 to 85 years old from the general population. The dermatology domain of the study consists of questionnaire survey questions, physical examination for signs of flexural dermatitis, physiology measurements as well as biological samples collection (blood and skin tape samples) for all participants. Data related to other medical domains, such as cardiovascular, respiratory, liver, endocrine, eye were also collected. They were in the form of questionnaire responses, signs from physical examination and laboratory results.

Studies 1 and 2 addressing the first two specific aims of the dissertation were based on a cross sectional study design to evaluate the prevalence, characteristics and disease associations/risk factors of AD as well as skin physiology parameters among this well-represented sample of adults of 30 to 85 years old from the HELIOS population cohort.

Studies 3 and 4 addressing the subsequent two specific aims of the dissertation were based on a matched case-control study design to evaluate the skin microbiome diversity and serum inflammatory biomarkers profile in a selected group of Chinese AD participants age- and gender-matched to a group of healthy controls. Due to resource constraints, it would not be practical to perform detailed skin microbiome and proteomics serum biomarkers analysis on the whole HELIOS population cohort. Therefore, the skin

microbiome and proteomic serum biomarkers analyses were limited to 300 of the study participants using a (1:1) matched case control design. Details of sample size calculations are discussed in the subsequent section 3.5. Taking into considerations ethnic differences and to further enhance our statistical power, only Chinese participants were included. One hundred and fifty AD cases were selected with 150 age- (within ± 5 years) and gender-matched controls. Definition criteria for AD cases are further discussed in section 2.3.

3.2. Study population

Participants were enrolled from the general population in the community as part of the LKC School of Medicine HELIOS study. The HELIOS study aims to recruit a representative sample of participants from the general population and the study team monitors the recruitment rates and situation. The cohort recruited men and women aged 30-85 years old. In order to facilitate the study of differences across ethnic groups, the HELIOS study oversampled Indian and Malay participants such that the distribution of participants is approximately 60% Chinese, 20% Indian, and 20% Malay. Details of the HELIOS study is advertised on various media and participants contacted the study team for participation on their own accord. No response rates could be calculated as no denominator was available.

Inclusion criteria

- Singaporean citizens and permanent residents aged 30-85 years.

Exclusion criteria

- Pregnancy (self-reported or by pregnancy test).
- Acute illness
- Unable or unwilling to give informed consent

3.3. Classification of AD: Disease/dependent factor

Atopic dermatitis was the main disease/dependent factor in this study. The screening assessment and diagnosis of atopic dermatitis among HELIOS study participants was based on the following

1) a self-reported diagnosis of atopic dermatitis
2) UK Working Party Diagnostic Criteria for Eczema (Itch plus 3 out of 5 minor criteria) [31]
3) Modified UK Working Party Diagnostic Criteria for Eczema based on questionnaire responses (Itch plus 2 out of 4 minor criteria) [53, 54]

The modified UK Working Party Diagnostic Criteria for Eczema was selected as the main criteria of diagnosing AD in our subsequent analyses to address our study aims SA1, SA2, SA3 and SA4. It was deemed to be not as over-sensitive as the self-reporting screening criteria, and yet not too stringent compared to the conventional UK Working Party Diagnostic Criteria that involves physical examination for presence of flexural dermatitis.

Mandatory criteria

- Itch (Questionnaire SK3)

AND

Minor criteria (4 items)

- Personal history of flexural dermatitis as above (Questionnaire SK3B)
- Personal history of dry skin in the last 12 months (Questionnaire SK2)
- Personal history of asthma or allergic rhinitis (Questionnaire items in respiratory domain)
- Onset of signs and symptoms under the age of 2 years (Questionnaire SK3A)

Questionnaire questions can be found in Annex B. The screening methodology of AD was adapted from the standardized field epidemiology training package developed by Prof Hywel Williams, University of Nottingham [44]. It included a physical examination component that examined for visible flexural dermatitis e.g. antecubital and popliteal fossae in the conventional UK Working Party Diagnostic Criteria.

This consists of a list of AD related survey questions administered by trained interviewers as well as physical examination by trained technologists.

The list of AD related survey questions can be found in dermatology domain questions of the HELIOS study as detailed in Annex A. It focused on the following areas:

- Past symptoms of itch
- Recent itch
- Age of itchy skin condition
- Flexural involvement
- Asthma
- Rhinitis
- Dry skin
- Self-reported eczema
- Location of eczema
- Impact of eczema
- Extent of eczema
- Family history of eczema

In addition, study participants were asked to self-report the severity of their AD as mild, moderate or severe in the questionnaire component. Visible flexural dermatitis over the following body areas were recorded by trained screening technologists.

- fronts of elbows,
- behind the knees,
- fronts of ankles,
- around the neck,
- around the eyes

A validation exercise of the accuracy of visible flexural dermatitis was conducted. Visible flexural dermatitis is one of the five minor criteria of the original UK Working Party Criteria but can be subjected to inaccuracies in field epidemiology studies.

To assess the accuracy of the screening technologists, a random sample of 50 participants was evaluated for presence of flexural dermatitis by a trained dermatologist in AD who was blinded to the results(Figure 3.1). It was planned for 25 participants labelled as no flexural dermatitis and 25 participants labelled as having flexural dermatitis.

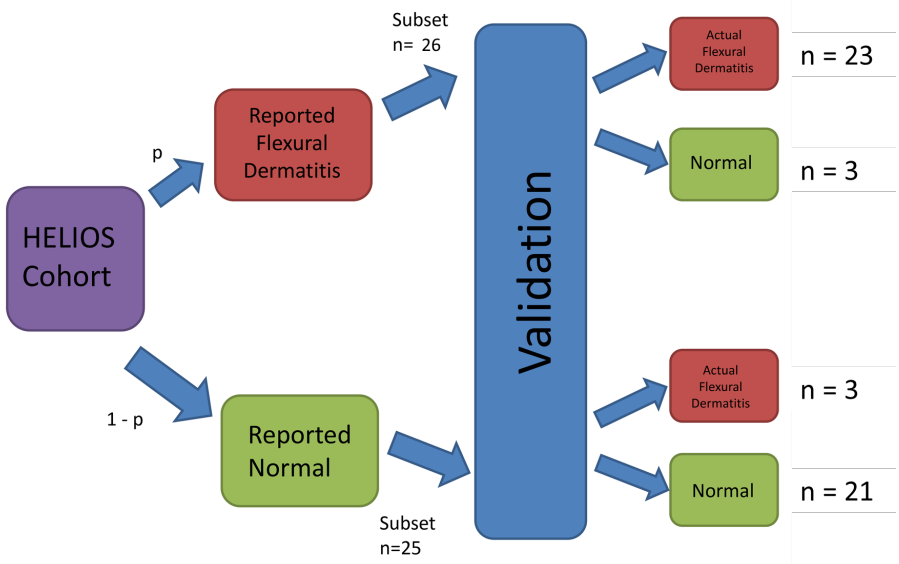


Figure 3.1. Validation exercise of the accuracy of visible flexural dermatitis.

Eventually, there were 24 normal controls and 26 participants with visible flexural dermatitis. Twenty-three out of 26 study participants were correctly assessed to have flexural dermatitis while 21 participants were correctly assessed not to have flexural dermatitis, resulting in a sensitivity and specificity of 88.5% and 87.5% respectively.

		Diagnosis of flexural dermatitis	
		Yes	No
Classification of flexural dermatitis	Yes	23	3
	No	3	21

Table 3.1. Sensitivity and Specificity analyses of recording flexural dermatitis

3.4. Exposure/Risk factors- Classification of obesity

Obesity in participants was identified via the measurement of

1) Body Mass Index (BMI) derived from the formula: weight divided by (height x height) in *S.I* units.

   According to the WHO Criteria for obesity classification, a BMI of ≥ 25.0 kg/m^2 implies overweight while a BMI of ≥ 30.0 kg/m^2 implies obesity [169]. Participants were classified into obesity categories for all analyses based on the WHO criteria in this thesis to be consistent with other international studies. A lower BMI cut-off of 27.5 kg/m^2 has previously been proposed for Asians; however, it mainly reflects a cut off for similar obesity-related diabetic and cardiovascular risks [170].

   Body weight and height are measured once wearing light clothing using computerized measuring instruments with automated data capture.

2) Waist and Hip circumference in cm

   A non-stretchable sprung measuring tape was used for waist and hip circumference measurements. Waist circumference was measured once at the mid-point between the iliac crest and the lowest rib, at the smallest circumference of the natural waist (if visible), and hip circumference was measured once at the maximum circumference of the buttocks, over the greater trochanters. Waist hip ratio (WHR) was then calculated based on these measurements.

3) Body fat percentage by bioelectrical impedance analysis [214]

Compared to muscle and body water, fat would impede electrical current flow more. Therefore, the body fat percentage could be calculated by measuring the current flow and using predictive equations, adjusting for age, sex, race, height and weight.

InBody 770 system was used to measure body impedance at the same time as measuring weight by passing a very weak, imperceptible electric current between limb leads. Using a computer algorithm including variables such as age, sex, weight and height, an estimate of body fat and fat free mass can be obtained.

4) Dual energy x-ray absorptiometry (DXA) [215]

Whole body DXA provides a measurement of total and proportionate bone, fat, and lean mass, and also gives the distribution between segments of the body (arms, legs, head, thorax, trunk, and pelvis) automatically within seconds of the scan. This information complements the standard anthropometric measurements collected and gives a more accurate measurement of body fat and its regional distribution. Whole-body DXA scans were performed by trained and certified radiographers using HorizonTM W densitometer (S/N 30052M, Hologic, Massachusetts, USA, QDR software version 13.6.0.5) in the HELIOS cohort. Information including Visceral fat (Kg) and total fat mass (Kg, %) are directly reported from the DXA Hologic scanner. Visceral fat is estimated by subtracting total fat with subcutaneous fat in the L4 region using an algorithm as part of the DEXA Hologic scanner, validated against both CT scan and MRI (known gold standards), reflecting DXA's accuracy in estimating visceral fat [215-217].

Participants lie on their backs on an X-ray table to undergo a body scan. The test involves the participant lying supine on the scanner for the whole-body scan and hip, while for the lumbar spine scan, the hips and knees are flexed and supported with a

cushion. The test involves a low-dose X-ray (Hologic Horizon W model or equivalent), equivalent to 3 hours of background radiation or ½ a chest X-ray.

Cardiovascular and metabolic syndrome diseases will be based on self-reported responses to the question of a history of the following conditions:

- Hypertension
- hyperlipidemia
- diabetes mellitus.

Blood pressure was measured three times in the right arm using the Omron 907 Professional Blood Pressure Monitor.

3.5 Sample size calculation

For studies 1 and 2, we calculated the sample size that we would expect to give us adequate statistical power to assess the associations between AD and obesity, and therefore the observed differences in skin physiology parameters.

Sample size was calculated based on the following parameters using the Quanto ver 1.2 software:

Power: 80%

Confidence level: 95%

Expected prevalence of overweight/obese in non-AD group: 40.1% (based on Singapore Health Survey 2010) [218]

Expected/Observed relative odds of AD for every unit (kg/m2) increase of BMI: 1.03

Expected prevalence of AD in Singapore: 10%

Expected Odds ratio (OR) of AD among overweight/obese participants: 1.30* (based on Zhang et al., 2015 data in Asia region) [11]

As the estimates of actual/observed relationship between AD and obesity could vary in different studies, we computed a range of sample size required to evaluate the associations of AD with obesity, both as a continuous measure (BMI) and categorical measure (BMI ≥ 30 kgm^2). Figure 3.2 shows the range of sample sizes required for the study. Figure 3.3. showed the sample size calculation for study 1 and 2

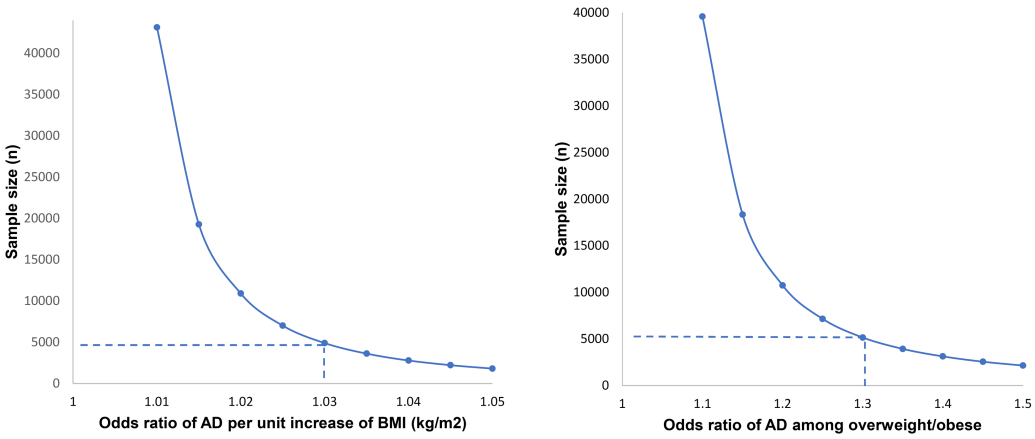


Figure 3.2. Sample size curves for associations between obesity and atopic dermatitis (AD). Sample size curves with continuous measure of adiposity (Body Mass Index, BMI) and a binary variable (Overweight/Obese, BMI ⩾ 27.5 kgm2) at 80% power and a type 1 error rate of 0.05. Left panel: For different values of odds ratio (OR) of AD per unit (kg/m2) increase in BMI, the dashed line shows sample size needed (n=4900) to detect OR of 1.03 per unit increase of BMI. Right panel: For different values of OR of AD among overweight/obese participants compared to lean, the dashed line shows sample size needed (n=5160) to detect an OR of 1.30.

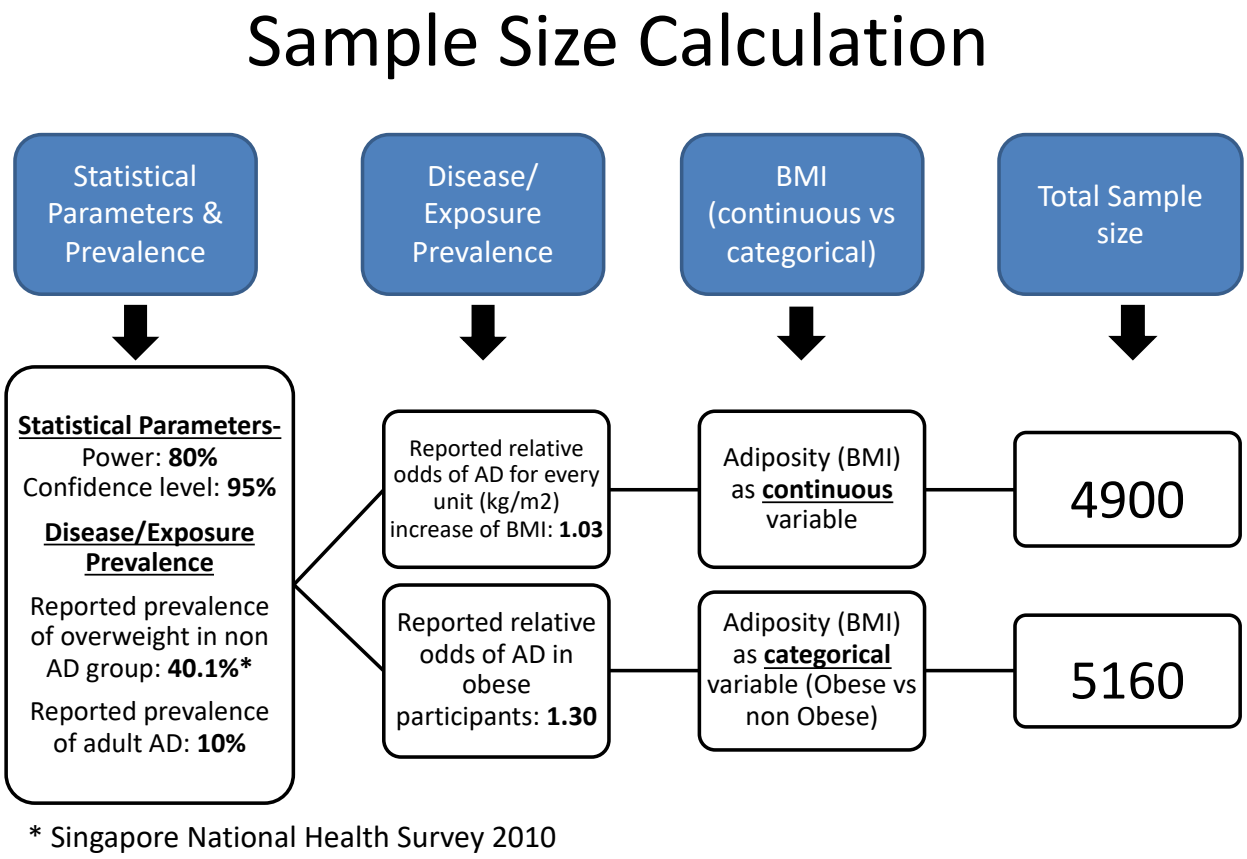


Figure 3.3. Sample size calculation for study 1 and 2.

For studies 3 and 4, these were exploratory studies to determine the skin microbiome and serum inflammatory biomarkers profile among participants with AD and controls. However, it is known that biomarkers with diagnostic utility are anticipated to have odds ratio for disease of >4.0 [219]. A sample size of 300 participants including 150 cases of confirmed AD and 150 age- and gender-matched controls without AD provides 80% power to identify a biomarker with odds ratio for AD >2.0 at P<5x10-5 (after Bonferroni correction), assuming that the proposed biomarker is raised in 25% of the control population.

4. Study 1: Prevalence of AD and its relationship with obesity

4.1. Overview and rationale

Study 1 addressed the specific aim 1 of the thesis to evaluate the prevalence of AD in a large local general population cohort in Singapore. It also aimed to evaluate the association of AD and obesity, which is the primary association of interest in this thesis. As part of a larger ongoing cohort study with a myriad of other meta-data and co-morbidities information collected, it also allowed association analyses of AD with other co-morbidities.

In the study cohort, obesity was measured and classified according to anthropometric, bio-impedance and dual x-ray absorptiometry measures. This served as a form of sensitivity analysis in our assessment of the relationship between AD and obesity, and provided insights on whether certain form of adiposity would be important in the association.

Further to obesity measures, classification of AD in this study was according to the modified UK Working Party Criteria (Itch and 2 out of 4 minor questionnaire criteria). Prevalence of AD according to the original UK Working Party criteria (Itch and 3 out of 5 minor criteria), self-reported history of eczema and flexural dermatitis were also available for comparison.

Findings of this study would improve our understanding on the disease burden of AD in an adult population cohort. It would also add to the growing body of literature on the relationship between AD and obesity.

4.2. Methods and Analysis

Study participants from the HELIOS cohort study from initiation (December 2017) till January 2020 were included in the analysis. AD and obesity classifications have been described in the aforementioned section. AD was diagnosed by the modified UK Working Party Criteria.

The population cohort was analyzed in a descriptive manner to yield its demographic, social economic status (SES) and selected co-morbidities of the study participants. These selected co-morbidities included asthma, allergic rhinitis, hypertension, hyperlipidaemia and diabetes mellitus. The cohort prevalence of AD and obesity as well as other relevant co-morbidities were described. In addition, prevalence of AD using various diagnostic criteria of AD were also summarized and presented in the analysis.

Univariate analysis of the relationship between AD and exposure factors was performed using chi-square tests. Multivariate analysis with logistic regression modelling was done with AD as the dependent variable, and with exposure variables of interests and confounding variables such as age, gender, ethnic group, education level and SES as the independent variables.

Logistic regression models with AD as the outcome variable were built sequentially by adding confounders to the regression model according to the following stepwise approach:

Model 1: Demographic variables: Age, Gender, Ethnic group

Model 2: Model 1 + Education level and Socio-economic status (by household income)

Model 3: Model 2 + Lifestyle factors: Alcohol consumption and Smoking history

Odds ratio (OR), 95% confidence interval (CI), and P values were calculated to test the null hypotheses of no association between AD and exposure variables of interest.

A 2-sided P value of <.05 was considered statistically significant. All statistical analysis was performed using the Statistical Package for the Social Sciences (v25; IBM SPSS, Armonk, NY, USA)

4.3 Results

4.3.1. Demographic profile

A total of 5,560 HELIOS study participants were included in the analysis. Their age ranged from 30.0 to 84.8 years old, with a median age of 50.4 years old. About 60% of the participants were female. Majority (75.2%) of participants were Chinese, followed by Indians (15.2%) and Malays (8.5%). The demographic characteristics of study participants are summarized in Table 4.1. Compared to the Singapore census 2010 population (limited to the same age group of 30 to 84 years old), HELIOS participants were more likely to be female, Indian, more highly educated, higher household income [220]. Compared to the National Population Health Survey (NPHS) 2019 study, HELIOS participants were more likely to consume any alcohol but less likely to be current smokers [221]. (Annex A, Table S.1)

Characteristics	**AD (n=492)**	**No AD (n=5068)**	**Total (n=5560)**	**P value**
Age (years, mean)	48.9	51.5	51.2	***<0.001****
Gender (%)				
Female	66.3	59.1	59.7	***0.002****
Ethnic Groups (%)				0.094
Chinese	78.3	74.9	75.2	
Malay	5.9	8.8	8.5	
Indian	15.2	15.2	15.2	
Others	0.6	1.2	1.2	
Education level (%)				***<0.001****

Primary School or no education	3.0	5.6	5.3	
O levels or ITE	19.1	24.4	23.7	
A levels or diploma	21.7	24.7	24.2	
Undergraduate/Graduate	56.1	45.4	45.9	
Household income ($)/month (%)				***0.005****
<2000	12.2	13.1	11.1	
2000 - 3999	15.1	16.1	13.5	
4000 - 5999	14.1	19.8	16.3	
6000 - 9999	24.0	23.9	20.3	
>= 10000	34.6	27.1	23.5	
Alcohol Consumption (%)	24.4	20.1	20.3	***0.024****
Current smokers (%)	9.3	8.0	8.1	0.292
Asthma (%)	25.9	4.7	6.4	***<0.001****
Allergic Rhinitis (%)	17.3	2.1	3.3	***<0.001****
Atopy[ł] (%)	38.1	6.3	8.9	***<0.001****
Diabetes Mellitus (%)	9.3	8.4	8.5	0.456
Hypertension(%)	21.6	18.6	18.6	0.111
Hyperlipidemia(%)	33.7	34.2	33.4	0.812

ITE: Institute of Technical Education
ł Atopy: ever had either asthma or allergic rhinitis
Table 4.1. Characteristics of study participants

4.3.2. Prevalence of AD

The overall prevalence of AD in the HELIOS cohort was 8.8%. The prevalence figures are provided in Figure 4.1. The youngest age strata (30 to 40 years old) had the highest AD prevalence of 12.1%, while the oldest age group (greater than 80 years old) had the lowest AD prevalence at 4.5%. This is not surprising given that AD predominantly affects the younger population. Besides younger age, female gender, higher education level and

monthly household income, alcohol consumption and having atopic diseases (asthma and allergic rhinitis) were significantly associated with an increased risk of AD.

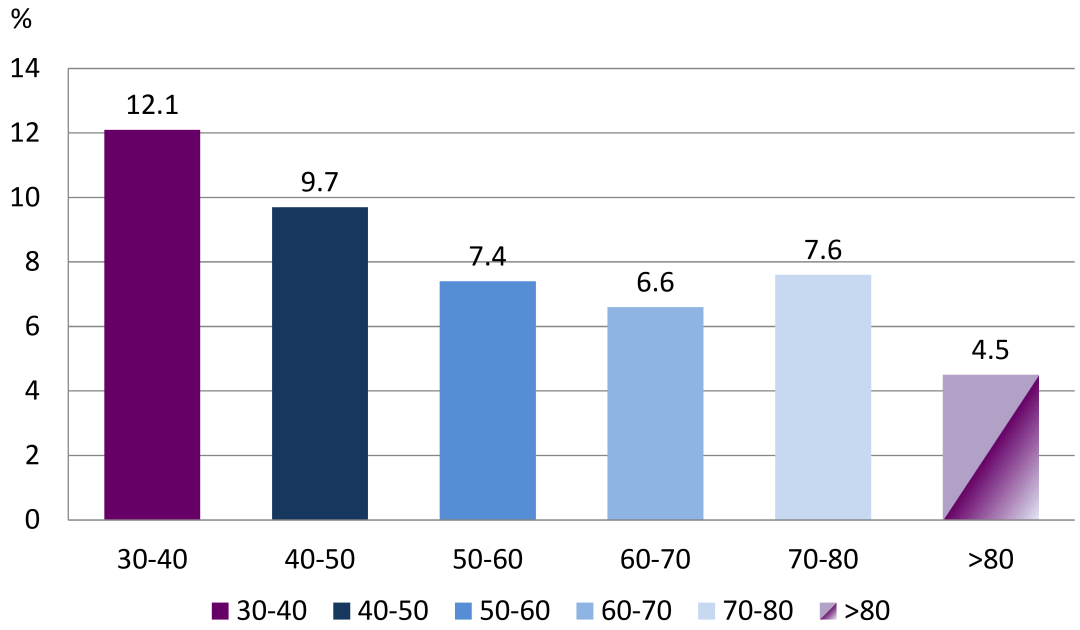


Figure 4.1. Prevalence of AD across the different age strata

The prevalence of AD according to various diagnostic criteria are provided as follows:

Diagnostic Criteria of AD	Prevalence (%)	Kappa statistic*	Agreement
Modified UK Working Party Criteria	8.8		
UK Working Party (UKWP) Criteria	2.8	0.46	Moderate
Self-reported eczema	19.0	0.32	Fair
Visible flexural dermatitis	3.2	0.23	Fair

* as compared to modified UK Working Party Criteria

Table 4.2. Prevalence of AD according to various diagnostic criteria.

The overall prevalence of AD according to the modified UK Working Party (UKWP) Criteria was 8.8% in our study cohort. About 19.0% of study participants reported a history of eczema. This was not surprising given that the prevalence of AD among children is

about 20% in Singapore. With the UKWP diagnostic criteria (where symptom of itch and 3 out of 5 minor criteria are required for diagnosis of AD to be made), the prevalence of AD was lowest at 2.8%. This possibly suggests that UKWP might be too specific and not sensitive enough in diagnosing AD in an Asian setting in Singapore.

The UKWP criteria for AD was in moderate agreement with the modified UKWP criteria with Kappa statistic of 0.46. This was not unexpected since the modified criteria focused on the questionnaire responses of the participants without the presence of flexural dermatitis on physical examination. Self-reported history of eczema and visible flexural dermatitis had fair agreement with the modified UKWP criteria. However, there might be some degree of recall bias among study participants for the single self-reported question on whether they ever have eczema.

4.3.3. Severity of AD

Only 230 out of 492 study participants with AD based on the modified UK Working Party Criteria reported their severity of their skin condition. This was based on their response to a survey question on how they perceive their skin condition to be (“Mild”, “Moderate” and “Severe”). Majority of participants with AD (52.6%) reported a mild severity while 10.4% of participants reported a severe disease severity. Participants with a more severe disease (moderate to severe AD) were significantly more likely to be younger, having a lower education level and were less likely to have allergic rhinitis or atopy (Annex A, Table S.2).

4.3.4. Relationship of AD with obesity

The odds ratio (OR) of AD among obese participants (BMI ≥ 30 kgm^2) was 1.23 after adjusting for age, gender and ethnicity (95% CI: 0.92 – 1.64; p = 0.159). The relationship of obesity as determined by BMI remained not significant in other expanded versions of the model. The OR of AD per unit increase in BMI was 1.02 (95% CI: 0.99 – 1.04; p = 0.189) after adjusting for age, gender and ethnicity. Table 4.3 shows the relationship of AD with obesity as measured by anthropometric measures such as BMI, waist hip ratio, body fat by bioimpedance as well as DXA imaging measures.

Increasing waist hip ratio (WHR) was significantly associated with an increased risk of AD. The OR of AD per unit increase of WHR was 4.67 (95% CI: 1.10 – 19.86; p = 0.037). This relationship remained significant when the multivariate model was expanded to include other confounding variables. Obesity as determined by WHR cut offs (Female ≥ 0.85 and Male ≥ 0.90) was significantly associated with an increased risk of AD when adjusted for age, gender and ethnicity (OR: 1.24; 95% CI: 1.01 – 1.51; p = 0.038).

Increasing total body fat as represented by total body fat percentage by bioimpedance and DXA scan was not significantly associated with an increased risk of AD. However, there was increasing OR of AD with increasing visceral fat mass (in kg) adjusted for age, gender and ethnicity (OR: 1.53; 95% CI: 1.05 – 2.23; p = 0.028) as well as in the expanded multivariate models.

	Relative odds of AD with obesity						
	Model 1			Model 2		Model 3	
	OR	95% CI	P value	OR	P value	OR	P value
Obesity (BMI ≥ 30.0)	1.23	0.92-1.64	0.159	1.15	0.379	1.16	0.377
BMI			0.366		0.678		0.664
Lean	1.00			1.00		1.00	
Overweight	0.99	0.79-1.24		0.99		0.97	
Obese	1.23	0.91-1.66		1.15		1.14	
BMI (kg/m^2)	1.02	0.99-1.04	0.169	1.01	0.463	1.01	0.511
Waist-Hip ratio (WHR)	4.67	1.10-19.86	***0.037****	5.13	***0.042****	4.65	0.057
Obesity by WHR Female ≥0.85, Male ≥0.90	1.24	1.01-1.51	***0.038****	1.22	0.074	1.20	0.098
Percentage body fat (%)-Bioimpedance	1.01	0.99-1.03	0.261	1.01	0.421	1.01	0.378
Total Fat Percentage (%)- DXA	1.01	0.99-1.03	0.298	1.01	0.240	1.01	0.255
Total Fat Mass (kg)-DXA	1.01	0.99-1.02	0.147	1.01	0.324	1.01	0.368
Visceral Fat Mass (kg)-DXA	1.53	1.05-2.23	***0.028****	1.55	***0.036****	1.52	***0.046****

Model 1: adjusted for age, gender, ethnicity
Model 2: adjusted for Model 1 + education level, household income
Model 3: adjusted for Model 2 + current smoking, drinking alcohol

Table 4.3. Relationship of AD with different measures of obesity in various logistic regression models.

The relationship of AD disease severity with various obesity was similarly evaluated based on the limited information available in only 230 AD participants. There was no statistical significance in the relationship between moderate to severe AD and obesity as determined by various measures. It was however observed that there was increasing OR of moderate to severe AD with increasing WHR adjusted for age gender, ethnicity, education level and monthly household income (Model 2) (OR: 137.73; 95% CI: 1.51 – 12547.36; p = 0.032) (Annex A, Table S.3).

4.4 Discussion

The prevalence of AD among the HELIOS cohort was 8.8% (n=5,560). This prevalence is comparable to known population prevalence of AD (10%) in Singapore that was observed from two published cohort studies on the local prevalence of AD [2, 3]. The first study was a population cohort of young adult males mainly aged 18 to 19 years old who underwent compulsory medical screening upon enlistment into the military service [3]. Diagnosis of AD were based on diagnosis codes and made by military medical officers during medical screening. As all Singaporean males are mandated by law to serve in the military, this prevalence of AD therefore represented a national prevalence among these young adults. The second study was a community population survey of a well-sampled representative population across a wide spectrum of age groups [2]. The study provided prevalence of AD across several age groups.

However, the prevalence of AD in this study was slightly lower than what was previously reported. This could possibly be attributed to two reasons. The median age of study participants of this HELIOS cohort was about 51.2 years. As it can be seen in the results, the prevalence of AD decreased with increasing age strata, which is consistent with what has previously been reported. The older age of the HELIOS

cohort may possibly account for its slightly lowered AD prevalence. Indeed, the overall AD prevalence when age adjusted for the Singapore Census 2010 population was calculated to be 9.2% [220]. Furthermore, the HELIOS cohort consists of participants who have volunteered to participate in the study. The demographic distribution of these participants might differ from that of the general Singapore population. As mentioned earlier in the methods section, the HELIOS study oversampled Indian and Malay participants to facilitate investigation of characteristics in minority ethnic groups. In the study cohort of this study, the proportion of Indians (15.2%) was higher than the Singapore population (Singapore Census 2010) proportion (8.8%) and might therefore affect the overall prevalence of AD. The overall AD prevalence adjusted for the ethnic group proportions of the Singapore Census 2010 population was calculated to be 8.6%.

AD was determined in this study using the modified UK Working Party Criteria. It has always been a challenge to classify AD which is a heterogenous skin disease in population cohort studies [56]. This has affected results of the relationships of AD and obesity as well as other cardiometabolic co-morbidities in various epidemiology studies. Although Hanifin and Rajka criteria has been considered a gold standard diagnostic criteria for AD, it can be cumbersome to be administered in large scale population cohort studies. Thyssen et al. have therefore proposed and implemented this AD diagnostic criteria in their AD association studies [54]. The modified criteria relied only on the questionnaire responses of the participants and are deemed to be more sensitive in diagnosing AD while being less sensitive to recall bias compared to a single self-reported AD question. It should be noted that the original UK working party criteria and its modified version in this thesis have been mainly validated in UK and other European countries among children. One of the minor criteria included a disease onset before age of 2. This could result in recall bias and ascertainment bias

against the older participants, resulting in younger AD patients more likely to be diagnosed than their older counterparts as they were more likely to recall or have improved healthcare access to general or specialist skin care. However, the prevalence of AD based on the modified diagnostic criteria in this thesis, has been comparable to observed AD prevalence in other local studies.

In this study, it was noted that participants with AD were more likely to be younger, female, having a higher socio-economic status in terms of monthly household income, education level attained and a strong personal history of atopic diseases. These observations are consistent with what has previously been reported in the literature [222]. Among those who reported severity of AD, it was interesting to note that those with a lower education level had a more severe disease. It provides suggestions that increasing awareness of the disease and its treatments could still be enhanced in certain patient groups. Those with a more severe disease were also less likely to have allergic rhinitis or atopy, reflecting more intrinsic type of AD in this cohort [223].

One of the strengths of this study have been the use of various methods to measure obesity including imaging methods. This provided an opportunity to investigate how various measures of obesity may affect the risk of AD. General obesity as defined by BMI as well as BMI as a continuous variable were not found to have a significant association with risk of AD. Similarly, total fat mass and percentage as measured by bioimpedance device and DXA imaging respectively were also not observed to have a significant association with AD in this study. However, increasing abdominal or visceral obesity as measured by waist hip ratio and visceral fat mass on DXA scan were found to be significantly associated with an increased risk of AD in various regression models. This is similar to what was reported in a Korean cross-sectional population cohort study of about 5,200 adults aged 19 to 40 years old. In that study, it

was reported that both general and abdominal obesity was considered a risk factor for AD in women but not in men [10]. In contrast, only abdominal or visceral obesity was associated with risk of AD in this study. This observation was limited predominantly in men when the analyses was performed with stratification according to gender. This was despite a higher risk of AD among female participants in this study. An important distinction was an older median age of this study cohort compared to the Korean cohort. It has been previously hypothesized that oestrogen in women as well as oestrogen produced in adipose tissue may contribute to the increased impact of abdominal obesity on the risk of AD among female participants [184, 185]. With a median age of 50.2 years, a large proportion of females in this study cohort would be post-menopausal. With a lack of oestrogenic hormones in the post-menopausal state, the risk of AD with abdominal obesity may be more enhanced. This might be why despite a higher overall proportion of female having AD in this study, the effect of increased WHR on risks of AD was observed to be significant in men.

Abdominal obesity has also been reported by Silverberg et al. as a risk factor for more severe AD. Central obesity was related to moderate to severe AD in children aged 4 to 17 years old [162]. WHR has been found to be a more accurate predictor of co-morbidities and mortality, particularly in older people than waist circumference or BMI [224, 225]. It likely reflects underlying disturbances of systemic inflammatory markers such as cytokines, stress and reproductive hormones [226]. Therefore, it is not surprising that visceral or abdominal obesity was related to increasing risk of AD in this study.

While the relationship of AD disease severity with various obesity measures did not reach statistical significance, it can be seen that some of the obesity measures such as BMI groups and waist hip ratio approached statistical significance. As only 230

participants provided disease severity information, this has affected the statistical power to detect a difference. However, the magnitude and direction of the effect size of the relationship of AD disease severity with various obesity measures were consistent with the relationship of AD risk with various obesity measures. These findings would have important implications given that obesity not only affects the risk of AD but also its disease severity. Therefore, targeting obesity might have both a primary and secondary prevention effect on AD.

Evaluations of the possible underlying mechanisms of this observation are discussed in the subsequent studies and sections.

5. Study 2: Extent to which obesity contributes to differences in skin physiology parameters

5.1. Overview and rationale

Skin epidermal barrier dysfunction is a typical manifestation of atopic dermatitis (AD). Its disrupted skin barrier function is characterized by an increased in trans-epidermal water loss (TEWL), lowered moisture content of skin surface corneocytes and a higher skin pH. TEWL is a common indicator of skin barrier function, it measures the amount of water lost from within the skin to the external atmosphere. Skin surface hydration refers to hydration values at the stratum corneum level. The measurement takes into account the changing thickness of the stratum corneum's dry layer. Skin surface pH refers to apparent skin surface pH. High skin pH generally reflects a bad skin barrier function. These three different skin physiology measures give a reflection of the overall skin barrier function of the person.

Beyond a mere chronic skin condition, AD has been reported to be associated with adiposity as well as other co-morbidities in various epidemiological studies. This is further supplemented by recent evidence that genetically determined increase in adiposity is related to an increased risk of AD [227].

Two studies of limited sample size have reported an increased TEWL in obese children and adults [12, 188]. However, another study reported conflicting evidence that rate of TEWL is lower in obese individuals, especially those with intra-abdominal obesity [189]. Another more recent study suggested that there might a paradoxical tendency that TEWL was lowest in overweight individuals but became highest in obese individuals [190]. There is therefore a need to clarify how adiposity affects skin barrier function, as skin barrier dysfunction could possibly be one of the mechanistic pathways linking obesity and AD.

5.2. Methods and Analysis

Skin physiology measurement consists of measuring TEWL by a vapometer, skin surface hydration by a MoistureMeter SC and skin surface pH by a pH meter. Three readings of each skin physiology measures are taken over the ventral forearms at the midpoint between elbow crease and ventral wrist crease. The area of measurement had to be free of any active skin lesions. Study participants were also acclimatised for at least 20min at an ambient temperature and room humidity to eliminate sweating. Measurements were taken three times in the same room with a standardized pre-set temperature and humidity setting of about 24.0 degree Celsius and 60% respectively. The average value of the three measurements were calculated and included for analysis.

TEWL is the main indicator of skin barrier function, it measures the amount of water lost from within the skin to the external atmosphere. Skin surface hydration refers to hydration values at the stratum corneum level. The measurement takes into account the changing thickness of the stratum corneum's dry layer. Skin surface pH refers to apparent skin surface pH. It is used to evaluate the state of diseased skin with acute or chronic changes. High skin pH coincides with bad skin barrier function.

Vapometer is equipped with closed cylindrical chamber that contains sensors for ambient relative humidity (RH %) and ambient temperature (T °C). When placed on the skin, water vapour from the skin collects in the chamber and over time, the humidity in the chamber increases, slowly at first, and thereafter linearly. Trans-epidermal water loss (TEWL) value is calculated from the information the instrument acquires based on this increase. Measurements are not affected by ambient airflows.

Figure 5.1. Vapometer

MoistureMeter SC is a sensitive instrument for measuring the skin surface hydration values at stratum corneum level. The measurement takes into account the changing thickness of the stratum corneum's dry layer. It is an all-in-one unit that comprises a sensitive round probe head and a built-in contact pressure sensor.

Figure 5.2. MoistureMeter

pH Meter is a sensitive instrument with a glass probe and a main machine for measuring apparent skin surface pH.

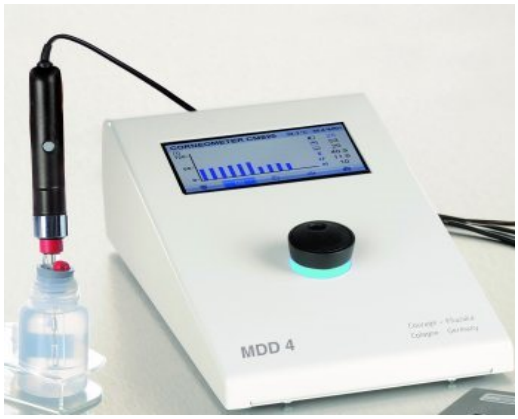


Figure 5.3. pH meter

Descriptive analysis was first performed to evaluate the characteristics of the study participants. Mean TEWL, skin surface moisture and pH values among the three

adiposity groups, namely lean, overweight and obese, were compared and presented. Skin physiology measurements noted to be outside the detection limits of the equipment were excluded from the analysis. The skin physiology measures were then assessed for normality and found to have skewed data distribution using Kolmogorov-Smirnov test and Normal Q-Q plots (Figure 5.4). Log transformation was performed to transform these skin physiology values to approximately conform to normality before further analysis. As part of sensitivity analysis, data values greater than 3rd quartile plus 3 times interquartile range as well as smaller than 1st quartile minus 3 times interquartile range were excluded to see if statistical outliers influenced the results.

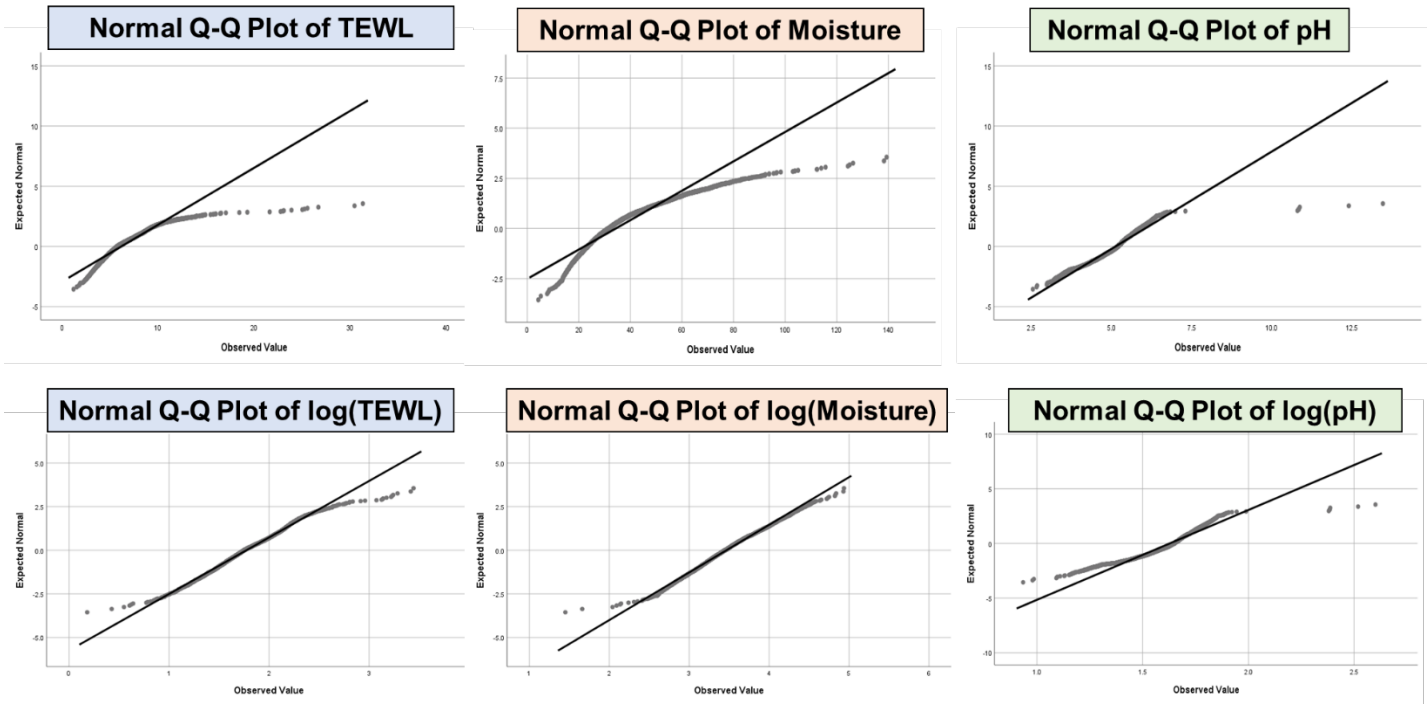


Figure 5.4. Normal Q-Q plots of skin physiology parameters before and after log transformation.

Linear regression analyses were performed with the log transformed skin physiology measurements as dependent variables and the different measures of adiposity as independent variables adjusted by age, gender and ethnicity. As AD is a known condition with characteristic skin barrier function, similar linear regression analysis with

AD as an independent variable was performed to assess if skin physiology measurements are consistent with the literature in our study.

To identify how the various measures of obesity might possibly affect skin physiology status, multiple linear regression analyses on the log transformed skin physiology measurements were performed as independent variables adjusted by age, gender and ethnicity. Results from these multiple linear regression analyses in the form of standardized β coefficients and p values were presented as volcano plots. Adjustment for multiple testing was performed using the Benjamini-Hochberg Procedure with the false discovery rate (FDR) set at 0.1. All statistical analysis was performed using the Statistical Package for the Social Sciences (v25; IBM SPSS, Armonk, NY, USA) and R statistical software (RStudio version 1.2.1335).

5.3. Results

A total of 5,490 participants had data on skin physiology measurements. Characteristics of participants have been presented in section 4.3.1. Relationship of demographic characteristics with skin physiology parameters are presented in the following table.

	Log (TEWL) (n=5487)			
	Beta	SE	Odds ratio (EXP(β))	P
Age (-10 years)	-0.030	0.003	0.970	<0.001
Female (Gender)	-0.106	0.008	0.899	<0.001
Ethnicity				<0.001
Chinese	Ref		1.00	
Malay	0.049	0.015	1.05	0.001
Indian	0.072	0.012	1.07	<0.001
Others	0.022	0.040	1.02	0.556
Log (Moisture) (n=5490)				
Age (-10 years)	0.0023	0.0042	1.00	0.565
Female (Gender)	-0.020	0.010	0.980	0.042
Ethnicity				<0.001
Chinese	Ref		1.00	
Malay	-0.128	0.017	0.880	<0.001
Indian	-0.156	0.014	0.856	<0.001
Others	0.036	0.046	1.04	0.439
Log (PH) (n=5317)				
Age (-10 years)	-0.010	0.0017	0.990	<0.001
Female (Gender)	0.34	0.004	1.40	<0.001
Ethnicity				0.864
Chinese	Ref		1.00	
Malay	-0.021	0.007	0.979	0.005
Indian	0.010	0.006	1.01	0.086
Others	0.013	0.020	1.01	0.493

SE: standard error

Table 5.1. Relationship of demographic characteristics with skin physiology parameters.

Age, gender and ethnicity are significant demographic factors affecting skin barrier function. Older participants were likely to have lower TEWL and higher skin surface moisture while female participants had a lower TEWL but a lower skin surface moisture. They would need to be adjusted for when evaluating the relationship of obesity measures with skin barrier function.

5.3.1 Impact of obesity on skin barrier function

There were increasing mean TEWL values across lean, overweight and obese participants. These were 6.05 ± 0.03, 6.25 ± 0.05, 6.76 ± 0.11 g/h/㎡ respectively (p

< 0.001) (Figure 5.5a). This relationship remained significant with regression analysis of log(TEWL), adjusting for age, gender and race (Beta = 0.023; p < 0.001). Regression analyses of log(TEWL), adjusting for age, gender and race performed for various measures of adiposity such as BMI, BMI > 30 kg/m2, and waist hip ratio yielded statistical significance as illustrated in Table 5.2 and corresponding volcano plot (Figure 5.6a). There were increasing TEWL with increasing adiposity.

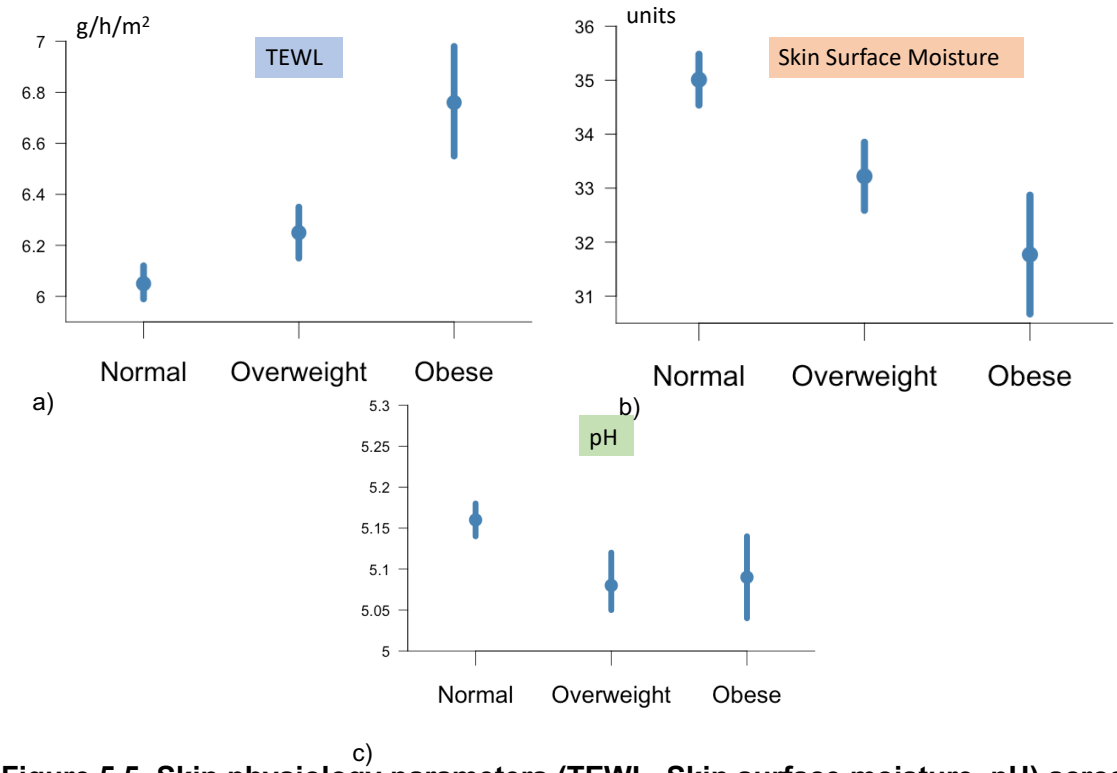


Figure 5.5. Skin physiology parameters (TEWL, Skin surface moisture, pH) across BMI groups.
The points represent mean values of these measurements. The bars represent 95% confidence intervals for mean.

There was a decreasing skin surface moisture with increasing BMI, with highest in the normal weight group at 35.01 ± 0.24 units compared to the overweight and obese groups (33.22 ± 0.32 and 31.7 ± 0.56 units respectively; p <0.001) (Figure 5.5b). This relationship remained significant with regression analysis of log(Moisture), adjusting for age, gender and race. (Beta = -0.034; p < 0.001). Regression analyses of log(Moisture), adjusting for age, gender and race performed for other measures of

adiposity such as BMI > 30 kg/m2, weight, waist and hip girth as well as total and visceral fat mass yielded statistical significance as illustrated in the volcano plot (Figure 5.6b). There were decreasing skin surface moisture with increasing adiposity.

Skin pH appeared to be more acidic and similar between overweight and obese groups but increased in the normal weight group (p <0.001) (Figure 5.5c). However, regression analysis of log(pH), after adjusting for age, gender and race showed a significant negative correlation with increasing BMI (Beta = -0.002; p < 0.001). Skin pH became increasingly acidic with increasing adiposity. This trend remained significant with other measures of adiposity such as BMI > 30 kg/m2, weight, waist and hip girth as well as visceral fat mass (Figure 5.6c).

	Log (TEWL) (n=5487)		**Log (Moisture) (n=5490)**		**Log (PH) (n=5317)**	
	Beta	P value	Beta	P value	Beta	P value
BMI						
Lean						
Overweight	0.001	0.905	-0.029	***0.012***	-0.011	**0.003**
Obese	0.059	***<0.001***	-0.058	***<0.001***	-0.013	***0.022***
BMI (kg/m^2)	0.003	***0.003***	-0.004	***<0.001***	-0.002	***<0.001***
Waist-Hip ratio (WHR)	0.189	***0.003***	-0.325	***<0.001***	-0.046	0.075
High WHR (Male ≥ 0.90) (Female ≥ 0.80)	0.024	***0.007***	-0.039	***<0.001***	-0.004	0.231
Total Fat Mass (kg)	0.000	0.721	-0.004	***<0.001***	0.000	0.075
Visceral Fat Mass (kg)	0.005	0.749	-0.094	***<0.001***	-0.012	***0.011***

*Adjusted for age, gender and ethnicity

Table 5.2. Relationship of various obesity measures on skin barrier function.

Sensitivity analysis excluding statistical outliers of skin physiology measures yielded similar results (Annex A, Table S.4). Various obesity measures remain significant with increasing water loss, decreasing skin surface moisture and more acidic pH with increasing obesity. Notably, waist-hip ratio variables were statistically not significant although the beta estimates were numerically similar to the main analysis.

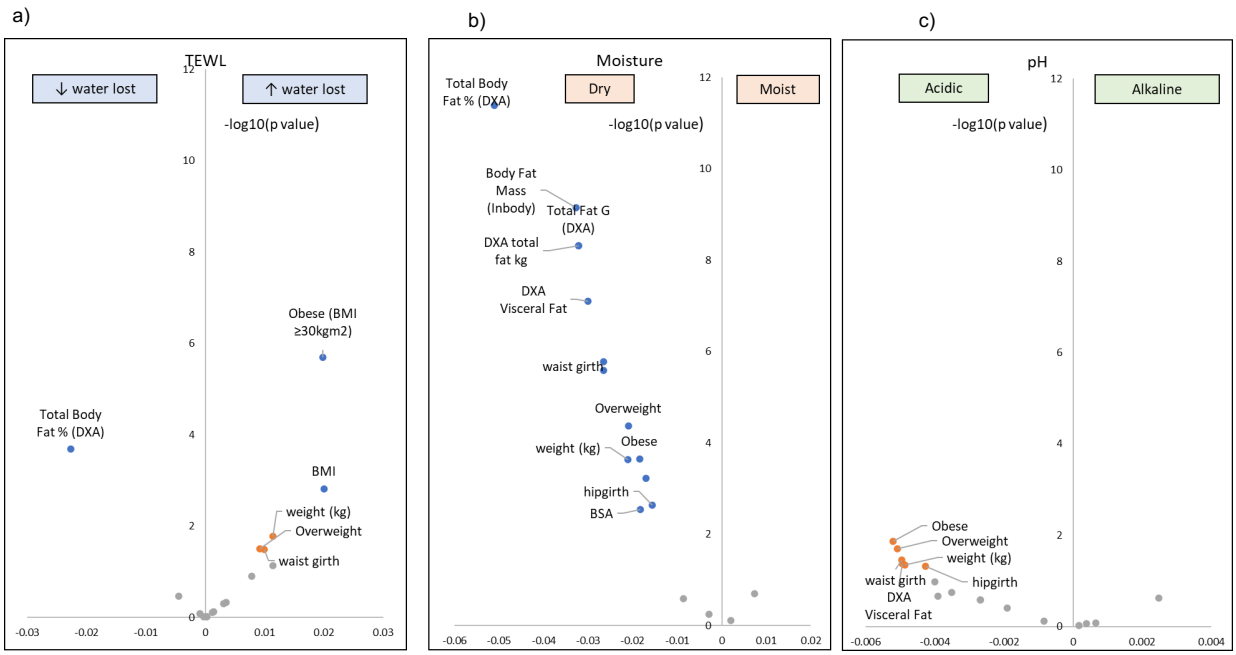
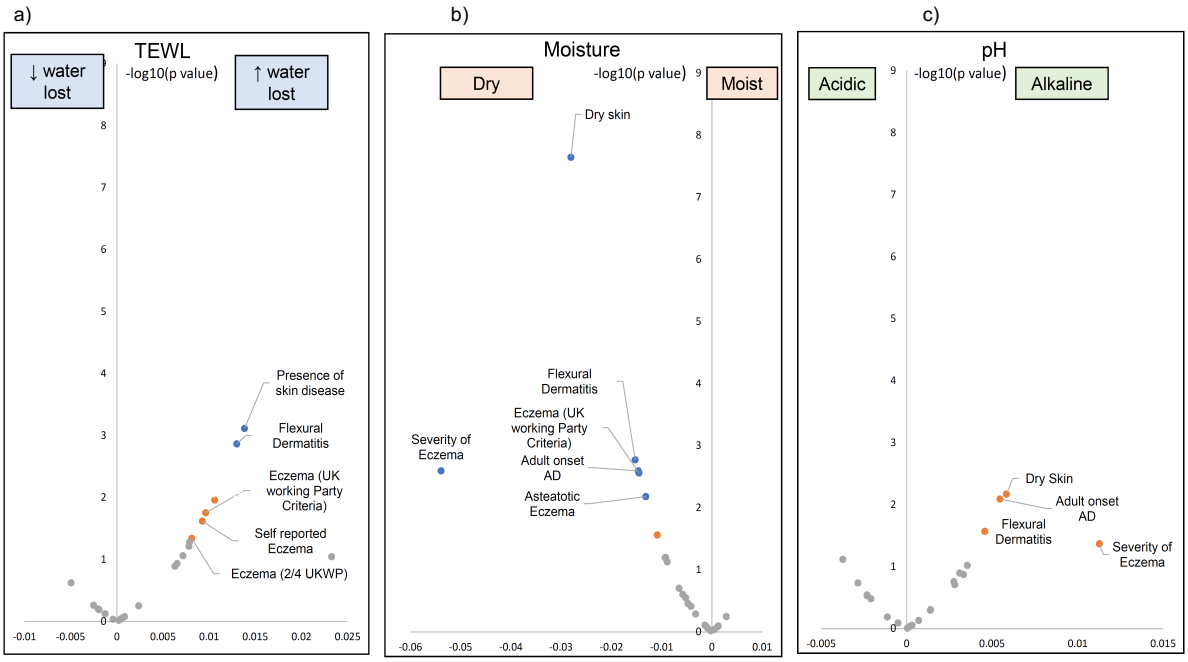


Figure 5.6. Volcano plots of obesity measures against skin physiology parameters. Standardized β coefficients and p values from multiple linear regression analyses of various obesity measures on the log transformed skin physiology measurements (adjusted by age, gender and ethnicity) are plotted as x-axis and y axis of the volcano plots. Data points in blue were significant after adjusting for multiple testing by B-H procedure. Those in orange have p value <0.05 but not significant after adjusting for multiple testing.

5.3.2 Skin barrier function in atopic dermatitis

TEWL was noted to be significantly higher while skin surface moisture was noted to be significantly reduced among participants with atopic dermatitis. Regression analysis of log(TEWL), after adjusting for age, gender and race was significant for atopic dermatitis (Beta = 0.029; p = 0.045). Regression analyses for self-reported eczema, visible flexural dermatitis as well as AD as diagnosed by the full UK Working Party criteria were similarly significant (Figure 5.7a). Regression analysis of log(Moisture), after adjusting for age, gender and race was significant for atopic dermatitis (Beta = -0.054; p = 0.002). Regression analyses for asteatotic eczema as well as AD diagnosed by UK Working Party criteria were similarly significant (Figure 5.7b). Skin pH was also noted to be more alkaline in participants with atopic dermatitis (Beta = 0.016; p = 0.027).



* Measures and types of eczema adjusted by age, gender and ethnicity

Figure 5.7. Volcano plots of eczema measures against skin physiology parameters. Standardized β coefficients and p values from multiple linear regression analyses of various measures and types of eczema on the log transformed skin physiology measurements (adjusted by age, gender and ethnicity) are plotted as x-axis and y axis of the volcano plots. Data points in blue were significant after adjusting for multiple testing by B-H procedure. Those in orange have p value <0.05 but not significant after adjusting for multiple testing.

5.4. Discussion

This study evaluated the skin physiology parameters of a large cohort of about 5,490 study participants from the general population. The skin physiology parameters measured were TEWL, skin surface moisture and skin pH. This study's main outcome of interest was how increasing adiposity might affect the skin barrier function as reflected by these parameters. It also evaluated the skin barrier status of participants with AD, a chronic skin condition characterized by disrupted skin barrier function. This served as a validity check for the skin physiology parameters measured.

There have been previous studies that evaluated how skin barrier function of adults and children was affected by increasing adiposity. However, they were of limited sample size and consisting of patients from a clinical cohort. Evidence available on how adiposity might affect skin barrier function were also conflicting. The study by

Monteiro Rodrigues et al. reported that there was a U shape relationship of TEWL with increasing BMI [190]. Those at the lowest strata and highest strata of BMI had the highest value of TEWL measured. In another study by Guida et al., it was reported that there was a decreased TEWL with increasing BMI [189].

In this study, several skin physiology parameters were evaluated concurrently among the study participants from the general population to reflect the status of skin barrier function. A disrupted skin barrier function would consist of increased TEWL, lowered skin surface moisture and alkaline skin pH. These parameters were measured in participants and compared to see how they would be affected by increasing measures of adiposity. Consistent across various measures of adiposity, it has been observed that there were increased TEWL and reduced skin surface moisture with increasing general and abdominal obesity. However, contrary to what would be expected of a disrupted skin barrier, increasing obesity was associated with an acidic skin pH. It was possible that the skin barrier dysfunction observed in obese individuals was not contributed by a change in skin pH.

Skin physiology parameters can be easily affected by several external factors. They include operator dependent factors, ambient environment temperature and humidity equipment as well as patients' factors. Efforts were taken to ensure that study participants were acclimatized to the room temperature and humidity (tight controlled and measured) for at least 20 minutes before measurement. A total of three readings were taken and mean value was included for analysis. Given that this study involved over 5000 study participants, it was not practical and possible to have the same operator for all the participants to reduce inter-operator variability. Measurements were however made by the same team of field technologists throughout the period of the study and there were also standardized protocols for training and regular equipment

checks. As part of validity check, skin physiology parameters were compared between participants with AD and without AD. As expected from the characteristics skin barrier features of AD, increased TEWL, lowered skin surface moisture and alkaline skin pH were consistently observed in AD participants with various diagnostic criteria of AD used. This gave some degree of confidence to the validity of the skin physiology parameters obtained.

These findings suggested that the skin barrier disruption observed with increasing obesity might be one of the mechanistic pathways linked to the development AD. However, it has not been previously evaluated how the state of obesity might lead to skin barrier disruption but with a reduced skin pH. It is plausible that the systemic inflammatory state of obesity results in a change of inflammatory cytokines milieu in the dermis and epidermis. These in turn affects the metabolic pathways of the skin, resulting in a disturbed profile of metabolites on the skin surface. A recent study by Mori et al. has provided some evidence in 93 American women supporting this hypothesis. The study reported that obesity was associated with a reduction of water content in facial skin associated with changes in lipid profile of the stratum corneum, specific reduction in ceramide content and increased inflammatory marker TNF-α in the skin surface [228]. Another study by Crew et al. has reported that the inflammatory state of obesity resulted in immunological changes in the dermal adipocytes and vasculature, directly affecting the metabolic changes in the adjacent epidermis [229]. Alternatively, obesity may lead to a disturbed skin microbiome profile that in turn also affects these metabolic pathways. Resultant metabolite pathways may result in a change in skin barrier function as well as a change in skin pH. It would be worthwhile to plan for future studies to investigate how obesity affects the skin surface natural moisturising factors and lipids.

A study by Loffler et al has previously hypothesized that the observed skin epidermal barrier changes might be related to various obesity induced physiological changes such as increased sweat gland activity, high blood pressure and physiological temperature regulating changes [188]. It demonstrated that obese participants showed significantly increased skin blood flow [188]. It is also not known if any underlying increased subcutaneous fat or abnormal dermal vasculature may have contributed to the barrier dysfunction. Future studies with skin imaging evaluating skin thickness and dermal vasculature may shed more light on these possible mechanisms.

While skin thickness or skin blood flow were not measured in our population cohort, blood pressure measurements were available among the participants. We analyzed if the skin physiology measures remained significantly associated with increasing BMI after adjusting for blood pressure readings. There remained a significant increased TEWL, reduced skin surface moisture and a more acidic pH with increasing BMI ($p = 0.037$, 0.000054, 0.002 respectively).

In summary, this study demonstrated that there was increased TEWL and reduced skin surface hydration, suggestive of skin barrier dysfunction in participant with increasing adiposity. This might be one of the mechanistic pathways linking obesity with the development of AD. However, it remains important to determine if the observed skin barrier dysfunction in obese individuals share similar mechanisms as that of barrier dysfunction in AD patients. As discussed, there could be various possible hypotheses on the skin barrier dysfunction in obesity. The presence of skin barrier dysfunction in obese individuals could also have further implications on other cutaneous complications or associations of obesity such as prolonged wound healing, acanthosis nigricans, skin tags or psoriasis. However, evidence in this area is currently lacking. Further studies would be important to evaluate further the underlying

mechanisms of these observed skin barrier changes with obesity and this could add knowledge to prevention and treatment of other obesity related cutaneous issues.

6. Study 3: Extent to which obesity affects the diversity of skin microbiome in AD participants.

6.1. Overview and rationale

Skin microbiome has been observed to interact with the skin epidermal barrier and immune system to give rise to atopic diseases such as asthma and atopic dermatitis (AD) [192]. AD skin is often found to have a decreased skin microbial diversity but increased abundance of *Staphylococcus aureus* during disease flares [230]. There is also corresponding decrease in specific bacterial populations of the *Streptococcus, Corynebacterium* and *Cutibacterium* [231, 232]. Emerging evidence has also reported that disruptions to these skin commensals microbes have important effects on the immune system [233, 234]. For example, exposure to *Staphylococcus epidermis* and *Corynebacterium* can result in an increase in antigen specific CD4+ T cells and Th17 programs. All the aforementioned evidence suggests an important role of skin microbiome on the development of AD. It would therefore be imperative to assess for any skin microbial disturbances in obese individuals that might provide clues explaining the observed relationship between obesity and AD.

While it is well established that the gut microbiome diversity is intimately related to the obese state, much less has been studied about the skin microbiome in obese individuals and how they might relate to the development of skin diseases. Studies to date have mainly been limited to animal studies [235]. A recent microbiome study in 2019 evaluated the skin microbiome profile from 995 skin sites and analyzed the 16S sequencing data across BMI categories of underweight, normal, overweight and obese [15]. Shannon diversity of skin microbiome was significantly lower in obese/overweight individuals compared to those underweight. Shannon diversity, an index of alpha diversity, reflects the diversity of a community microbial composition in terms of

abundance and evenness at species level [236]. The relative abundance of *Corynebacterium* was significantly correlated with increasing BMI. Therefore, there are important changes in microbial diversity and specific commensal bacterial species with increasing obesity. However, comprehensive evidence is still lacking. While there are preliminary studies in mice and skin microbiome data in humans using 16S sequencing, further studies are needed. With whole metagenomic sequencing available in microbiome, it is therefore now possible to comprehensively evaluate the skin microbiome profile of obese individuals and assess how it might correlate with the risk of developing AD.

With whole metagenomic sequence data available, it would also allow functional profiling of the skin microbiome sequence data available. Functional profiling allows quantification of metabolic pathways contributed by microbial communities on the skin. This is beyond the usual taxonomic profiling that evaluates what microbes are present in the skin, and provides a much closer link between local molecular activities with disease states.

In this study, the taxonomic and functional profiling of skin microbiome were assessed in close to 300 study participants. The skin microbiome profiles were analyzed across AD status as well as measures of obesity to address the study aim of how skin microbiome changes in obesity might affect the risk of developing AD.

6.2. Methods and Analysis

6.2.1 Study design

It was not possible to perform skin microbiome analysis for the entire HELIOS cohort although skin tapes for microbiome were collected for all participants. Due to resources constraints, skin microbiome analysis was performed in 300 of the study

participants using a (1:1) matched case control design. To avoid the possible confounding by ethnic differences in skin microbiome as well as to further enhance statistical power, only Chinese participants were included. One hundred and fifty AD cases were selected with 150 age (within ± 5 years) and gender matched controls. Participants with known history of systemic inflammation, immunosuppression, systemic infections were excluded from the study. Any participants with recent oral antibiotics usage within the past 4 weeks were excluded from the cohort.

The sample size of 300 was calculated based on the sample size calculation for the serum inflammatory markers study elaborated in the next chapter (Chapter 7; Study 4). This study design would provide analysis results for skin microbiome and serum proteomic markers within the same cohort of 300 participants.

Skin microbiome collection, processing, sequencing and analysis were performed according to current recommendations and guidelines [237, 238]. These are discussed in the subsequent paragraphs.

6.2.2 Skin microbiome collection

Skin microbiome were collected using the tape strip method from the participants' skin over both the left and right ventral forearm area between elbow crease and ventral wrist crease. The area of measurement had to be free of any active skin lesions. The area of the skin microbiome collection was also away from the spot where skin physiology measurements were earlier made. This was done deliberately to avoid any possible external influence of previous skin equipment over the skin surface.

Participants were advised to avoid any washing of the sampled sites for a minimum of 6 hours or any topical emollients use over the area for 12 hours prior to study visit. Screening questions on prior washing and use of topical products were asked and

recorded with majority of participants following the recommendations. Upon arrival at the study site, efforts were made to ensure no further washing or application of topical products right before the samples were collected.

A 22 mm adhesive tape disc as shown in Figure 6.1 was used for skin microbiome collection. Each tape was pressed down multiple times with a thumb to apply firm, even pressure, until saturation and not sticky. This was done for approximately 1 minute, allowing for approximately 50 re-applications at the same skin location. During the reapplication and peeling process, the tape disc would become saturated with skin surface material including skin microbiome and skin corneocytes. Each tape disc was then bent with the adhesive surface curved internally and placed into a labelled 1.8 ml microfuge tube with 70% ethanol wiped forceps. These were then stored under -80 degree Celsius till further DNA extraction was done based on established recommendations [239, 240].

It has been previously shown that skin microbiome collection via tape stripping collection has high concordance with other methods of collection such as swabs, scrape or skin biopsies in terms of relative abundance of microbial taxa [194]. Skin microbiome collection was performed over the inner forearm as it represented a moist site. Skin areas could be divided into three groups based on their physiological characteristics, namely dry, moist or sebaceous. They have been noted to have similar composition of microbial communities [239, 240]. As the usual areas of AD involvement consist of the flexural and moist areas, the inner forearm was chosen to represent the moist area of the skin. This location would also minimize the impact from external environment's influence.

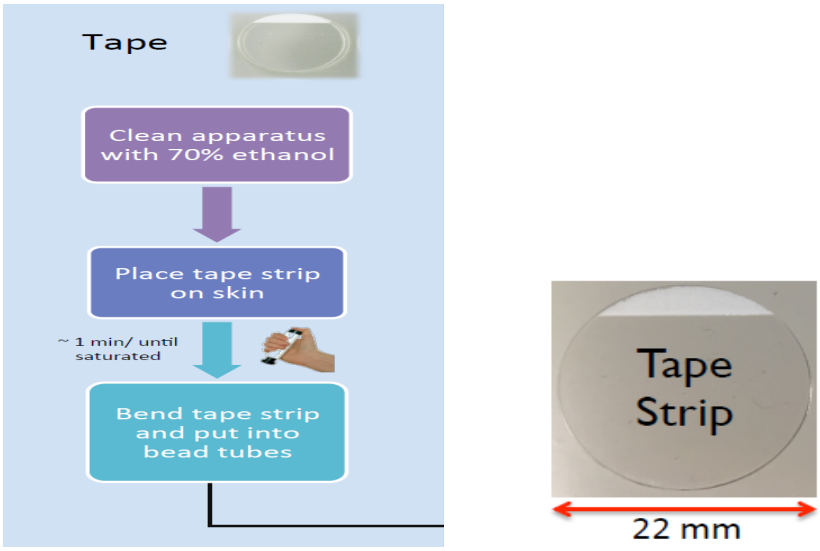


Figure 6.1. Method of sampling for skin microbiome collection.

6.2.3 Samples processing

Only one sample (collected over the left forearm) per selected study participant was utilized for analysis. Stages of samples processing included DNA extraction, library construction, read processing followed by whole metagenomic sequencing.

DNA extraction

500 mL of ATL Buffer (Qiagen) was added to the D-Squame tape discs (CuDerm Corporation) in Lysing Matrix E tubes (MP Biomedicals). These were then thoroughly mixed with bead-beating in a FastPrep-24 Instrument (MP Biomedicals) at 6.0 m/s for 40 s. Centrifugation of the mixture was performed at 16,000 ´g for 5 min. Proteinase K (Qiagen) was then added to 200 mL of the supernatant and incubated at 56 °C for 15 min. DNA was extracted using the EZ1 Advanced XL Instrument (Qiagen) and EZ1 DNA Tissue Kit (Qiagen). This was quantified using the Qubit dsDNA HS Assay Kit (Life Technologies) and stored at -20 °C before the subsequent steps.

Library construction and read pre-processing

26 mL of extracted DNA were constructed using NEB Ultra II FS Kit: E7805 (New England Biolabs) and barcode adaptors according to the manufacturer's protocol. Custom index primers with PCR cycles of 12 were used for enrichment of DNA libraries. These were then quantified using the Agilent DNA High Sensitivity Kit (Agilent Technologies) on an Agilent 2100 Bioanalyzer (Agilent Technologies). Samples were then normalized and pooled, followed by paired-end sequencing (2 X 150 bp reads) on an Illumina HiSeq X Instrument at the Novogene AIT Sequencing Platform (Genome Institute of Singapore).

300 samples were processed but only 294 samples provided sufficient extracted DNA for library amplicons to be created for the subsequent step of sequencing.

The samples were distributed at random with respect to the case and control status in terms of plates and lane runs. Laboratory technicians were blinded to the case status throughout the analysis. A clustering analysis in the form of a principal coordinates analysis of Bray-Curtis distance was done. This was according to the different runs that the samples were processed to assess for any batch/clustering effects. There were no differences or any clustering effects according to their runs.

Blank samples as controls

Eight blank tape discs serving as blank control samples were processed and run in parallel to the skin samples of interest under the same study conditions. As skin samples typically consist of very low biomass, having blank samples as controls would be pertinent to identify any contamination during extraction, library preparation and sequencing [237]. Blank control samples were mapped to evaluate the extent of

contamination and the microbial flora that would be associated with contamination during the process.

6.2.4. Whole metagenomic sequence analysis pipeline

DNA obtained by whole metagenomic shotgun sequencing was subjected to a series of steps before data analysis on the relative abundance of various microbes at species level. Functional profiling was also performed. Figure 6.2 illustrates the analysis pipeline.

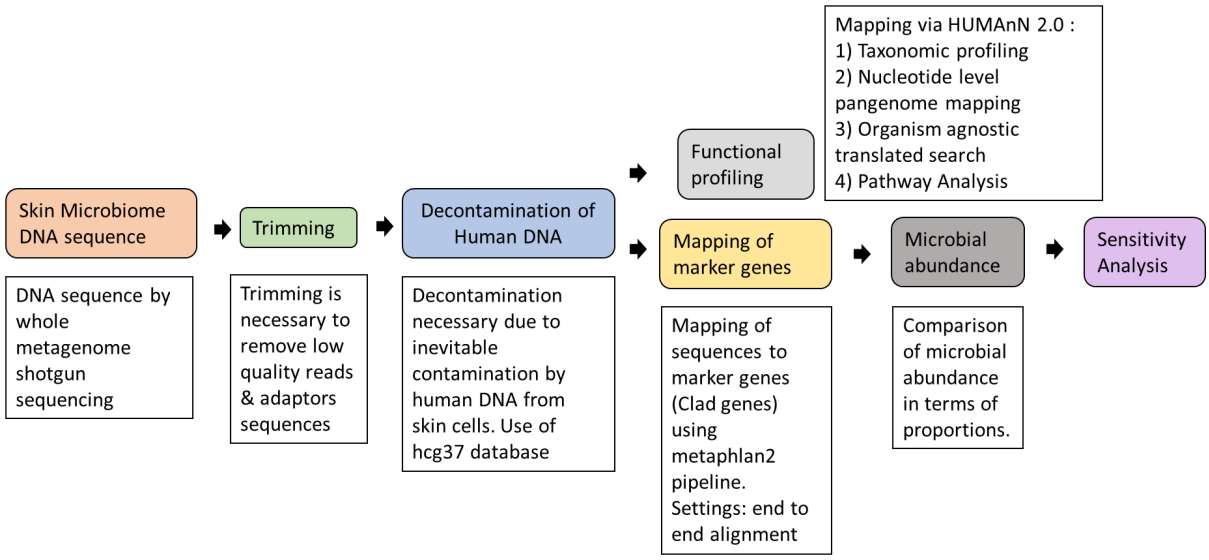


Figure 6.2. Analysis pipeline for whole metagenomic sequence processing.

Quality control and trimming of raw reads

Paired end sequence reads in FASTQ file format compressed in “gzip” were subjected to trimming to remove low quality reads and adaptors sequences. Quality control with FASTQC software was performed before and after trimming to check for quality scores, presence of low-quality reads, adaptors contamination and duplicates as a quality check [241, 242].

Trimming was performed with the multithreaded command line tool, Trimmomatic V0.39.3 using the following parameters: “2:30:10 LEADING:3 TRAILING: 3 SLIDING WINDOW: 4:20 MINLEN:50”. It performed the following actions in order:

A) It looked for seed matches (16 bases) but allowed a maximum of 2 mismatches. These seeds will be extended and clipped when the paired end reads a score of 30 was reached.

B) It removed leading and trailing low quality or N bases below quality of 3.

C) The reads were scanned with a 4-base wide sliding window, cutting when the average quality per base drops below 20.

D) Reads that were less than 50 bases long after these steps were dropped.

Decontamination with KneadData v0.7.3

Reads after quality control and trimming were subjected to “decontamination”. This step would be necessary as skin corneocytes would have been collected as part of the skin biomass during tape stripping. It is necessary to remove any human DNA from the skin corneocytes. This was performed using the KneadData pipeline v0.73 [243]. The KneadData pipeline consists of Trimmomatic for quality filtering, Tandem Repeat Finder, FASTQC and Bowtie2 to align the reads to a provided list of reference database of sequences to be removed. An end-to-end alignment setting was invoked for Bowtie2 alignment.

Microbiome profiling with MetaPhlAn v2.7.7

For whole metagenome shotgun profiling, MetaPhlAn v2.7.7 (default parameters) was used to determine bacterial, viral and eukaryotic community abundances. MetaPhlAn (Metagenomic Phylogenetic Analysis) is a tool that estimates the relative abundance

by mapping reads to a catalogue of marker genes (Clad genes) [244]. These clad gene sequences are unique and can identify microbial clades at species or higher taxonomic levels. The relative abundance of each microbes at species or higher levels were provided in percentages. MetaPhlAn has previously been deemed by a comparison study of various metagenomic classifiers tools to be recommended as a single tool that is fast and limits false positives [245].

Functional profiling with HUMAnN 2.0

While it is important to understand the relative abundance of microbial communities in the environment, it is also pertinent to evaluate the functional profile of the microbial communities present. The underlying molecular activities would provide clues to how certain environment or clinical phenotypes might be linked to the underlying microbial communities. Changes in certain metabolic pathways or functions may be contributed by a shift in the microbial abundances or dysregulation of certain pathways brought about by a group of microbial species.

HUMAnN 2.0 is a pipeline of tools that performed functional profiling in an efficient and accurate manner [246]. It reports the presence or absence of microbial pathways in a community based on metagenomic sequence data, and describes the potential metabolic functions of the microbes in that sample. It consists of several tools that first identify the underlying microbial species in that community via MetaPhlAn2 and then performs nucleotide level pangenome mapping using Bowtie2 to functionally annotated species pangenomes provided by ChocoPhlAn. Any unmapped reads are subsequently subjected to a translated search in the universal protein reference database using the Diamond tool. Finally, the HUMAnN tool integrates the organism specific gene hits and the protein families hits to determine the pathway abundance of

various metabolic pathways according to the MetaCyc pathway collection [247]. The pathways abundance quantified in reads per kilobase are then renormalized to copies per million, with the values constrained to sum to 1 million. A list of the metabolic pathways is provided in Annex B.

6.2.5. Statistical analysis

Differentially abundant taxa at the kingdom level between AD and controls as well as obese (BMI≥ 30kgm^2) and non-obese were first analyzed using the non-parametric Wilcoxon rank-sum test (two-sided). Results were then illustrated with box plots.

Ecological diversity measures.

Ecological diversity of the underlying community is often measured by both alpha diversity within sample as well as beta diversity between samples. Alpha diversity measures provide information about the composition of the community taking into account the relative abundances of various species. In this study, the Shannon's diversity index, the Simpson's diversity index, richness and evenness were compared across the various samples [248]. The Shannon's diversity index accounts for both abundance and evenness of the species present but it is influenced by the number of species and rare species. This is compared to inverse Simpson index that gives more weightage to the species evenness and common species. Richness refers to the total number of species in that sample or community while evenness is a measure of how equal the community is numerically among the species.

Beta diversity in the form of Bray-Curtis dissimilarity was computed for all the samples and compared according to the metadata groups [249]. Aforementioned diversity analyses and Principal Coordinates Analysis (PCoA) of the species composition performed based on Bray–Curtis dissimilarity were done using the R package 'vegan'.

Principal Coordinates Analysis (PCoA) visualized the data of the beta diversity distance matrix in PCoA plots, allowing the identification of any patterns of clustering within the samples.

Linear discriminant analysis Effective Size (LEfSe)

Relative abundance of individual microbes was also subjected to analysis by Linear discriminant analysis Effective Size (LEfSe) [250]. LEfSe first performed a non-parametric factorial Kruskal Wallis sum rank test to detect species with significant differences in relative abundance according to the metadata group of interest. Linear discriminant analysis (LDA) was then performed to estimate the effect size of the difference in abundance. LDA is a dimensionality reduction method to maximize the separability between the groups. The effect size was calculated by averaging the differences between the original unmodified class means with differences between the projected class means along the first discriminant axis. The value would then be log-transformed and scaled in the [1, 10^6] interval to give a score between 0 and 6. Relative abundances of pathway analysis from HUMAnN2 were similarly subjected to LEfSe analysis to identify any discriminant pathways of interest.

Multiple linear regression

Multiple linear regressions, adjusting for age and gender were also performed to identify significantly different microbial species between AD and controls as well as between obese (BMI ≥ 30 kgm^2) and non-obese. P values were subsequently subjected to multiple testing correction using the Benjamini–Hochberg (B-H) procedure at false discovery rate (FDR) threshold of 0.1. Volcano plots were then plotted to identify any microbial species with the most meaningful change with each

exposure of interest. Relative abundances of pathway analysis from HUMAnN2 were also subjected to multiple linear regressions adjusted for age and gender.

6.3. Results

6.3.1. Demographic profile

Only 294 samples have sufficient DNA extracted from skin tapes for library amplicons preparation to be performed for the whole metagenomic sequencing.

Characteristics	AD (n=146)	Controls (n=148)	P value
Age (years, mean)	52.1	52.7	0.676
Female (%)	67.1	67.6	0.935
Obesity by BMI (%)	8.9	7.4	0.828
Obesity by WHR (%)	40.4	38.5	0.739

Table 6.1. Demographic profile of skin microbiome study.

The age and gender characteristics between AD and controls appeared largely similar. There were also similar proportions of obese participants in both AD and control groups.

6.3.2. Overview of skin microbiome in AD and obese participants

More than 90% of the skin microbiome were identified to be from the bacteria kingdom, followed by eukaryote and viruses. (Figure 6.3) There were similar proportions of bacteria, eukaryote and viruses among AD and controls. The proportion of eukaryote appears to be significantly higher in obese compared to non-obese participants ($p = 0.010$). To investigate if this observed difference in proportions was related to sampling issues, the relationships of library concentration and the number of reads with BMI

were analyzed respectively. Both DNA library concentration and number of reads did not have a significant relationship with increasing BMI adjusted for age and gender (p=0.132 and p=0.377 respectively) (Annex A, Figures S.1 and S.2)

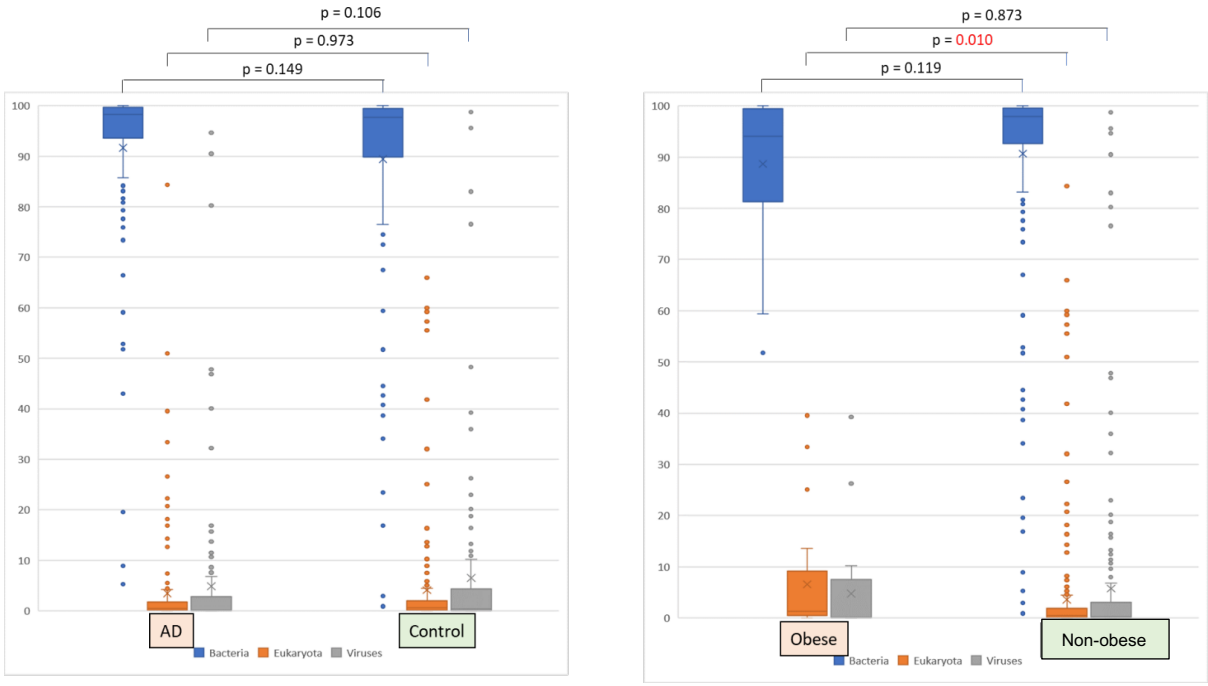


Figure 6.3. Boxplots illustrating the relative abundance at taxonomic kingdom levels for AD vs controls and obese vs non-obese.
Boxplot in blue represents bacteria, orange represents eukaryote and grey represents viruses kingdoms.

There were altogether 736 microbial species identified from relative abundance profiling. The top 200 species are illustrated on a cladogram (Figure 6.4) as a form of classification of the microbial organisms. As can be seen from the cladogram, many of the known skin commensals bacteria present were found on the skin of the study participants. These included *Corynebacterium* species, *Propionibacterium (Cutibacterium) acnes*, *Staphylococcus* and *Streptococcus* species.

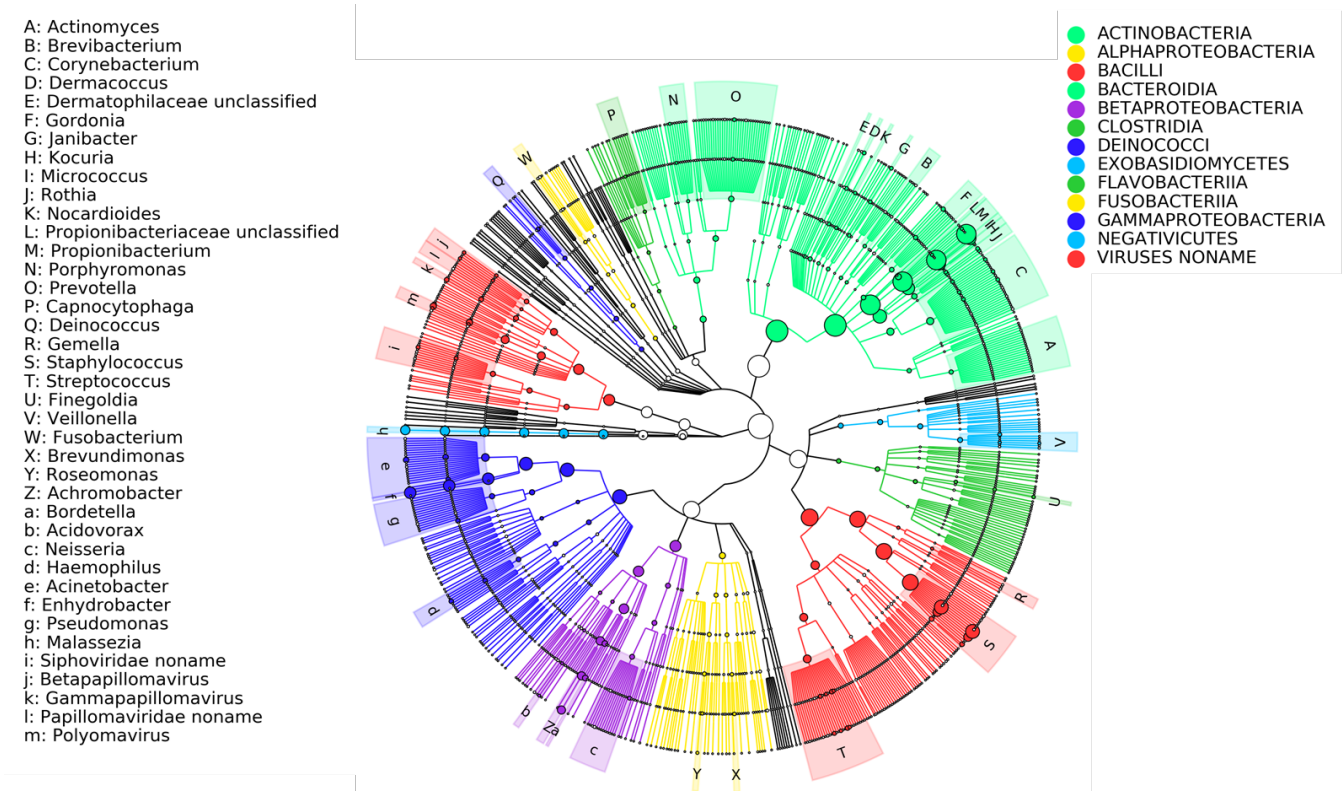


Figure 6.4. Cladogram of top 200 microbial species detected in the skin microbiome study.
It provides a general classification of the organisms present on the skin surface of the study cohort.

6.3.3. Characteristics of blank controls

Blank tapes were submitted as blank control samples to identify any microbial contaminants that might be introduced from the environment during samples processing. There was a total of eight blank controls processed.

Blank Controls	Estimated total number of reads from known clades	Total number of reads from		
		Bacteria	Eukaryote	Viruses
1	1858	1858	0	0
2	10669	10664	0	5
3	102102	98853	3237	12
4	30712	30712	0	0
5	17714	17709	0	5
6	20495	20495	0	0
7	34356	34356	0	0
8	45547	45377	0	170
Median	25604	25604	0	3

Table 6.2. Estimated total number of reads from known clads in blank control samples.

Table 6.2 illustrates the estimated total number of reads from known clades for the eight blank controls. The median total number of reads contributing to known microbial clades was about 25,604. This amounted to less than 0.1% of the total number of reads for an average skin microbiome samples, suggesting that contamination during extraction process, library preparation and sequencing was likely to be minimal.

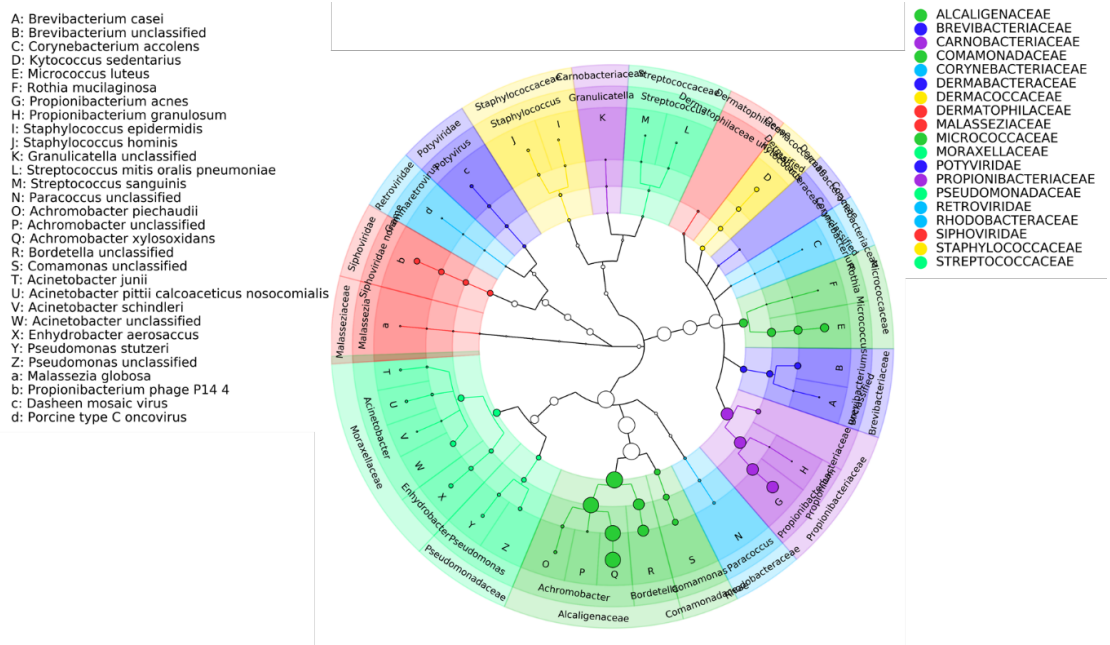


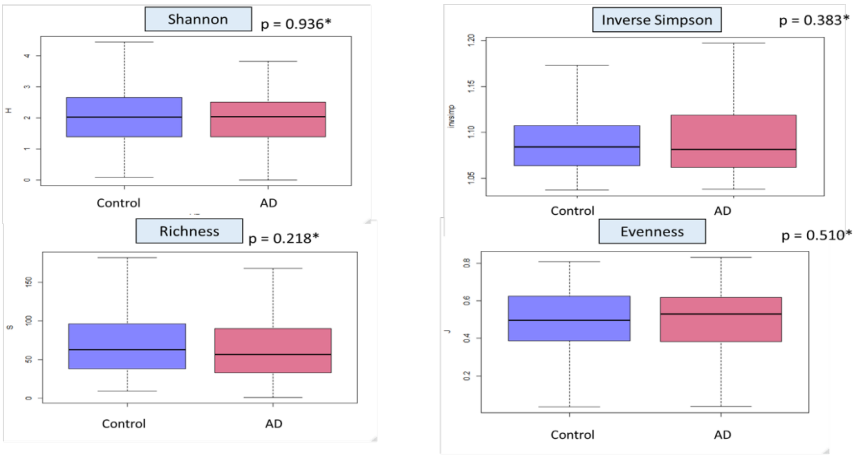
Figure 6.5. Cladogram of microbial species detected in the blank controls.

Majority of the microbes identified from the blank controls were from the bacteria kingdom followed by viruses.

6.3.4. Alpha and beta diversity among AD and obesity

AD

Alpha diversity measures such as Shannon diversity index, Inverse Simpson index as well as the richness and evenness were similar between AD and their controls. (Figure 6.6) Although the median Shannon diversity index and richness appeared to be lowered in those with AD, none achieved statistical significance after adjusting for age and gender.



AD	Estimate	P value
Shannon	-0.00813	0.936
Inverse Simpson	0.00393	0.383
Species	-5.83	0.218
Evenness	0.0143	0.510

* adjusted for age and gender

Figure 6.6. Boxplots of alpha diversity measures between AD and controls.
These measures were similar between the two groups.

Beta diversity in terms of Bray-Curtis dissimilarity was calculated to quantify any dissimilar compositions between the samples. This dissimilarity index was then reduced in dimensions and presented in a principal coordinates analysis (PCoA) plot to illustrate any clustering patterns.

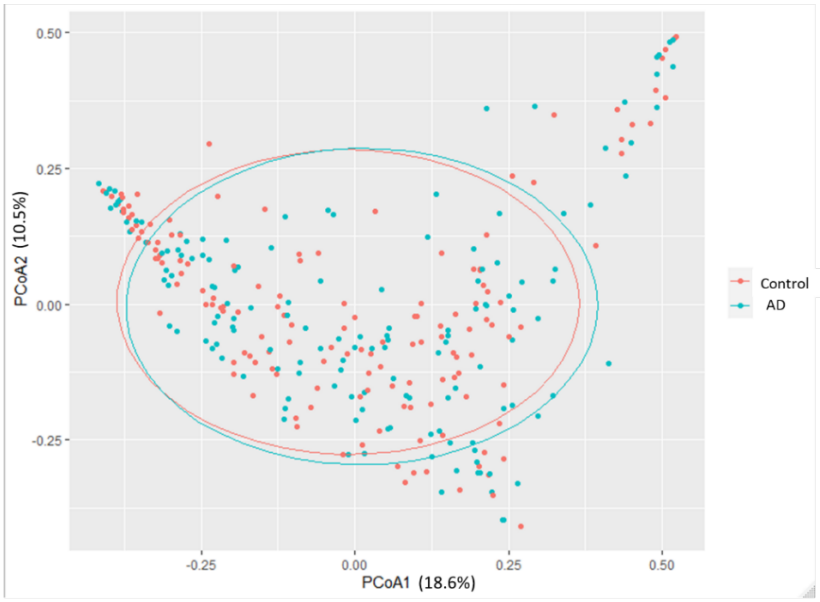
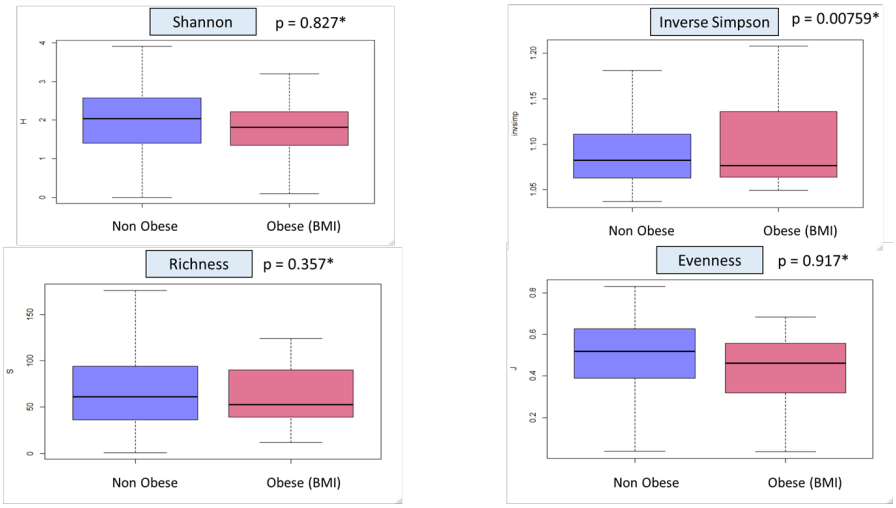


Figure 6.7. Principal coordinates analysis (PCoA) plot.
PCoA plot of Bray-Curtis distance between individual skin samples (n=294) to reflect the beta diversity between the different skin samples. Data points in blue indicate participants who have AD. Those in orange indicate control participants. The respective standard deviational ellipses are also shown in the plot.

As shown in Figure 6.7, the data points on the PCoA plot overlapped each other. There was no difference in terms of Bray-Curtis dissimilarity between AD and controls.

Obese

Alpha diversity measures such as Shannon diversity index, richness and evenness appeared to be reduced in participants who were obese (BMI≥30kgm^2). However, none achieved statistical significance (Figure 6.8). Inverse Simpson was slightly but significantly increased in obese participants.



BMI	Estimate	P value
Shannon	-0.00289	0.827
Inverse Simpson	0.00152	0.00759
Richness	-0.568	0.357
Evenness	-0.000294	0.917

* adjusted for age and gender

Figure 6.8. Boxplots of alpha diversity measures between obese (BMI≥30kgm2) and non-obese.
These measures were similar between the two groups.

Alpha diversity measures were also compared across obesity groups defined by WHR measures (data not shown). There were no significant differences between most alpha diversity measures between those who were obese and those not obese as defined by WHR. Inverse Simpson index appeared slightly but significantly increased in obese participants with increased WHR.

In terms of beta diversity, the principal coordinates analysis (PCoA) plots (Figure 6.9) also failed to illustrate any clustering patterns between obese and non-obese defined by both BMI and WHR adiposity measures.

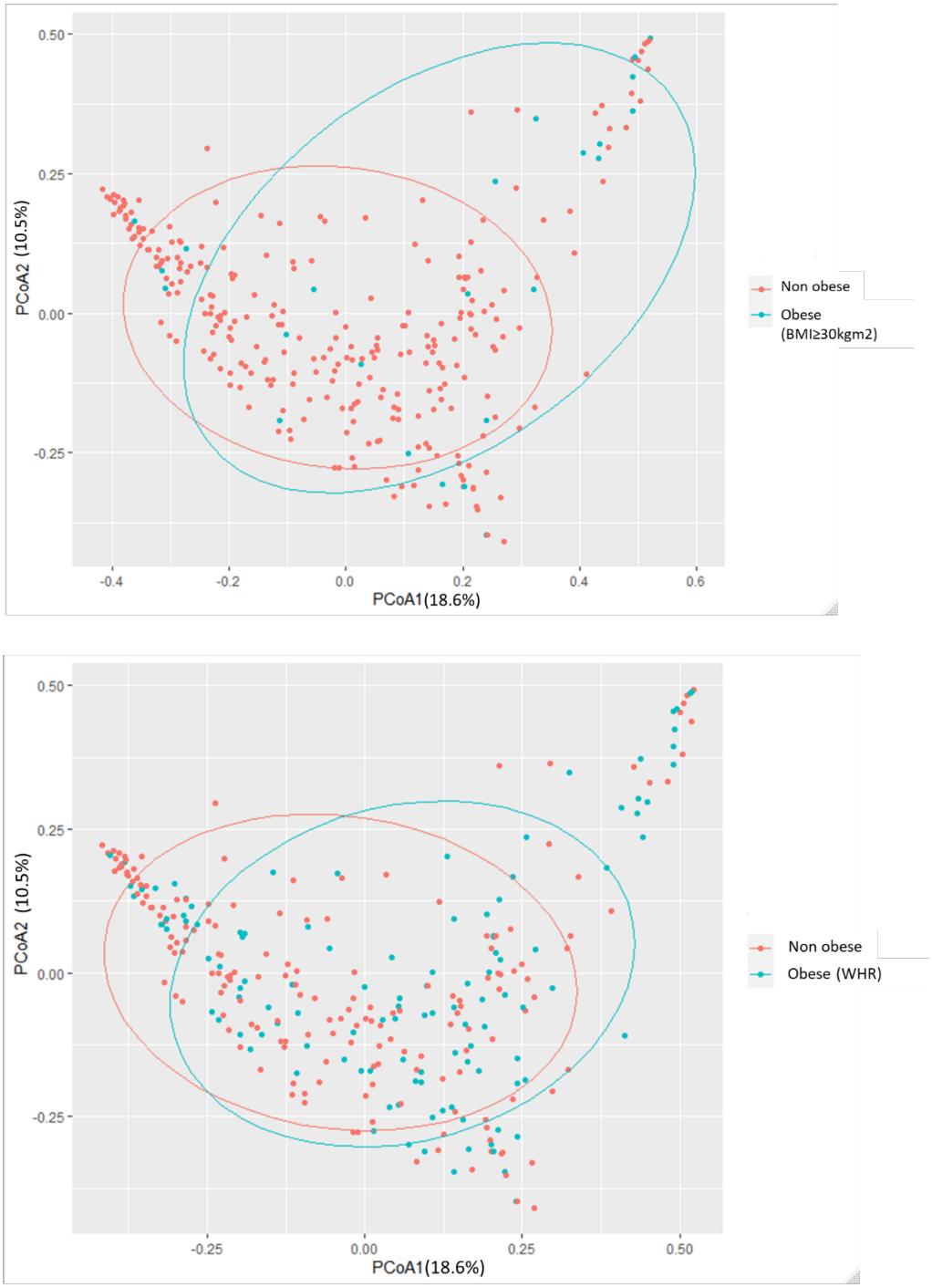


Figure 6.9. Principal coordinates analysis (PCoA) plots of obesity.
PCoA plots of Bray-Curtis distance between individual skin samples (n=294) to reflect the beta diversity between the different skin samples. Data points in blue indicate participants who were obese (based on BMI ≥. 30kgm^2 or WHR). Those in orange indicate the non-obese participants. The respective standard deviational ellipses are also shown in the plot.

6.3.4. Linear discriminant analysis Effective Size (LEfSe) in AD and obesity

Linear discriminant analysis (LDA) was performed at microbial species level to identify species with consistent differences in relative abundance between AD and controls as well as obese BMI$\geqslant$30kgm^2 and non-obese. Significant species were then ranked according to their effect sizes in terms of LDA in this LEfSe analysis [250].

AD

A total of 22 microbial species were found to be significantly different by the non-parametric factorial Kruskal-Wallis (KW) sum-rank test. However, only seven discriminative bacterial species had an absolute LDA score of greater than 2. These are illustrated in the following LEfSe plot for AD and controls. The seven bacterial species were *Enterobacter hormaechei* (for AD) and *Prevotella veroralis, Scardovia wiggsiae, Lactobacillus gasseri, Streptococcus mutans, Deinococcus wulumuqiensis*, and *Corynebacterium matruchotii* (for controls). *Enterobacter hormaechei* is a gram-negative bacterium known to cause nosocomial infections. Some of the bacterial species such as *Prevotella veroralis, Scardovia wiggsiae* and *Streptococcus mutans* are commonly found in the oral cavity.

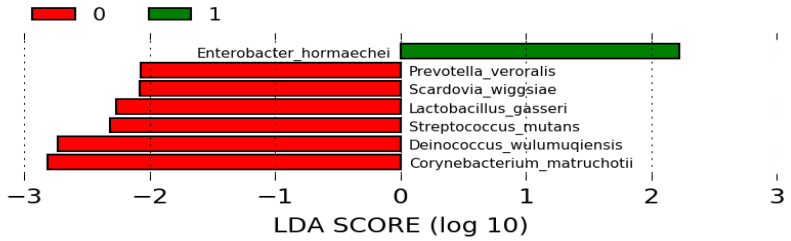


Figure 6.10. Linear discriminant analysis Effective Size (LEfSe) plot for microbial species with consistent difference in relative abundance between AD and controls.
This plot illustrates which microbial species that were statistically significant and explained the greatest differences between AD and control. The green histogram bars illustrate those species more abundant in AD while those red bars illustrate those more abundant in controls.

Obese

A total of 111 microbial species were found to be significantly different by the non-parametric factorial Kruskal-Wallis (KW) sum-rank test. However, only 97 discriminative microbial species had an absolute LDA score of greater than 2. (Figure 6.11) Notably, commonly known skin flora such as *Staphylococcus hominis, Streptococcus dysgalactiae* and several *Corynebacterium* species such as *Corynebacterium jeikeium, Corynebacterium pseudogenitalium, Corynebacterium tuberculostearicum Corynebacterium sp HFH0082, Corynebacterium massiliense, Corynebacterium aurimucosum* and *Corynebacterium striatum* were found to be increased among obese participants (BMI≥30kgm^2). *Propionibacterium (Cutibacterium) acnes*, a common skin bacteria, was found to be reduced in participants who were not obese. Taxonomic representation of those differentially abundant microbial species is illustrated in the following cladogram (Figure 6.12). This is based on representation using the Ribosomal Database Project (RDP) taxonomy [251].

Differences in microbial species were also evaluated in participants who were obese (abdominal obesity) based on WHR criteria. Forty-two microbial species were significantly different between obese (WHR) and non-obese. Twenty-seven discriminative microbial species have an absolute LDA score of greater than 2. Some species were similar to those identified in obese participants based on BMI criteria. These include *Corynebacterium* species and *Staphylococcus hominis*. *Propionibacterium (Cutibacterium) acnes* was similarly found to be significantly abundant in those non-obese participants.

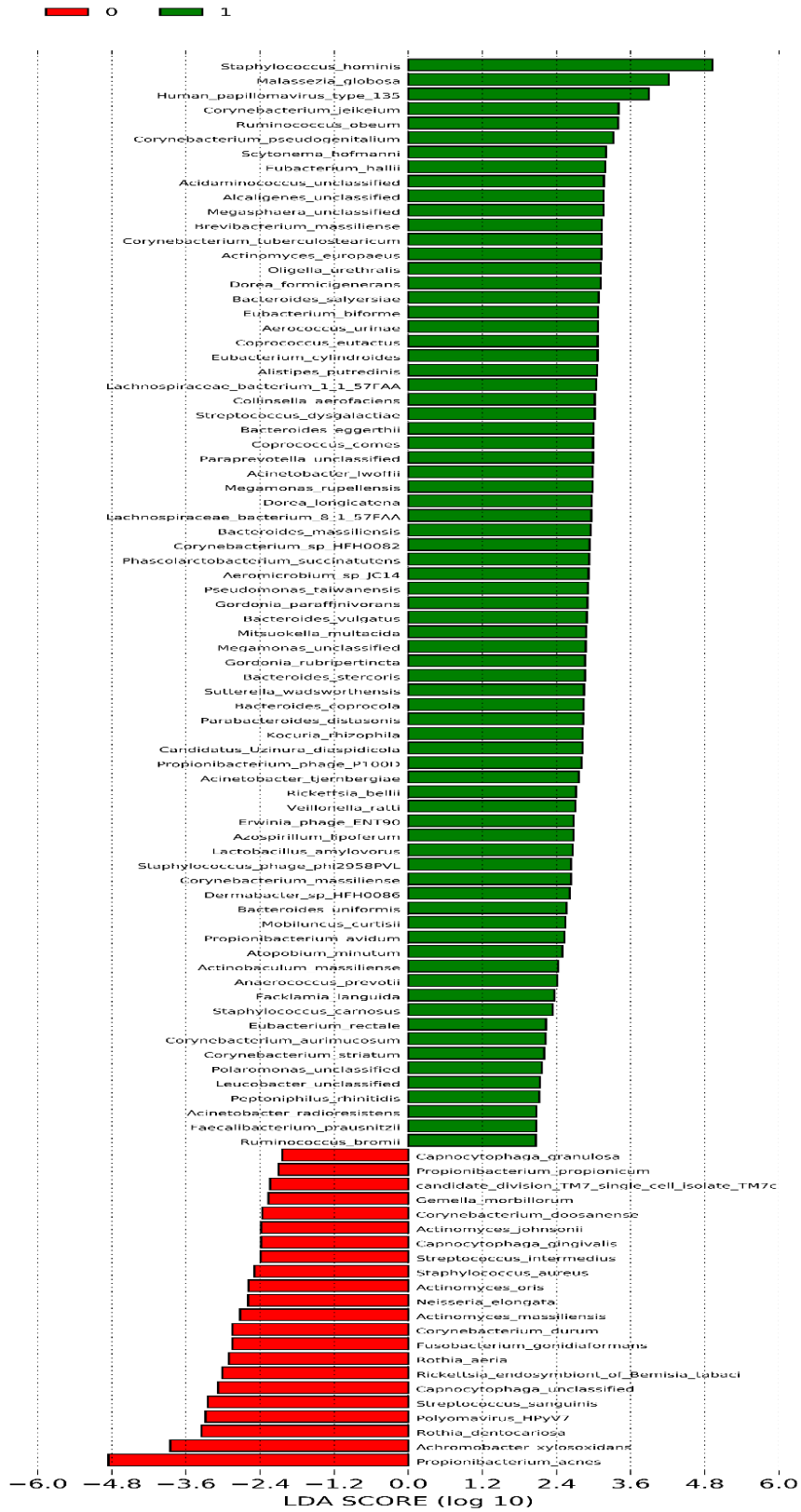


Figure 6.11. Linear discriminant analysis Effective Size (LEfSe) plots for microbial species with consistent difference in relative abundance between obese (BMI≥30kgm^2) and non-obese.

This plot illustrates which microbial species that were statistically significant and explained the greatest differences between obese and lean. The green histogram bars illustrate those species more abundant in obese while those red bars illustrate those more abundant in non-obese.

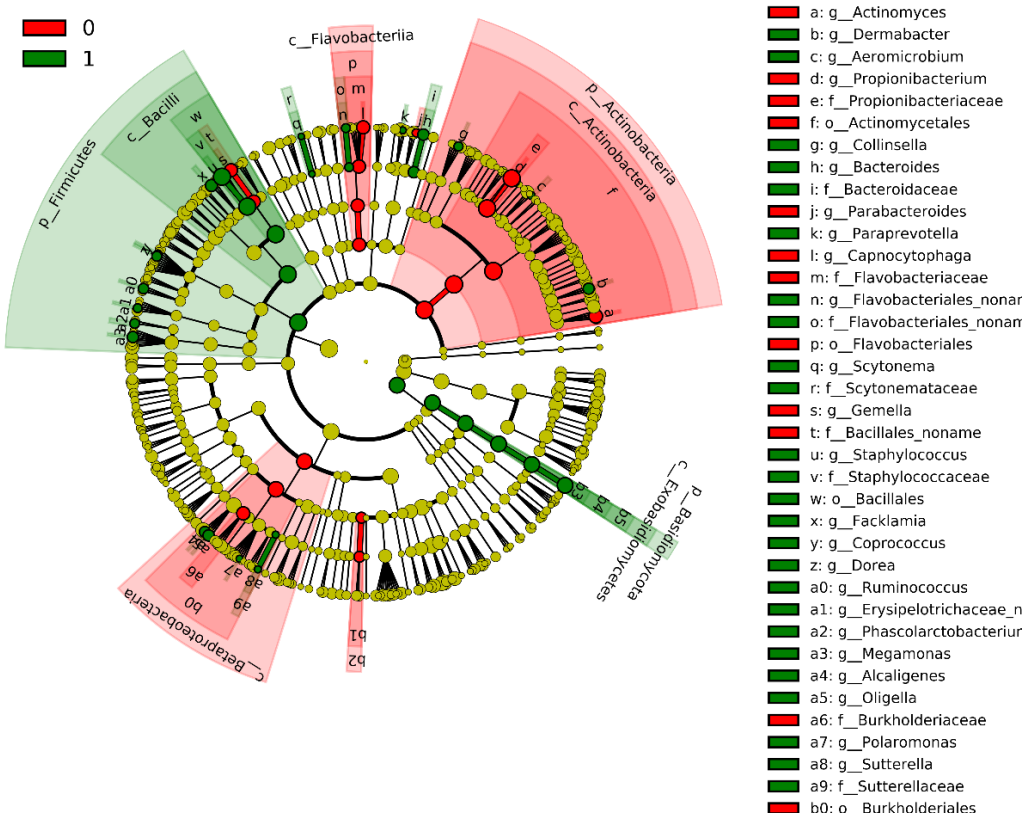


Figure 6.12. A cladogram illustrating the taxonomic representation of statistically consistent differences between obese (BMI ≥ 30kgm^2) and non-obese.
Green represents significant microbial taxa in obese while red represents that of non-obese. Each taxon's abundance is represented by the diameter size of the circle.

6.3.5. Multiple linear regression of skin microbiome species adjusting for age and gender

To account for possible confounding by age and gender, regression analysis adjusting for age and gender was performed for skin microbiome at species level with either AD or obesity. Relative abundances of skin microbiome species were expressed in proportions ranging from 0 to 1.0. Multiple linear regressions adjusted for age and gender were also performed to adjust for possible confounding. Volcano plots of resultant -log10(p values) against standardized effect estimates were plotted to identify any microbial species with the most meaningful change. It combines a measure of statistical significance with the magnitude of the change.

AD

Most microbial species did not differ significantly in relative abundances among participants with AD on multiple linear regression adjusting for age and gender. Only four bacteria species were significant after adjusting for age and gender as seen in Figure 6.13 volcano plot. Notably, the relative abundance of *Staphylococcus epidermidis* was significantly elevated in AD participants compared to controls (Odds ratio: 2.50, p = 0.0261). However, none reached statistical significance after multiple testing correction by B-H procedure.

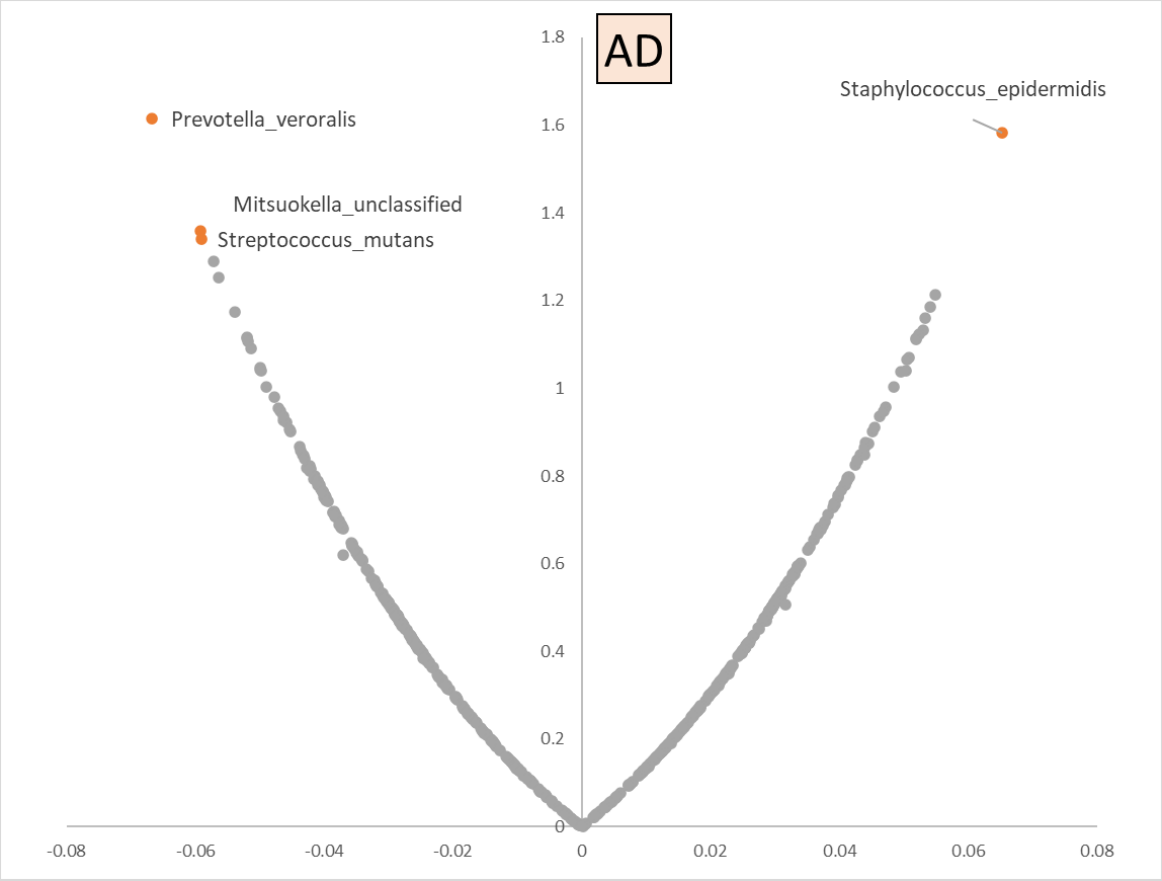


Figure 6.13. Volcano plots of skin microbiome species among AD vs controls.
Standardized β coefficients and p values from multiple linear regression analyses of all identified microbiome species on atopic dermatitis (AD) adjusted by age and, gender) are plotted as x-axis and y axis of the volcano plots. Data points in orange have p value <0.05 but not significant after adjusting for multiple testing. There were no data points in blue that signify significance after adjusting for multiple testing by B-H procedure.

Obesity

A total of 37 microbial species differed significantly in relative abundances with increasing BMI on multiple linear regression adjusting for age and gender and with multiple testing correction by B-H procedure at FDR threshold of 0.1. Majority of these significant microbial species increased in relative abundance with increasing BMI. Notably, multiple species of the *Corynebacterium* genus were significantly increased with increasing BMI. *Staphylococcus hominis* and *Streptococcus dysglactiae* were also increased with increasing BMI. Some significantly increased bacterial species (e.g *Eubacterium* spp, *Coprococcus* spp, *Prevotella buccalis*) are commonly found in the oral cavity or the gut. Only three out of the 37 microbial species had a decrease in relative abundance with increasing BMI. They were *Propionibacterium (Cutibacterium) acnes*, *Leptotrichia* unclassified and *Actinomyces massiliensis*. Details of these significant microbial species are provided in the following table 6.3 and volcano plot (Figure 6.14)

SN	Species	Estimate	P value	Corrected P value
1	*Staphylococcus hominis*	1.017	0.00001	0.00554
2	*Corynebacterium jeikeium*	0.838	0.00020	0.03627
3	*Propionibacterium (Cutibacterium) acnes*	-0.916	0.00013	0.03627
4	*Eubacterium rectale*	0.835	0.00019	0.03627
5	*Corynebacterium genitalium*	0.824	0.00025	0.03628
6	*Brevibacterium massiliense*	0.763	0.00067	0.05812
7	*Corynebacterium aurimucosum*	0.707	0.00164	0.05812
8	*Corynebacterium pseudogenitalium*	0.807	0.00071	0.05812
9	*Corynebacterium sp HFH0082*	0.694	0.00201	0.05812
10	*Corynebacterium tuberculostearicum*	0.767	0.00084	0.05812
11	*Collinsella aerofaciens*	0.685	0.00245	0.05812
12	*Bacteroides salyersiae*	0.685	0.00245	0.05812
13	*Parabacteroides distasonis*	0.685	0.00245	0.05812
14	*Paraprevotella unclassified*	0.685	0.00245	0.05812
15	*Prevotella buccalis*	0.767	0.00067	0.05812
16	*Prevotella copri*	0.689	0.00230	0.05812
17	*Candidatus Uzinura diaspidicola*	0.685	0.00245	0.05812

18	*Scytonema hofmanni*	0.685	0.00245	0.05812
19	*Streptococcus dysgalactiae*	0.685	0.00245	0.05812
20	*Eubacterium hallii*	0.685	0.00245	0.05812
21	*Coprococcus comes*	0.685	0.00245	0.05812
22	*Coprococcus eutactus*	0.685	0.00245	0.05812
23	*Dorea formicigenerans*	0.685	0.00245	0.05812
24	*Dorea longicatena*	0.685	0.00245	0.05812
25	*Eubacterium biforme*	0.685	0.00245	0.05812
26	*Phascolarctobacterium succinatutens*	0.685	0.00245	0.05812
27	*Megamonas unclassified*	0.692	0.00225	0.05812
28	*Megasphaera unclassified*	0.685	0.00245	0.05812
29	*Mitsuokella multacida*	0.685	0.00245	0.05812
30	*Sutterella wadsworthensis*	0.685	0.00245	0.05812
31	*Pseudomonas alcaligenes*	0.728	0.00118	0.05812
32	*Bacteroides vulgatus*	0.677	0.00270	0.06201
33	*Facklamia languida*	0.670	0.00297	0.06614
34	*Megamonas rupellensis*	0.661	0.00353	0.07634
35	*Campylobacter hominis*	0.652	0.00388	0.08164
36	*Actinomyces massiliensis*	-0.644	0.00435	0.08889
37	*Leptotrichia unclassified*	-0.637	0.00466	0.09271

Table 6.3. Significant microbial species with increasing BMI ranked based on measure of statistical significance.

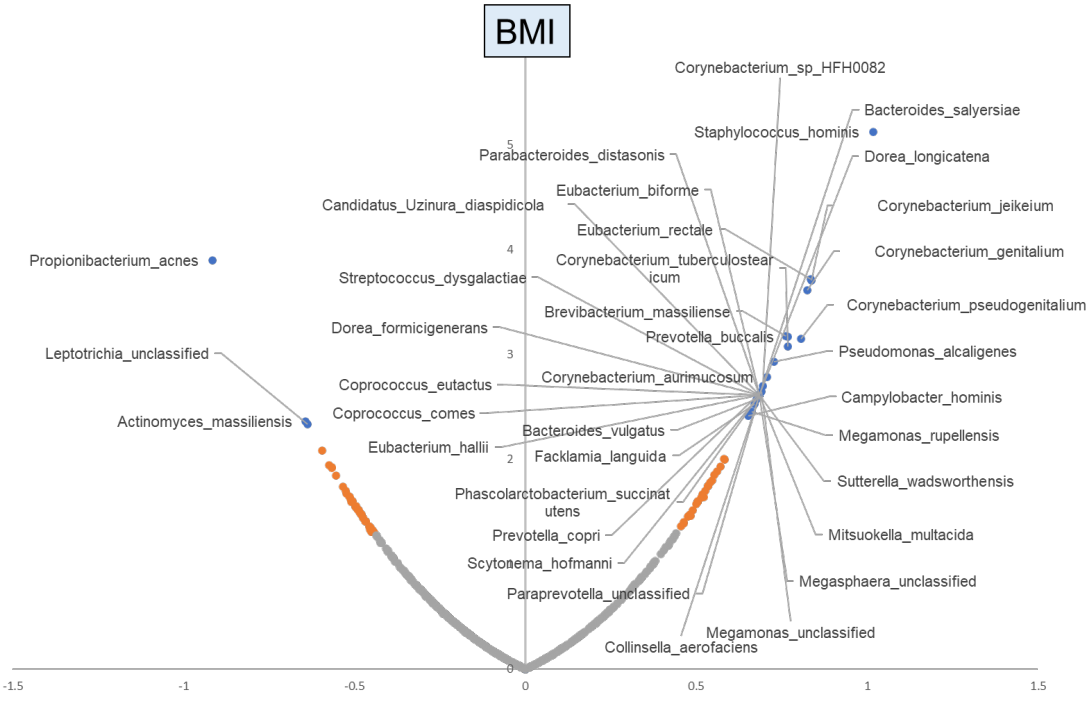


Figure 6.14. Volcano plots of BMI against skin microbiome species.
Standardized β coefficients and p values from multiple linear regression analyses of all identified microbiome species with increasing BMI adjusted by age and, gender) are plotted as x-axis and y axis of the volcano plots. Data points in blue were significant after adjusting for multiple testing by B-H procedure. Data points in orange have p value <0.05 but not significant after adjusting for multiple testing.

To assess how the relative abundance of microbial species were affected by abdominal obesity, multiple linear regression was also performed with increasing waist hip ratio (WHR) adjusted for age and gender. Majority of microbial species did not achieve statistical significance after confounders adjustment and multiple testing correction as seen in Figure 6.15. *Malassezia globosa* was noted to be increased with increasing WHR but did not meet the FDR threshold.

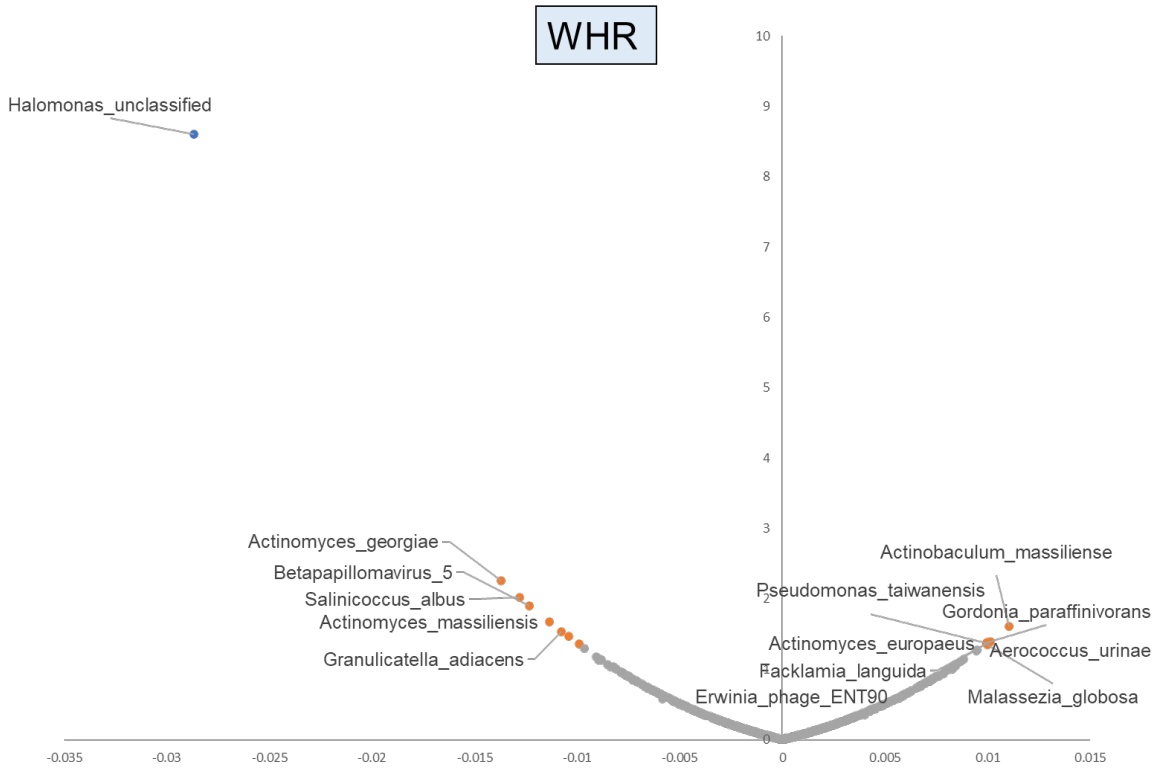


Figure 6.15. Volcano plots of WHR against skin microbiome species.
Standardized β coefficients and p values from multiple linear regression analyses of all identified microbiome species with increasing BMI adjusted by age and, gender) are plotted as x-axis and y axis of the volcano plots. Data points in blue were significant after adjusting for multiple testing by B-H procedure. Data points in orange have p value <0.05 but not significant after adjusting for multiple testing.

6.3.6. Sensitivity analysis of skin microbiome analysis

Sensitivity analysis was done with DXA scan data (Total fat and visceral fat). Not all the participants have complete DXA scan data. About 41 out of the 300 participants had missing DXA scan data. Most microbial species did not reach statistical

significance after multiple testing correction. However, species with significant p values ($p<0.05$) before B-H correction were largely similar to what is observed in the microbiota analysis with increasing BMI. Notably, Corynebacterium species and Malassezia globosa were noted to be increasing with increasing DXA fat measures. Propionibacterium acnes was noted to be decreasing with increasing DXA fat measures (Figure S.3 and S.4).

Sensitivity analysis was also performed to account for influence by samples with outlier number of reads. Outliers in terms of pre-mapping reads were identified and they are defined as samples with reads greater than 3 times interquartile range above 75th percentile. The boxplot (Figure S.5) illustrates the distribution of the reads for the 294 samples and as noted, there were 12 samples which were outliers as defined. As first part of the sensitivity analysis, these 12 outliers were removed and the remaining 282 samples were analyzed to compare diversity measures and individual microbiome species between AD vs controls as well as Obese vs Non obese. There were no significant differences of various alpha diversity measures. Beta diversity PCoA plots were also similar to the original plots presented (Figure S.6). While there were reduced number of significant microbiome species (after FDR correction) with increasing BMI, key commensal species such as Cutibacterium (P*ropionibacterium) acnes, staphylococcus hominis* and various *Corynebacterium* species were similarly identified in this sensitivity analysis (Figure S.7). *Streptococcus mutans* and *Mitsuokella* species were similarly significantly reduced (before FDR correction) in AD cases (Figure S.7). As second part of the sensitivity analysis, a down sampling of reads was performed for the 12 outliers. They were down sampled to the median number of reads among the 294 samples. This was calculated to be 12254863 number of reads. Again, there were no significant differences of various alpha diversity

measures. Beta diversity PCoA plots were also similar to the original plots presented (Figure S.8). Key commensal species such as *Propionibacterium acnes, staphylococcus hominis* and various *Corynebacterium* species were also identified to be significant with increasing BMI in this sensitivity analysis (Figure S.9). *Staphylococcus epidermis* was similarly significantly reduced (before FDR correction) in AD cases (Figure S.10).

6.3.7. Functional profiling of skin microbiome in AD and obesity

Functional profiling was performed to evaluate the relative abundance of metabolic pathways based on metagenomic gene sequences of the resident skin microbiome. It provided a snap-shot of the metabolic activities attributed to skin microbiome. Linear discriminant analysis (LDA) was also performed for metabolic pathways abundance to identify pathways with consistent differences in abundance between AD and controls as well as obese BMI $\geqslant$ 30kgm2 and non-obese. Significant pathways were then ranked according to their effect sizes in terms of LDA in this LEFSe analysis [250].

AD

A total of nine metabolic pathways were found to be significantly different by the non-parametric factorial Kruskal-Wallis (KW) sum-rank test. However, only five discriminative metabolic pathways had an absolute LDA score of greater than 2.0. They are illustrated in the following LEfSe plot for AD and controls. Only the super pathway of branched amino acid biosynthesis was significantly enhanced in those with AD. Significant pathways enhanced in controls were mainly that of various pathways of ubiquinol biosynthesis (prokaryotic).

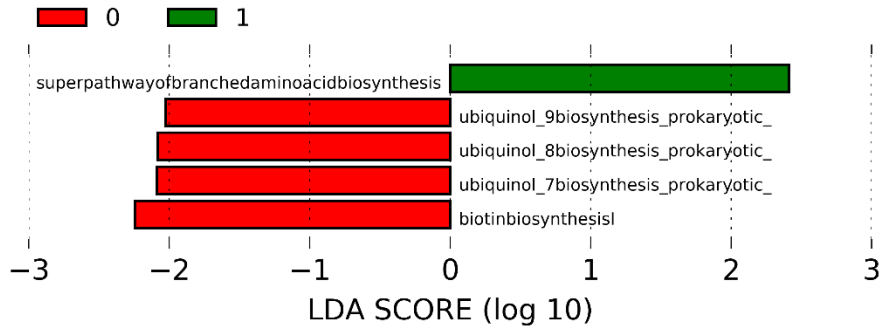


Figure 6.16. Linear discriminant analysis Effective Size (LEfSe) plot for metabolic pathways with consistent differences in abundance between AD and controls.
This plot illustrates which metabolic pathways that were statistically significant and explained the greatest differences between AD and control. The green histogram bars illustrate those pathways that were enhanced in AD while those red bars illustrate those more enhanced in controls.

Multiple linear regression of the abundance of these metabolic pathways with adjustment of age and gender revealed a total of five metabolic pathways which were significantly enhanced in participants with AD. These were various pathways of menaquinol biosynthesis (super pathway of demethylmenaquinol-8 biosynthesis, superpathway of menaquinol-8 biosynthesis I, superpathway of menaquinol-7 biosynthesis), ppGpp biosynthesis and myo-, chiro- and scillo-inositol degradation pathways. The super pathway of cytosolic glycolysis (plants), pyruvate dehydrogenase and TCA cycle as well as pyruvate fermentation to propanoate I pathways were reduced in AD. However, none remained significant after multiple testing correction. These results are illustrated in Figure 6.17.

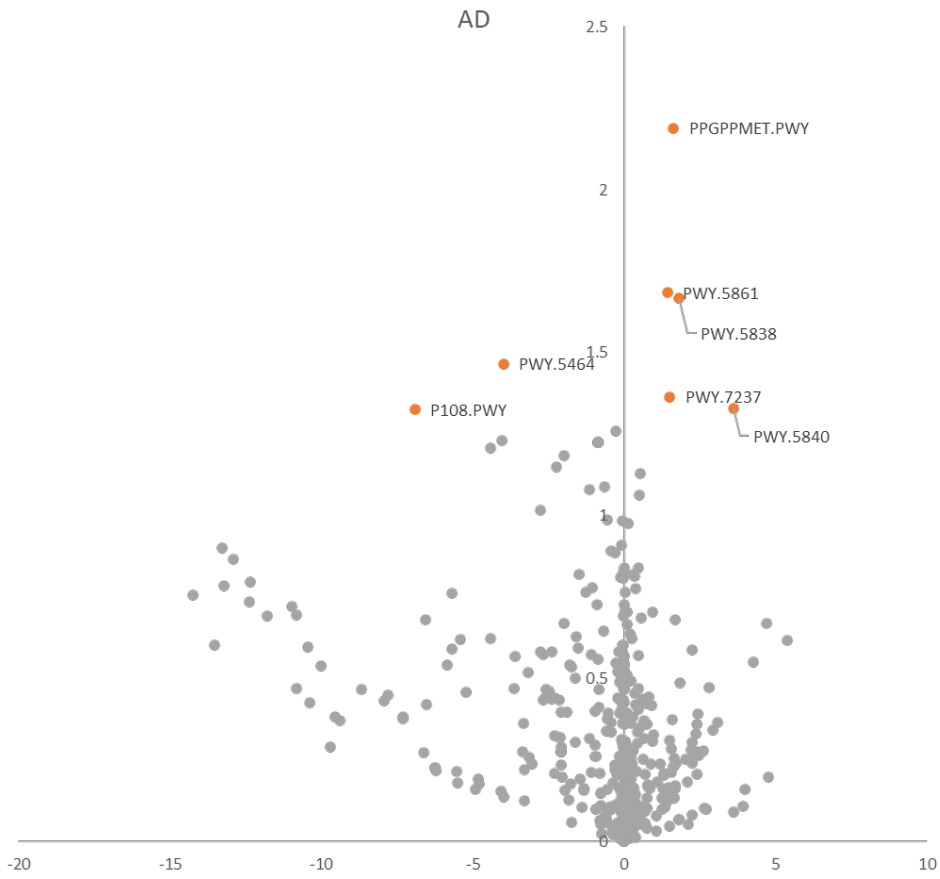


Figure 6.17. Volcano plots of microbiome metabolic pathways in AD.
Standardized β coefficients and p values from multiple linear regression analyses of all identified metabolic pathways on atopic dermatitis (AD) adjusted by age and, gender) are plotted as x-axis and y axis of the volcano plots. Data points in orange have p value <0.05 but not significant after adjusting for multiple testing. There were no data points in blue that signify significance after adjusting for multiple testing by B-H procedure. PPGPPMET.PWY: ppGpp biosynthesis, PWY.5861:super pathway of demethylmenaquinol-8 biosynthesis, PWY.5838: superpathway of menaquinol-8 biosynthesis I, PWY.5840: superpathway of menaquinol-7 biosynthesis, PWY.7237: myo-, chiro- and scillo-inositol degradation, PWY 5464: super pathway of cytosolic glycolysis (plants), pyruvate dehydrogenase and TCA cycle, P108.PWY: pyruvate fermentation to propanoate I

Obese

A total of 89 metabolic pathways were found to be significantly different by the non-parametric factorial Kruskal-Wallis (KW) sum-rank test between obese (BMI≥30kgm^2) and non-obese. However, 76 discriminative metabolic pathways had an absolute LDA score of greater than 2.0. These are illustrated in the following LEfSe plot for obese and non-obese.

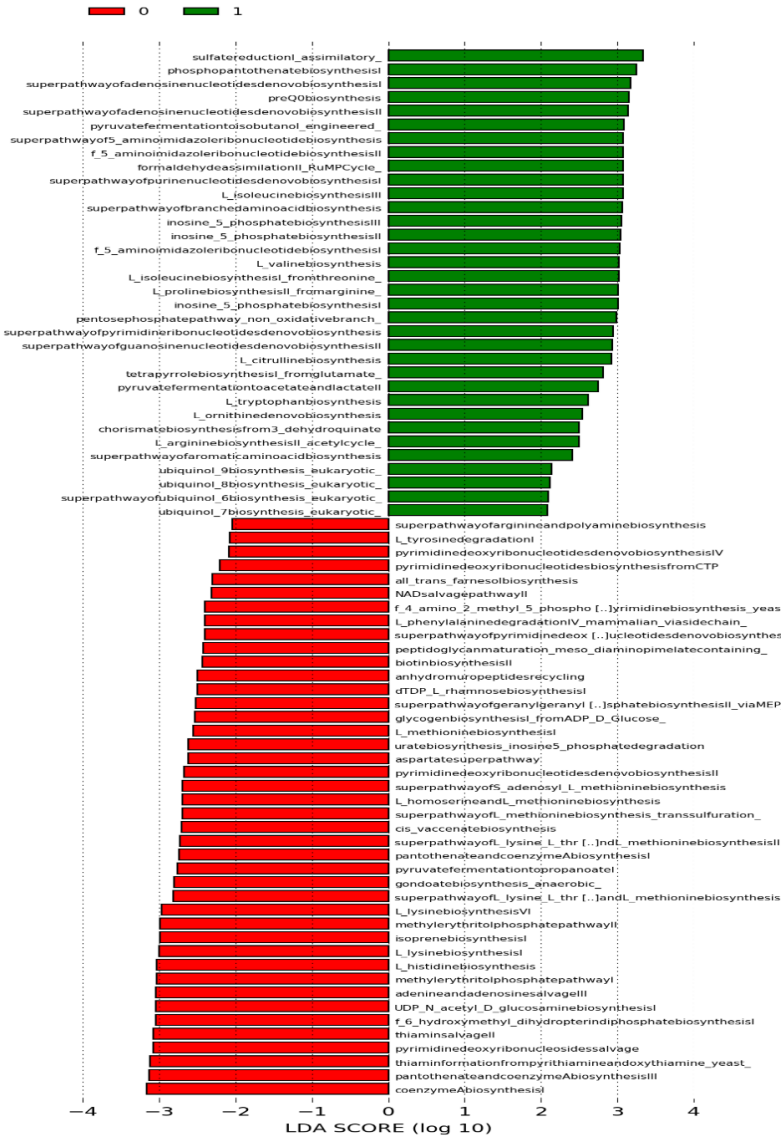


Figure 6.18. Linear discriminant analysis Effective Size (LEfSe) plots for metabolic pathways with consistent difference in relative abundance between obese (BMI≥30kgm^2) and non-obese.

This plot illustrates which metabolic pathways that were statistically significant and explained the greatest differences between obese and non-obese. The green histogram bars illustrate those pathways more abundant in obese while those red bars illustrate those more abundant in non-obese.

Some of the pathways that were significantly enhanced in obese individuals were inosine 5 phosphate biosynthesis pathways and ubiquinol biosynthesis for eukaryotic pathways. Super pathway of branched amino acids and sulfate reduction I assimilatory pathway.

Multiple linear regression of the abundance of these metabolic pathways with adjustment of age and gender revealed a total of 49 metabolic pathways which were significantly different with increasing BMI after multiple testing correction by B-H procedure. Twenty-five of these metabolic pathways were significantly enhanced with increasing BMI while 24 of these pathways were found to be reduced with increasing BMI. The formaldehyde assimilation II (RuMP Cycle) pathway was significantly enhanced with increasing BMI, with the largest effect measure and measure of significance while the coenzyme A biosynthesis I pathway was significantly reduced with increasing BMI.

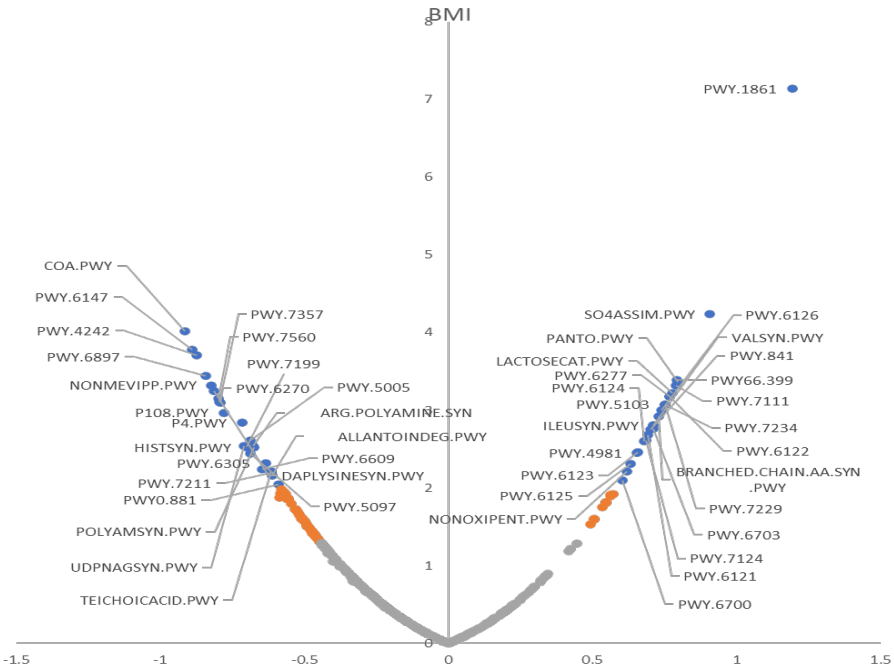


Figure 6.19. Volcano plots of microbiome metabolic pathways with increasing BMI.
Standardized β coefficients and p values from multiple linear regression analyses of all identified metabolic pathways with increasing BMI adjusted by age and, gender) are plotted as x-axis and y axis of the volcano plots. Data points in blue were significant after adjusting for multiple testing by B-H procedure. Data points in orange have p value <0.05 but not significant after adjusting for multiple testing.

As part of sensitivity analysis, multiple linear regression was similarly performed with increasing waist hip ratio (WHR) adjusting for age and gender. Eight metabolic pathways were significantly different with increasing WHR but none remained significant after multiple testing correction. These were gluconeogenesis III, glycolysis

VI (metazoan), superpathway of L-methionine biosynthesis (by sulfhydrylation), methanol oxidation to carbon dioxide, mannosylglycerate biosynthesis I, superpathway of allantoin degradation in yeast, ureide biosynthesis and superpathway of L-methionine biosynthesis (by sulfhydrylation).

6.4. Discussion

This study examined the skin microbiome using whole metagenomic sequencing in close to 300 participants and identified differences in the microbiome communities across AD and controls as well as obese and non-obese participants. Using whole metagenomic sequencing allowed evaluation of microbial communities beyond bacteria kingdom to include eukaryotes and viruses. This improved our understanding in skin microbiome, although majority of the mapped microbial species still belong to the bacteria kingdom. These microbial genetic sequences also allowed functional profiling of the skin microbiome and shed some light on metabolic activities on-going at the skin surface.

In this study, there were no significant differences in alpha diversity measures and Bray-Curtis dissimilarity index between AD and controls. This is in contrast to what have been reported in the literature that microbial diversity tends to be reduced in patients with AD. It has previously been demonstrated in various studies that diversity would decrease during times of AD flares as these patients' skin become colonized with *staphylococcus aureus*. There would also be reduced abundances of *Streptococcus, Corynebacterium* and *Propionibacterium* at times of flare [128]. However, a recent local study had also observed that the abundance of *staphylococcus aureus* remained normal in between flares and microbial diversity would remain stable during these in between disease flare states [194]. Majority of

these participants with AD in this population cohort study were likely to have a milder disease severity and in between disease flares. Having a disease flare or a worse severity might preclude their participation in this general population cohort study. This might therefore be one of the reasons why there were no observed differences in microbial diversity between AD and controls.

Similarly, there were no obvious differences in alpha diversity measures or Bray-Curtis dissimilarity (beta diversity) between obese versus non-obese individuals. Literature regarding skin microbiome in obesity has been sparse. A recent study reported that the Shannon diversity index was reduced in obese individuals compared to underweight individuals while there were no differences between normal weight and obese/overweight individuals [15]. The principal coordinates plot (PCoA) of Bray-Curtis distance however showed some clustering between obese (BMI $\geq$ 30kgm^2) and non-obese samples with some degree of overlap. This lack of statistical significance could possibly be attributed to a small sample size of obese individuals within this case control cohort.

In terms of differences in the relative abundance of specific microbial communities between AD and controls, it was noted in LEfSe analysis that *Enterobacter hormaechei* was significantly higher in AD cases and had an absolute LDA score of greater than 2.0. It is a gram-negative anaerobe and has been reported to cause nosocomial infections in hospital settings. Its significance in AD patients remained relatively unknown. It was found only in 11 out of the 294 samples sequenced and this skewed distribution might have contributed to its statistical significance. Multiple linear regression with adjustment for age and gender was also performed with AD as an independent variable and the relative abundance of various microbial species as continuous outcome. It was noted that the relative abundance of *Staphylococcus*

epidermis was significantly increased in AD compared to controls with an odds ratio of 2.50 and p =0.0216. *Staphylococcus epidermis* has been widely regarded as a beneficial commensal organism that in a way mitigates the harmful effects of *Staphylococcus aureus* [252, 253]. However, it is also known to cause serious nosocomial and implant infections [254, 255]. Emerging evidence has suggested that *Staphylococcus epidermis* may play an important pathological role in AD and its abundance has been linked with AD disease severity [256, 257]. This has been attributed to specific protease EcpA produced by *S. epidermis* in atopic skin [257]. Further analysis and studies should be done to evaluate this observation given that it did not reach statistical significance after multiple testing correction. Furthermore, *S. aureus* was not observed to be significantly more abundant in this cohort of AD participants.

It was apparent that various species of the *Corynebacterium* genus were significantly increased in obese individuals ($BMI \geq 30 kgm^2$) or with increasing BMI in LEfSe and multiple linear regression analysis respectively. These species are known to be skin commensals and have been previously reported in a recent skin microbiome study where *Corynebacterium* relative abundance increased significantly with BMI [15]. This was consistent with another animal study where obese mice on high fat diet were related to an increased *Corynebacterium* colonization [235]. It has been hypothesized that mycolic acid expressed by these bacterial species might promote skin inflammation specifically the Th-17 axis via IL-23 activation [234]. This was the basis for the hypothesis of how *Corynebacterium* might mediate the increase risk of psoriasis in obese individuals as psoriasis is mainly a Th-1/Th-17 inflammatory skin disease. Anti-IL23 has been a recent biologic therapy for treatment of psoriasis. Although AD remains mainly a Th-2 inflammatory skin disease, a recent comparative

study described an Asian AD immunotype that combined Th-2 and Th-17 immunotypes [69]. Th-17 immune activation as a result of increased *Corynebacterium* species might be one of the several mechanistic pathways linking obesity with AD.

Other skin commensals microbial communities that were significantly different between obese and non-obese included increased abundances of *Staphylococcus hominis, Streptococcus dysgalactiae, Malassezia globosa* and reduced abundances of *Propionibacterium (Cutibacterium) acnes* and *Staphylococcus aureus*. Multiple linear regression adjusting for age and gender yielded similar observations in *Staphylococcus hominis, Streptococcus dysgalactiae* and reduced abundances of *Propionibacterium (Cutibacterium) acnes* with increasing BMI. *Malassezia globosa* is a common skin commensal yeast that has been reported to be associated with the development of seborrheic dermatitis, another common inflammatory skin condition affecting the face and neck. Evidence has suggested its possible role in development of atopic dermatitis although there has been a study to suggest that protease, MgSAP1 secreted by *Malassezia globosa* could reduce specific virulence factors of *S. aureus* and therefore contribute to skin health [258-260]. The observed increased relative abundance of *M globosa* in LEfSe analysis with a LDA score of 4.0 in obese group could have implications on links between obesity and AD. However, it was interesting to note that the difference in *Malassezia globosa* was based on samples taken over the inner arms, a moist skin site. This study illustrated an increased relative abundance of several skin commensal bacteria that have been known to contribute to skin health [128, 261]. For example, specific strains of *Staphylococcus hominis* has been shown to attenuate the harmful effects of *S. aureus* on the skin epidermis [257, 262]. *Cutibacterium acnes*, a common skin commensal bacterial in sebaceous skin sites, similarly have protective effects against S. aureus but its abundance was reduced in

obese individuals sampled at the inner forearm, generally considered a moist skin site [263, 264]. An observed increased abundance of these skin commensals and concurrent reduction in *S. aureus* in obesity may confer a form of protective effects from epidermal skin injury from *S. aureus*. Therefore, the specific microbial communities characterized in obese individuals appeared to have different impact related to the risk of developing AD.

Finally, having whole metagenomic sequences of microbiome allowed metabolic pathway analysis to be performed. Super pathway of branched amino acids was enhanced in skin microbiome of both AD and obese individuals from LEfSe analysis. Branched chain amino acids include leucine, isoleucine, and valine. Plasma levels of these branched amino acids are known to be elevated in obese individuals as oxidation of these molecules play a role in fatty acid oxidation which in turn contribute to the mechanisms of obesity [265]. However, the significance of this pathway from a skin microbiome perspective remained unknown. Similarly, metabolic products of these microbiome pathways may affect skin barrier function.

Microbiome pathway analysis relies on mapping genetic sequences to known database collections of pathways. While it gives a snapshot and sensing of what pathways might exist on the skin, this is prone to certain interpretation bias and pitfalls. It gives a rough inference on the activity of the pathways based on the levels of reads of the relevant pathway sequences but it relies on the assumption that enzymatic activities and pathways expression are reflected by sequence reads mapping to pathways [246]. The relationships of intermediate products and their interactions cannot be adequately reflected by this sequence reads. Secondly, identity of these pathways relies on known definitions. As seen in the results, a large proportion of reads remained unmapped or unintegrated. This represents a significant portion of

unknown that may play important roles in skin surface metabolism. Finally, with the plethora of pathways mapped, it presents a statistical challenge on how to evaluate enhance and inhibition as well as their interactions. Therefore, it is important to recognize these limitations when interpreting this microbiome pathway analysis data.

In summary, there were no observed differences in microbiome diversity in AD cases and obese individuals compared to controls. There were, however, differences in relative abundances of specific microbial species among obese individuals that may play a role in skin inflammation. Various microbiome metabolic pathways appeared enhanced in obesity, but one needs to be cognizant of its limitations during interpretations.

7. Study 4: Serum proteomics profile in AD and obesity

7.1. Overview and rationale

AD is a chronic skin disease with disrupted skin barrier function and systemic inflammation. It is widely known to be a systemic disease with Th-2 activated inflammation and elevation of Total Ig E, IL4, IL13 and CCL17 [266]. Similar to AD, obesity is a chronic metabolic disease with systemic inflammation and associated with multiple co-morbidities. While there have been established evidence that obesity is a risk factor for other metabolic and cardiovascular diseases, evidence to support its links with AD and atopy have only been emerging [11].

The hypothesis was the inflammatory profile of obesity could lead to or augment the systemic inflammation observed in AD, providing an explanation to the observed relationship between obesity and AD. The aim of Study 4 was therefore to investigate the serum proteomic profile in AD using a multiplexed platform and the extent obesity underpins the inflammatory condition in AD. It was a matched case control study in a selected cohort of 300 study participants from HELIOS.

With the availability of multiplexed proteomic platforms with better sensitivity and specificity, it would be possible to evaluate the serum proteomic profile of AD and obesity in a high throughput manner. This allowed evaluation of known and lesser-known proteomic biomarkers. It would also further improve our understanding of the profile of AD and obesity as well as establish how obesity may affect the underlying inflammatory profile in AD.

7.2. Methods and Analysis

Similar to the study design in the skin microbiome study, proteomic analysis was performed in 300 of the study participants using a (1:1) matched case control design. Taking into considerations ethnic differences and to further enhance our statistical power, only Chinese participants were included as it was the majority ethnic group in the HELIOS cohort. This would avoid any potential confounding due to ethnic factors. One hundred and fifty AD cases were selected with 150 age (within ± 5 years) and gender matched controls. Participants with known history of systemic inflammation, immunosuppression and systemic infections were excluded from the study.

Serum samples were already drawn from HELIOS participants, centrifuged and subsequently stored at -80 degree Celsius until further processing. A targeted proteomic profiling using the Proximity Extension Assay (PEA) technology (OLINK) platform was performed on stored serum samples of these selected AD cases and controls samples to identify highly sensitive and highly specific biomarkers [267]. The three panels kit utilized were the Olink® cardiovascular II and III panels as well as the Olink® inflammation panel. A list of the proteomic biomarkers included in these panels are provided in Annex C. The selection of these proteomic biomarkers panels were based on other similar studies conducted in USA and Europe that evaluated the serum inflammatory biomarkers in the relationship between AD and cardio-metabolic comorbidities [268-270].

The serum biomarkers included established and exploratory biomarkers of the Olink multiplex panels. These included, but are not limited, to the following: Serum eosinophil cationic protein (ECP), TARC/CCL17, Serum sE-selectin, Cutaneous T-cell attracting chemokine (CTACK/CCL27), CD30, IL18, Macrophage derived chemokine (MDC), IL-22, osteopontin, IL31, IL33, IL4, IL13, and IL5. Cytokines of the Th1 and

Th17 pathway were also included given the observation that patients with AD has an elevated Th1 and Th17 profile. Biomarkers such as metabolic markers in obesity and cardiovascular diseases were also included. In addition, serum Total Ig E levels were analyzed separately among the 300 participants. The analysis of these biomarkers would allow assessment of how the presence of obesity could influence the characteristics and level of the various biomarkers.

OLINK® multiplex assay platform uses a PEA technology with oligonucleotide-labelled antibody probe pairs that bind to their respective targets [267]. Upon binding both members of the antibody pairs at the target site, the paired DNA reporter molecules bound to the antibodies give rise to DNA amplicons. These DNA amplicons are specific to each respective antigen. The amplicons are then subsequently quantified using a Fluidigm BioMarkTM HD real-time PCR platform [267, 271]. Raw data in the form of Ct (cycle threshold) values are normalized in three steps to generate the NPX (Normalized Protein Expression) unit, an Olink's arbitrary unit. Intensity normalization is then performed to adjust the NPX values so that median values of all samples would be the same for all plates.

All proteomic data were in NPX units on log2 scale and lowly expressed assays were replaced with limit of detection (LOD) values. These LOD values were based on the background signal, estimated from the negative controls included on every plate, plus three standard deviations. There were two samples with missing values and hence removed from the final analysis. This resulted in 149 AD cases with 149 matched controls for comparison.

Data were analyzed in the following manner: Multiple linear regressions were performed to identify significantly different serum biomarkers between AD and controls

as well as Obese (BMI ≥ 30 kgm^2) and not obese. P values were subsequently subjected to multiple testing correction using the Benjamini–Hochberg (B-H) procedure at false discovery rate (FDR) threshold of 0.1. Each volcano plot for AD or obesity was plotted to identify any biomarkers with the most meaningful change. It combines a measure of statistical significance from a statistical test with the magnitude of the change. Multiple linear regressions adjusted for age and gender were also performed to adjust for possible confounding.

To assess for influence of increasing BMI on AD, the following analysis steps were performed (Figure 7.1). Multiple linear regression analyses were performed with BMI as a continuous outcome and individual biomarkers as independent variables adjusted by age and gender. A list of statistically significant ($p<0.05$) biomarkers were identified and summarized. Reference was made to known serum biomarkers important in disease pathogenesis in AD. Subsequent multiple linear regression analyses adjusted by age and gender were then performed with AD status as the binary outcome and identified biomarkers from the previous step as independent variables. Significant biomarkers adjusted for multiple testing correction by B-H procedure were evaluated.

Principal component analysis was also performed to evaluate for any observed clustering base on AD and obesity status. Finally, partial least squares discriminant analysis (PLS-DA), a form of supervised clustering analysis, was performed to identify serum biomarkers that might be important in discriminating between AD versus control, as well as obese versus non-obese. PLS-DA aims to find a linear regression model by projecting the predicted variables and the observed variable onto a new space. A PLS model will find the multi-dimensional direction in X space that explains the maximum multidimensional variance direction in the Y space [272]. Variable importance in projection (VIP) scores estimate the importance of each variable in the model.

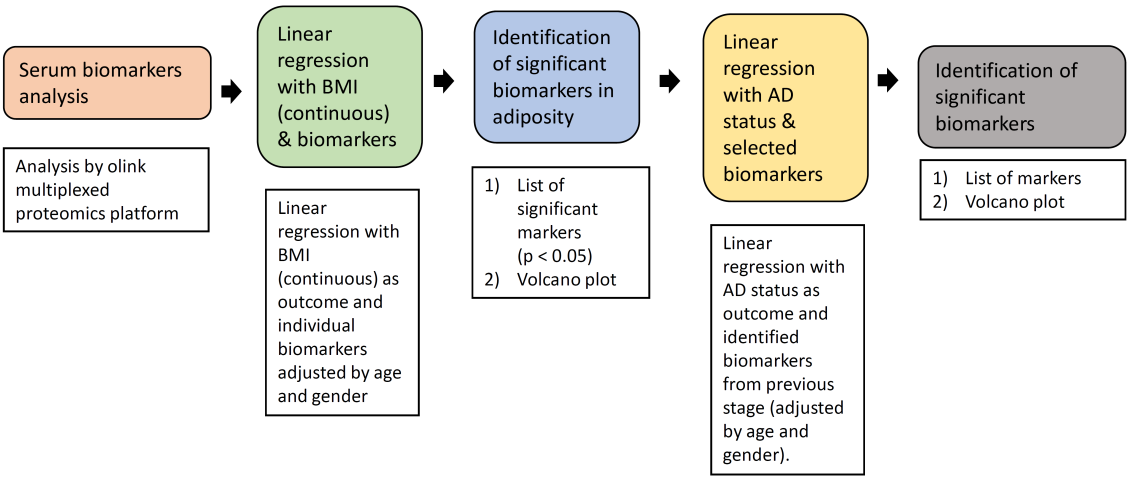


Figure 7.1. Analysis steps of serum proteomics biomarkers from Olink multiplexed platform.

Of the 149 AD cases included in the proteomic analysis, only 66 AD cases provided information about the severity of their skin condition. A sensitivity analysis was performed to evaluate if levels of particular biomarkers were significantly different in more severe AD cases compared to mild AD cases as well as non-AD controls. Results were summarized in volcano plots.

7.3. Results

7.3.1 Demographic profile

As mentioned in methods section, two samples had missing data and therefore only 149 AD cases and 149 matched controls were included in the final analysis. Majority of the participants included in this study were female (67.8%). Only 66 participants out of the 149 AD cases provided AD severity information. Majority (59.1%) were of mild severity and only about 9.1% had severe AD.

Characteristics	AD (n=149)	Controls (n=149)	P value
ǂ Age (years, mean)	52.0	52.5	0.726
ǂ Female (%)	67.8	67.8	1.000
Obesity by BMI (%)	8.1	7.4	0.828
Obesity by WHR (%)	37.6	35.8	0.751
Severity of AD (%) (n=66)			
Mild	59.1 (39)		
Moderate	31.8 (21)		
Severe	9.1 (6)		

ǂ Matched variables

Table 7.1. Characteristics of participants included in the Olink analysis.

7.3.2 Overview of serum proteomic profile in AD and obesity

There were 19 serum biomarkers found to be different between AD and controls at p value < 0.05. However, none remained statistically significant after correction for multiple testing by B-H procedure. Fifty serum biomarkers were found to be statistically different between obese vs non-obese after correction for multiple testing. (Figure 7.2)

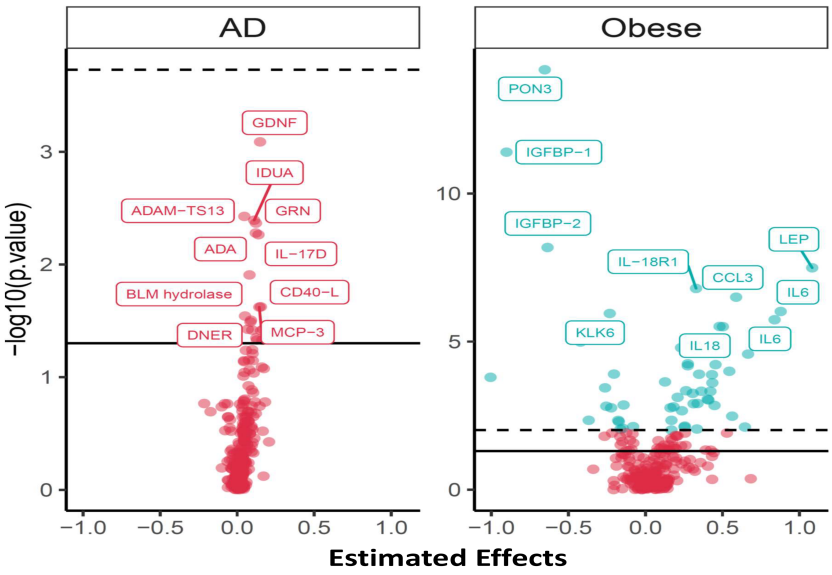


Figure 7.2. Volcano plots illustrating the effect size and measure of statistical significance of the Olink biomarkers.

7.3.3. Serum proteomic profile of AD

There were 11 serum biomarkers found to be different between AD and controls at p value < 0.05% after adjusting for age and gender. However, none remained statistically significant after correction for multiple testing by B-H procedure. These 11 serum biomarkers were IL-17D, MCP3, GDNF, GRN, BLM hydrolase, ADA, IDUA, ADAM TS13, DNER, LIF and TR.AP. Their effect estimates in AD participants and their measure of statistical significance are summarized in Table 7.2 and volcano plot (Figure 7.3).

SN	Biomarkers	Estimate	P value	Corrected P value
1	IL.17D	0.0582	0.00287	0.310
2	GDNF	0.0677	0.00335	0.310
3	GRN	0.0562	0.00348	0.310
4	BLM.hydrolase	0.0407	0.00808	0.455
5	ADA	0.0596	0.00852	0.455
6	IDUA	0.0457	0.0128	0.569
7	ADAM.TS13	0.0149	0.0195	0.694
8	LIF	0.0464	0.0212	0.694
9	DNER	0.0324	0.0234	0.694
10	MCP.3	0.0661	0.0353	0.817
11	TR.AP	0.0479	0.0389	0.817
12	MB	0.0534	0.0507	0.817
13	IL.22.RA1	0.0367	0.0508	0.817
14	TFPI	0.0388	0.0511	0.817
15	CCL24	0.0717	0.0520	0.817
16	MCP.4	0.0601	0.0530	0.817
17	IL.2RB	-0.0471	0.0544	0.817
18	NRTN	-0.0526	0.0551	0.817
19	CTRC	0.0659	0.0721	0.955
20	HO.1	0.0382	0.0738	0.955
21	CST5	0.0507	0.0751	0.955
22	GDF.2	0.0301	0.0832	0.992
23	Total IgE	0.181	0.0898	0.992
24	SCF	0.0243	0.0903	0.992
25	VSIG2	0.0418	0.0937	0.992

Table 7.2. Top 25 biomarkers for AD ranked based on measure of statistical significance.

A) Principal Component Analysis

As there were 267 unique serum biomarkers, principal component analysis (PCA) was performed as a form of unsupervised clustering analysis to evaluate if there were any distinct clusters between AD and controls. As illustrated in the PCA plot (Figure 7.4), principal component 1 explained 16.4% of the variance while principal component 2 explained about 6.6%. There was no obvious clustering observed based on the Olink biomarkers investigated. The blood proteomic profile of the AD cases and controls in this study was largely similar.

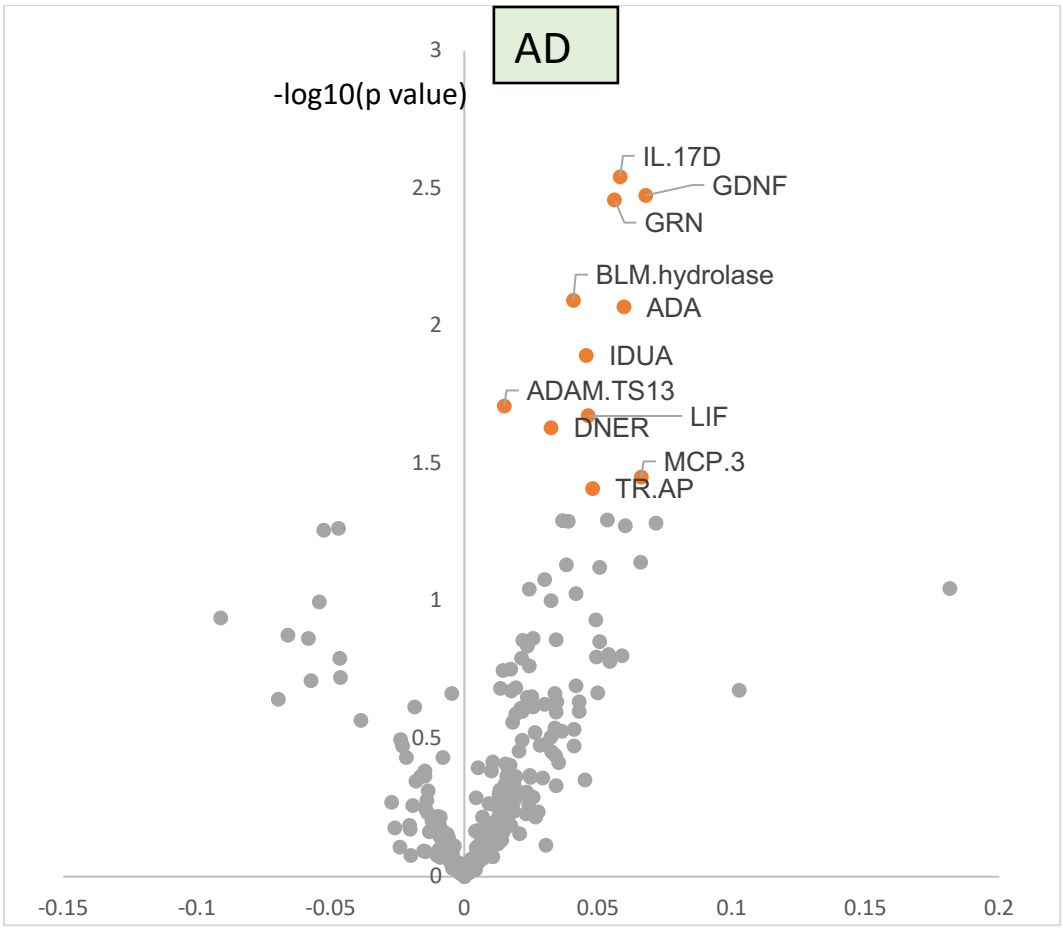


Figure 7.3. Volcano plot: Relationship of serum biomarkers and AD adjusted for age and gender.

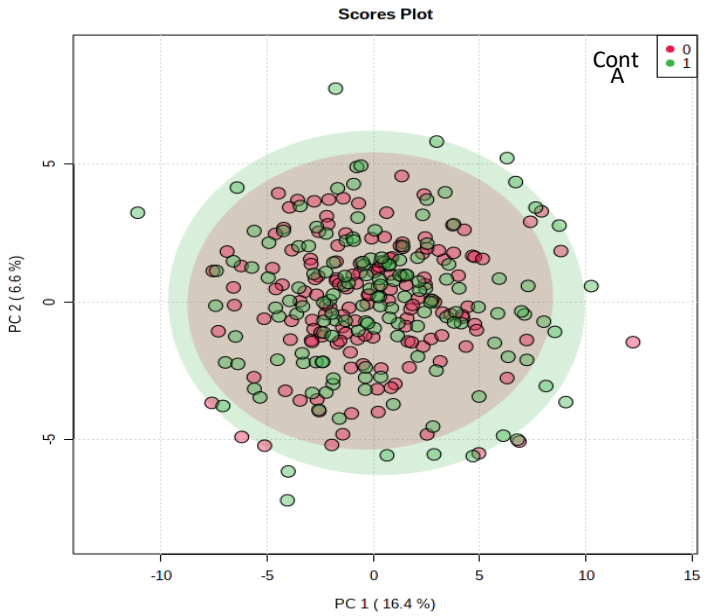


Figure 7.4. Principal component analysis plot of biomarkers for AD versus Control.

B) Partial least squares (PLS) discriminant analysis.

Partial least squares (PLS) discriminant analysis was performed to identify serum biomarkers that might be important in discriminate between AD versus control. Variable importance in projection (VIP) scores calculated for those projected in the model would estimate the importance of each serum biomarker in discriminating between AD and control.

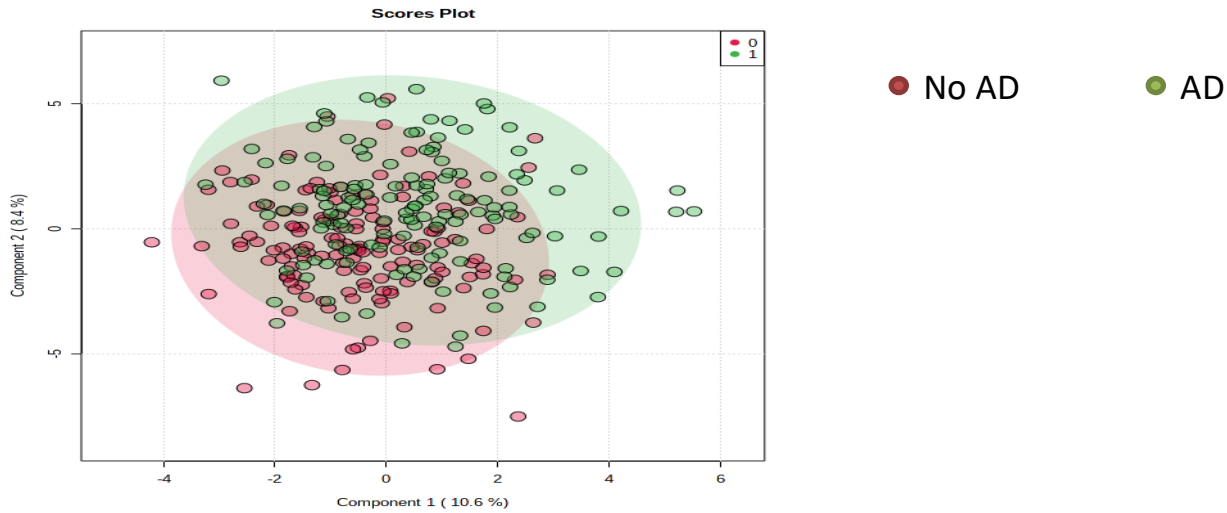


Figure 7.5. Supervised clustering plot: Partial least squares discriminant analysis of AD vs controls.

The PLS discriminant analysis plot showed a large degree of overlap between the AD and control clusters. There was no obvious clustering on PLS discriminant analysis.

The VIP projection scores of the various biomarkers are summarized in the VIP scores plot of the top 50 biomarkers with a VIP score greater than 1 (Figure 7.6). The VIP score measured the importance of each biomarker in the PLS-DA model in distinguishing between AD and controls. A cut-off value of 1 is often used for selecting relevant variables/biomarkers [273].

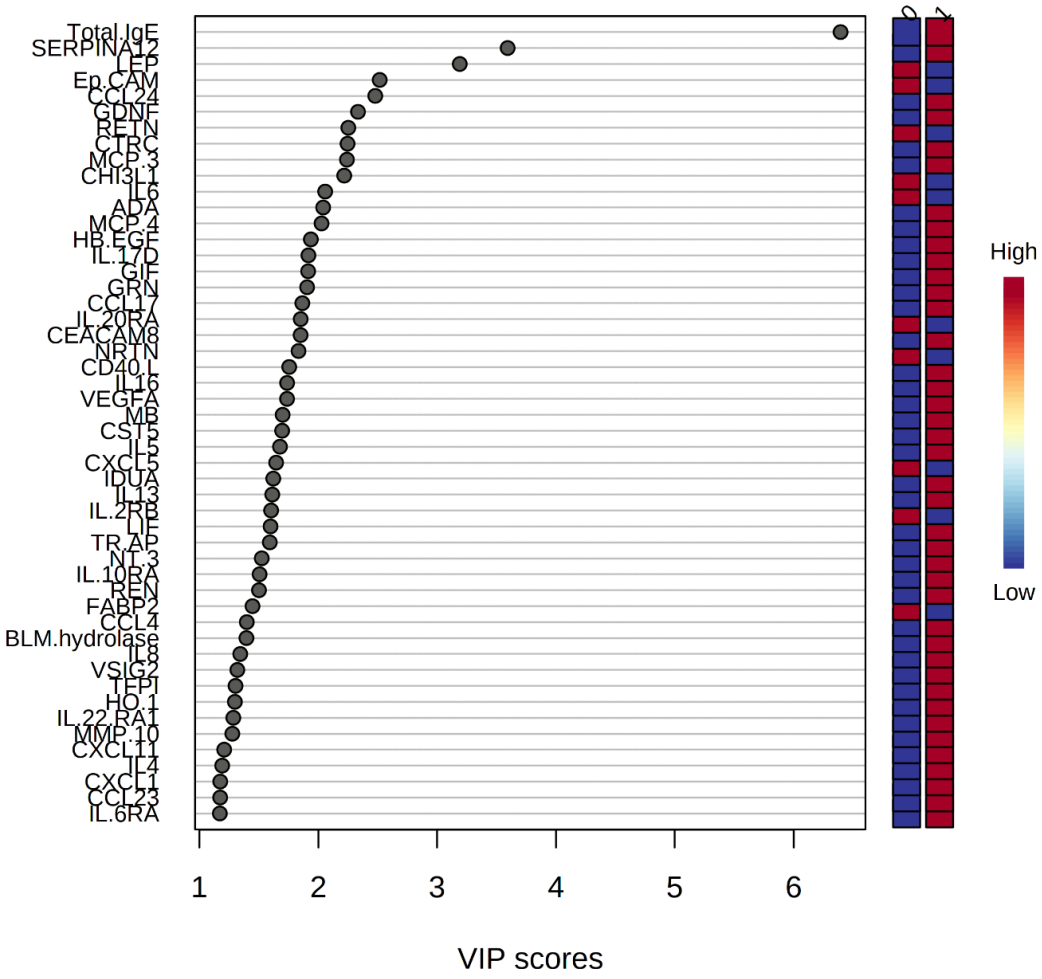


Figure 7.6. VIP scores of top 50 biomarkers of the PLS-DA component 1.
The first column with heading "0" indicates control while heading "1" indicates AD. The colours (red vs blue) in the respective cells indicates whether the biomarker is elevated and lowered respectively.

From the VIP scores of the serum biomarkers of component 1 of the PLS-DA model, it can be seen that Total IgE was the most important marker in the model distinguishing AD and controls. Other biomarkers of note included known Th-2 inflammatory markers for AD such as IL-4, IL-13, CCL17, IL2RB as well as Th-17 and Th-22 markers. Leptin, a widely known systemic marker for obesity, was also elevated with a VIP score greater than 3.0. However, it was important to note that while these markers were found to be important in the model distinguishing AD and controls, none of them were statistically significant different between AD and controls in their respective regression analyses with multiple testing correction.

C) Sensitivity analysis - analysis of serum biomarkers and severity of AD

Only 66 out of the 149 AD cases provided information on their disease severity. Multiple linear regression adjusting for age and gender was performed to compare serum biomarkers between moderate to severe AD vs mild AD cases. A total of 14 biomarkers had a p value of 0.05 and below in their respective linear regression (Figure 7.7) Known biomarkers associated with AD included MCP.1, CX3CL1 and SELE. However, none of them achieve statistical significance after multiple testing correction by B-H procedure. A further sensitivity analysis was performed to compare serum biomarkers between those with moderate to severe AD with non-AD controls to provide more contrast. A total of 10 biomarkers were significant with p value < 0.05 but all were not significant after multiple testing correction. They were similar to some of the biomarkers detected in the comparison of moderate to severe cases to mild ones.

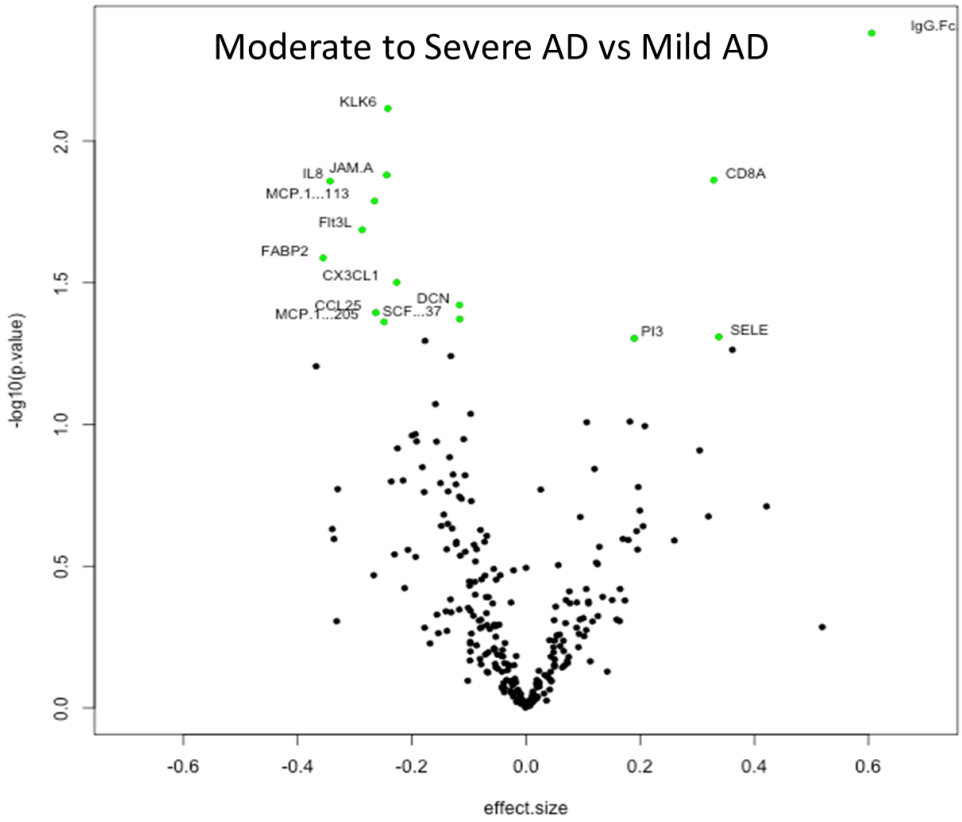


Figure 7.7. Volcano plot: Relationship of serum biomarkers and severity of AD (moderate to severe vs mild AD) adjusted for age and gender.

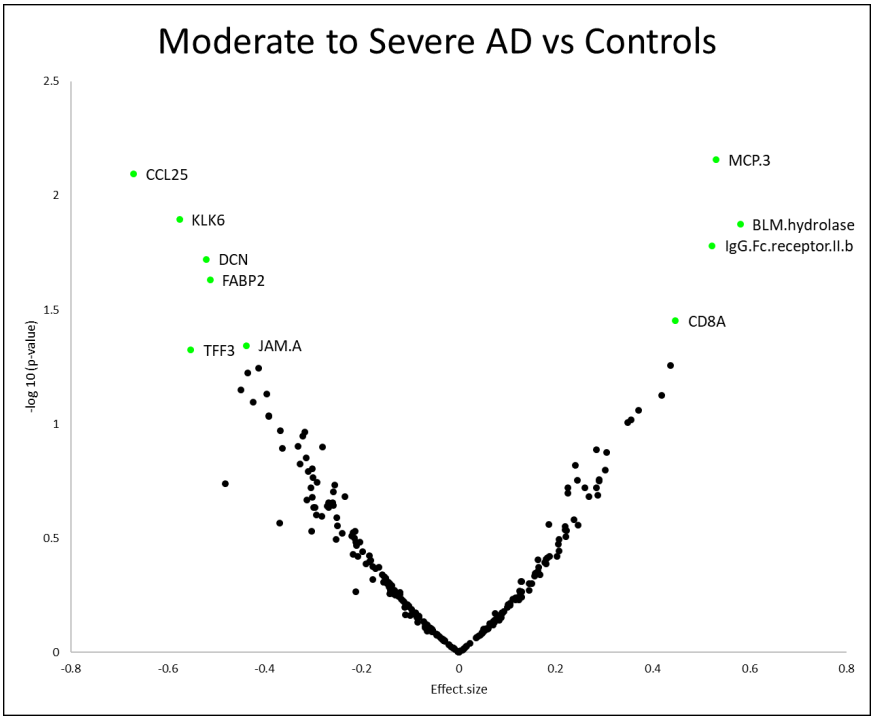


Figure 7.8. Volcano plot: Relationship of serum biomarkers and severity of AD (moderate to severe AD vs Controls) adjusted for age and gender.

7.3.4. Serum proteomic profile in obesity (BMI)

A total of 98 serum biomarkers were statistically significant with increasing BMI after adjusting for age and gender with multiple testing correction with B-H procedure. These are illustrated in the following volcano plot (Figure 7.8) and the top 25 biomarkers according to measure of statistical significance are summarized in Table 7.3.

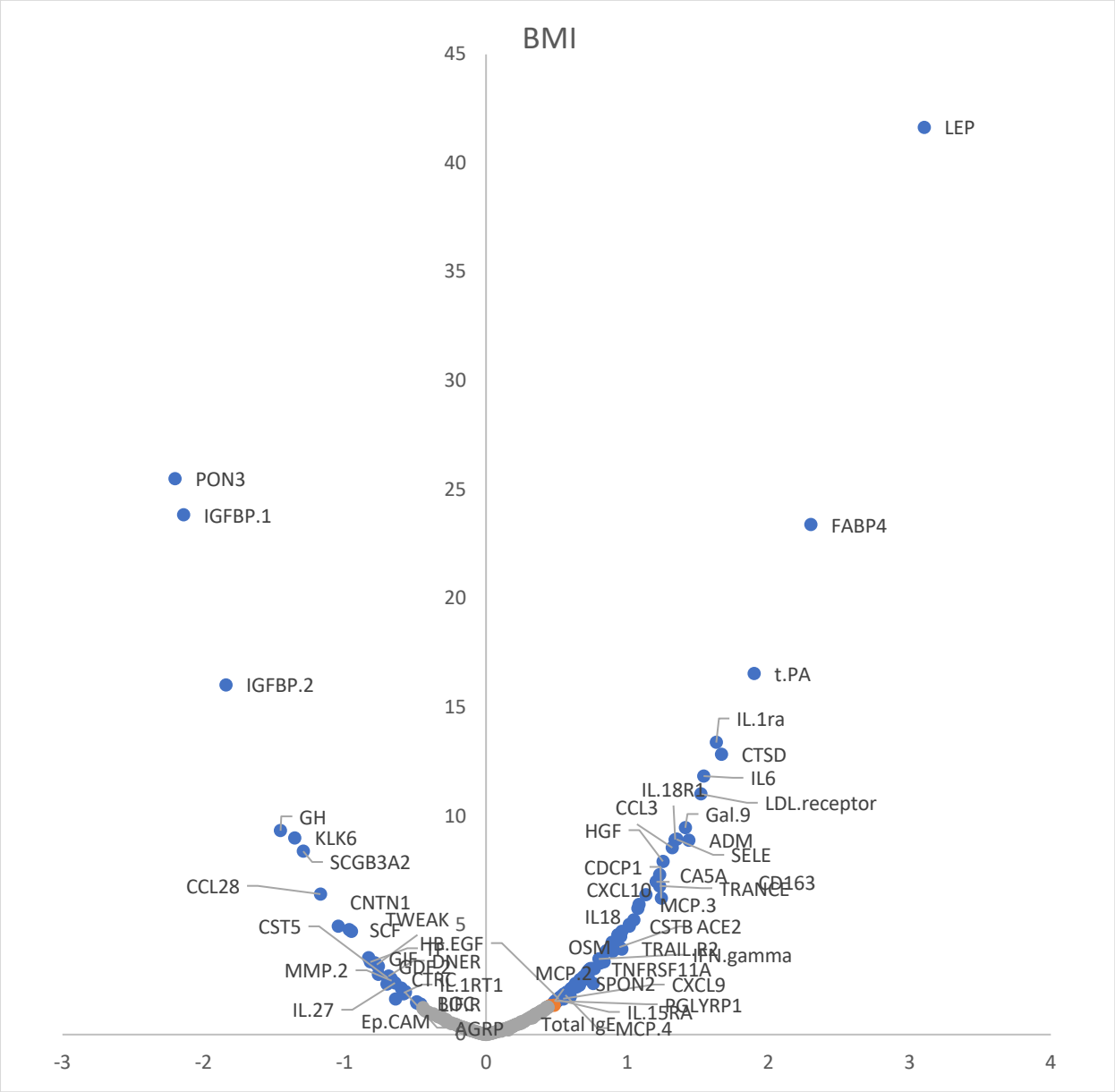


Figure 7.9. Volcano plot: Relationship of serum biomarkers and obesity (increasing BMI) adjusted for age and gender.

	Estimate	P value	Corrected P value
LEP	3.10321	2.20E-42	5.87E-40
PON3	-2.2008	2.99E-26	3.99E-24
IGFBP.1	-2.1429	1.41E-24	1.25E-22
FABP4	2.299984	3.95E-24	2.64E-22
t.PA	1.900239	2.65E-17	1.42E-15
IGFBP.2	-1.84203	8.71E-17	3.87E-15
IL.1ra	1.630671	3.93E-14	1.50E-12
CTSD	1.665905	1.35E-13	4.52E-12
IL6	1.540491	1.36E-12	4.02E-11
LDL.receptor	1.523098	9.18E-12	2.45E-10
Gal.9	1.411297	3.21E-10	7.80E-09
GH	-1.45514	4.37E-10	9.73E-09
KLK6	-1.35543	9.22E-10	1.89E-08
ADM	1.437112	1.18E-09	1.97E-08
SELE	1.350121	1.09E-09	1.97E-08
IL.18R1	1.339663	1.13E-09	1.97E-08
CCL3	1.319432	2.64E-09	4.15E-08
SCGB3A2	-1.2947	3.85E-09	5.71E-08
HGF	1.252806	1.12E-08	1.58E-07
CA5A	1.230385	4.59E-08	6.13E-07
CD163	1.206899	9.72E-08	1.24E-06
TRANCE	1.230264	1.50E-07	1.82E-06
CCL28	-1.17268	3.56E-07	4.13E-06
CXCL10	1.133688	3.88E-07	4.32E-06
CDCP1	1.240378	5.53E-07	5.91E-06

Table 7.3. Effect estimates of top 25 biomarkers based on measure of significance form multiple linear regression of biomarkers against increasing BMI adjusted by age and gender.

Leptin, LDL receptor, insulin binding protein 1 and other markers of systemic inflammation such as IL-6, IL1ra, and tPA were found to be significant with increasing BMI. These are known serum biomarkers for obesity.

A) Principal Component Analysis

Principal component analysis (PCA), based on obesity (BMI ≥ 30kgm^2), showed a fair amount of overlap of the two clusters (obese and non-obese) with some degree of separation.

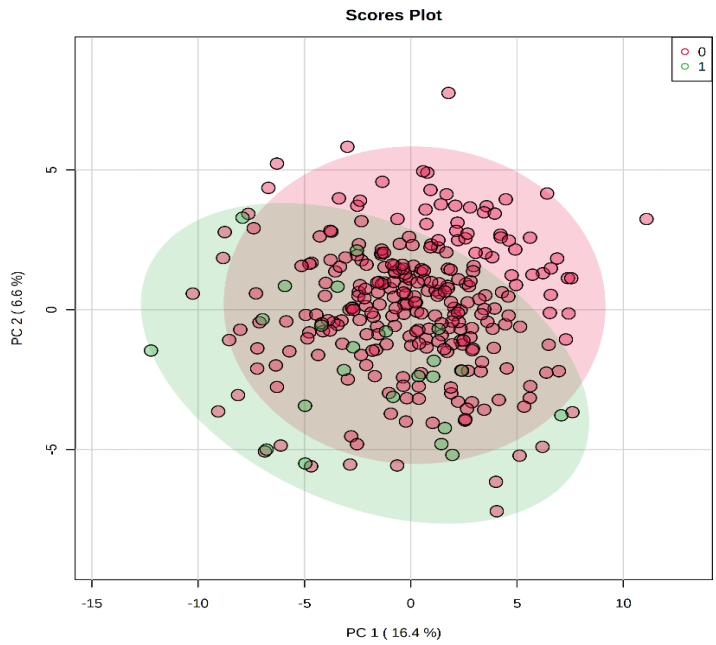


Figure 7.10. Principal component analysis plot of biomarkers for Obese (BMI≥30kgm2) versus Control.

B) Partial least squares (PLS) discriminant analysis.

Partial least squares (PLS) discriminant analysis was similarly performed to identify serum biomarkers that might be important in discriminate between Obese versus non-obese. The PLS discriminant analysis plot allowed visualization of any clusters.

Under supervised clustering, it can be seen that there were two clusters on the PLS-DA plot with some degree of overlap. Component 1 of the PLS-DA model explained about 11.5% of the variance of the biomarkers data while component 2 explained about 10.1%. VIP scores of individual biomarkers were calculated. Notably, growth hormone (GH), insulin growth factor binding protein (IGFBP) 1 and 2, leptin, IL-6 were important biomarkers in distinguishing obesity. Of note, total IgE, a known serum marker of atopy and AD had a VIP score of greater than 4 in this PLS-DA model. Total IgE was noted to be higher with increasing BMI (adjusted for age and gender and was significant after multiple testing correction (Beta: 0.519, p = 0.0215; corrected p = 0.0568).

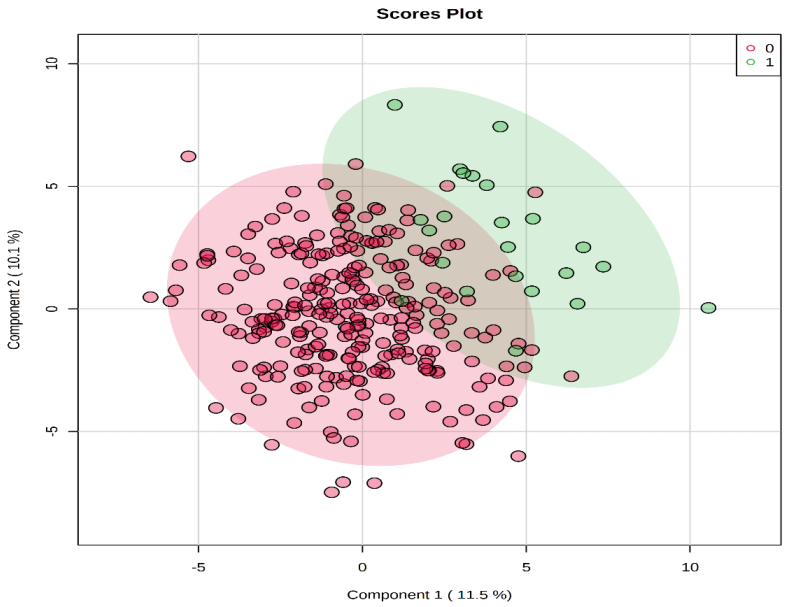


Figure 7.11. Supervised clustering PLS-DA plot: Partial least squares discriminant analysis of Obese (BMI≥30kgm2) versus Control.

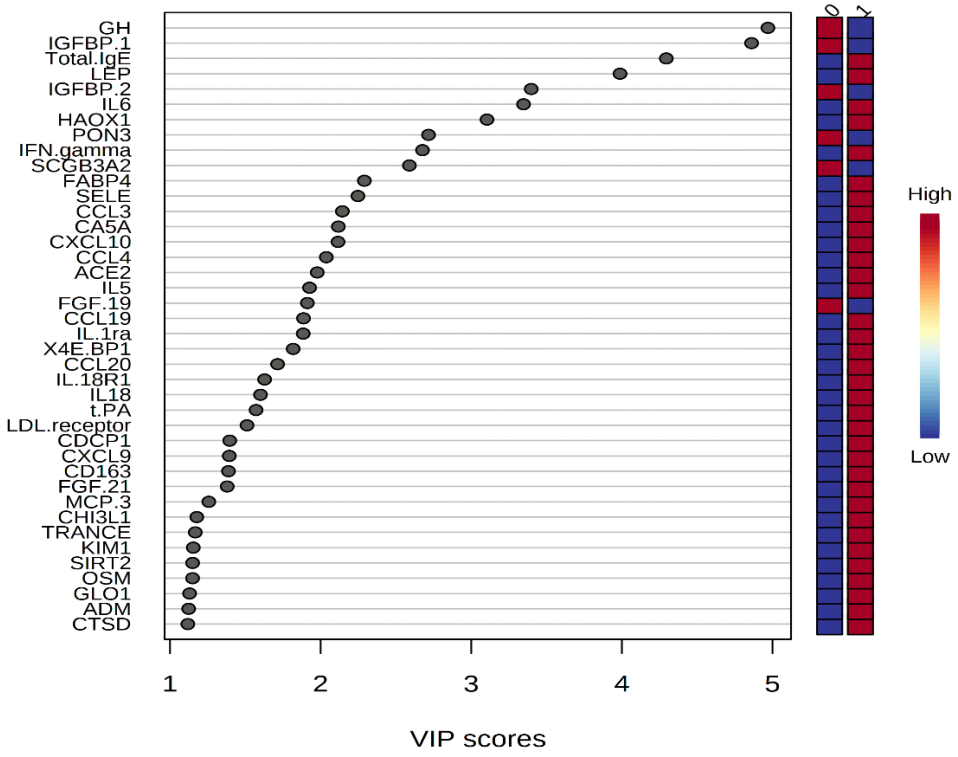


Figure 7.12. VIP scores of top 40 biomarkers of PLS-DA component 1 with VIP > 1.
The first column with heading “0” indicates control while heading “1” indicates AD. The colours (red vs blue) in the respective cells indicates whether the biomarker is elevated and lowered respectively.

7.3.5. Serum proteomic profile in abdominal obesity (WHR)

Forty-seven serum biomarkers were significantly associated with increasing WHR after adjusting for age and gender with multiple testing correction with B-H procedure. These are illustrated in the following volcano plot (Figure 7.12) and the biomarkers with increasing WHR are summarized in Table 7.4.

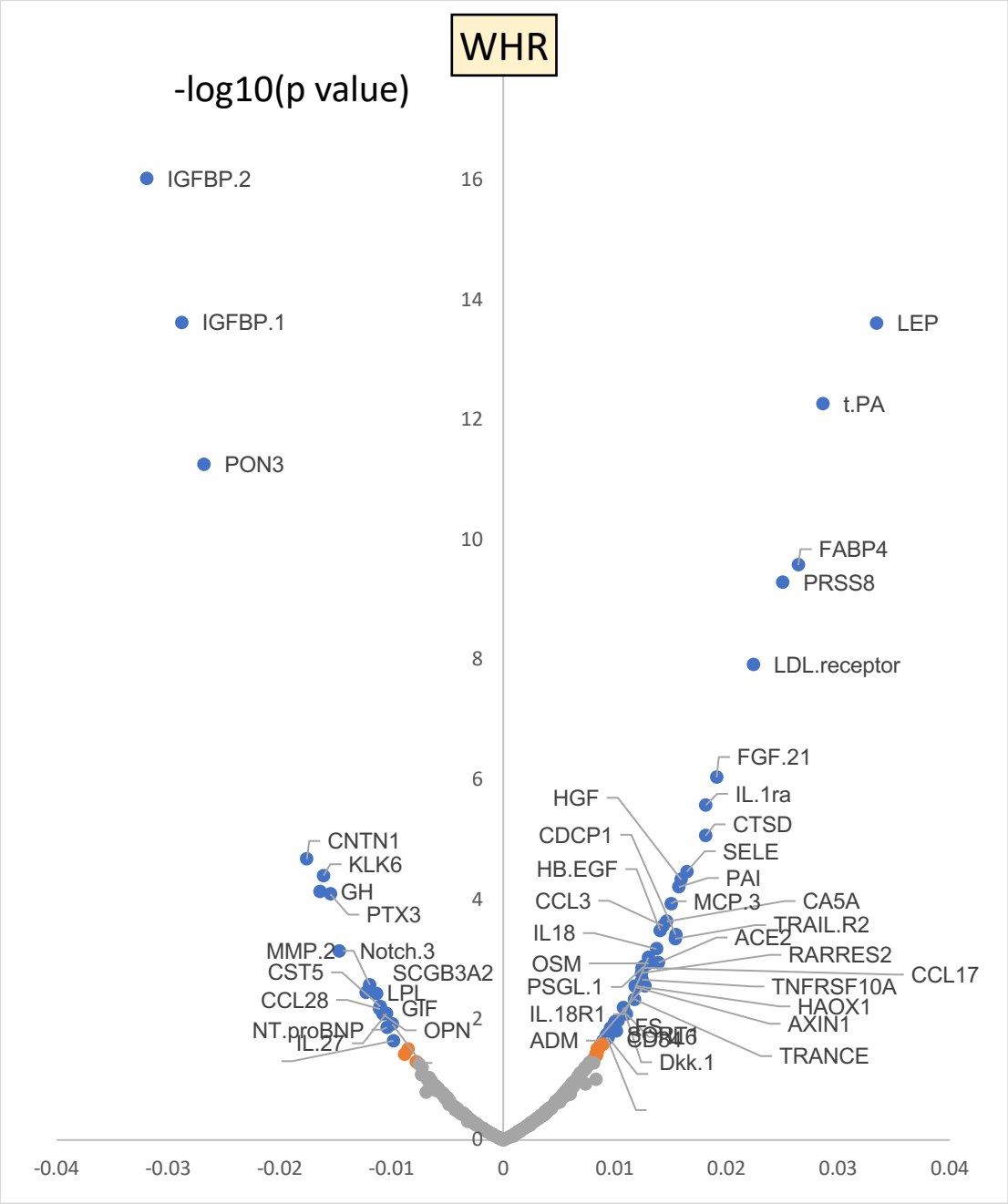


Figure 7.13. Volcano plot: Relationship of serum biomarkers and obesity (increasing WHR) adjusted for age and gender.

SN	Biomarkers	Estimate	P value	Corrected P value
1	IGFBP.2	-0.0319	9.38E-17	2.50E-14
2	LEP	0.0334	2.42E-14	2.15E-12
3	IGFBP.1	-0.0288	2.35E-14	2.15E-12
4	t.PA	0.0286	5.38E-13	3.59E-11
5	PON3	-0.0268	5.52E-12	2.95E-10
6	FABP4	0.0264	2.61E-10	1.16E-08
7	PRSS8	0.0250	5.10E-10	1.95E-08
8	LDL.receptor	0.0224	1.20E-08	4.01E-07
9	FGF.21	0.0191	9.05E-07	2.69E-05
10	IL.1ra	0.0181	2.63E-06	7.01E-05
11	CTSD	0.0181	8.45E-06	0.000205
12	CNTN1	-0.0176	2.08E-05	0.000463
13	SELE	0.0164	3.36E-05	0.00069
14	KLK6	-0.0161	3.90E-05	0.000745
15	HGF	0.0159	4.56E-05	0.000811
16	PAI	0.0157	6.07E-05	0.00101
17	GH	-0.0164	7.24E-05	0.00114
18	PTX3	-0.0155	7.97E-05	0.00118
19	MCP.3	0.0150	0.000114	0.00161
20	CA5A	0.0146	0.000228	0.00304
21	CCL3	0.0143	0.000268	0.00341
22	HB.EGF	0.0141	0.000321	0.00390
23	CDCP1	0.0154	0.000379	0.00440
24	TRAIL.R2	0.0154	0.000441	0.00491
25	IL18	0.0137	0.000654	0.00698
26	Notch.3	-0.0147	0.000711	0.00730
27	IL.18R1	0.0130	0.000904	0.00894
28	ACE2	0.0139	0.00110	0.0105
29	OSM	0.0130	0.00114	0.0105
30	PSGL.1	0.0126	0.00128	0.0114
31	CCL17	0.0124	0.00134	0.0116
32	RARRES2	0.0124	0.00165	0.0137
33	TNFRSF10A	0.0124	0.00212	0.0172
34	ADM	0.0127	0.00271	0.0201
35	HAOX1	0.0118	0.00270	0.0201
36	MMP.2	-0.0119	0.00260	0.0201
37	AXIN1	0.0119	0.00285	0.0206
38	LPL	-0.0123	0.00350	0.0246
39	SCGB3A2	-0.0114	0.00366	0.0251
40	TRANCE	0.0117	0.00450	0.0301
41	CST5	-0.0110	0.00594	0.0387
42	Dkk.1	0.0107	0.00621	0.0395
43	GIF	-0.0110	0.00636	0.0395
44	CCL28	-0.0111	0.00654	0.0397

Table 7.4. Effect estimate of biomarkers ranked based on measure of significance form multiple linear regression of biomarkers against increasing WHR adjusted by age and gender.

Leptin, LDL receptor, insulin binding protein 1 and other markers of systemic inflammation such as IL-6, IL1ra, tPA, CCL3 were found to be significant with increasing abdominal obesity (WHR). These are known serum biomarkers associated with obesity. Notably CCL17, an important Th-2 biomarker for AD was observed to be significantly positively correlated with WHR after adjusting for age and gender and with multiple testing correction by B-H procedure (Beta: 0.0124, p = 0.00134; corrected p = 0.0116).

A) Principal Component Analysis

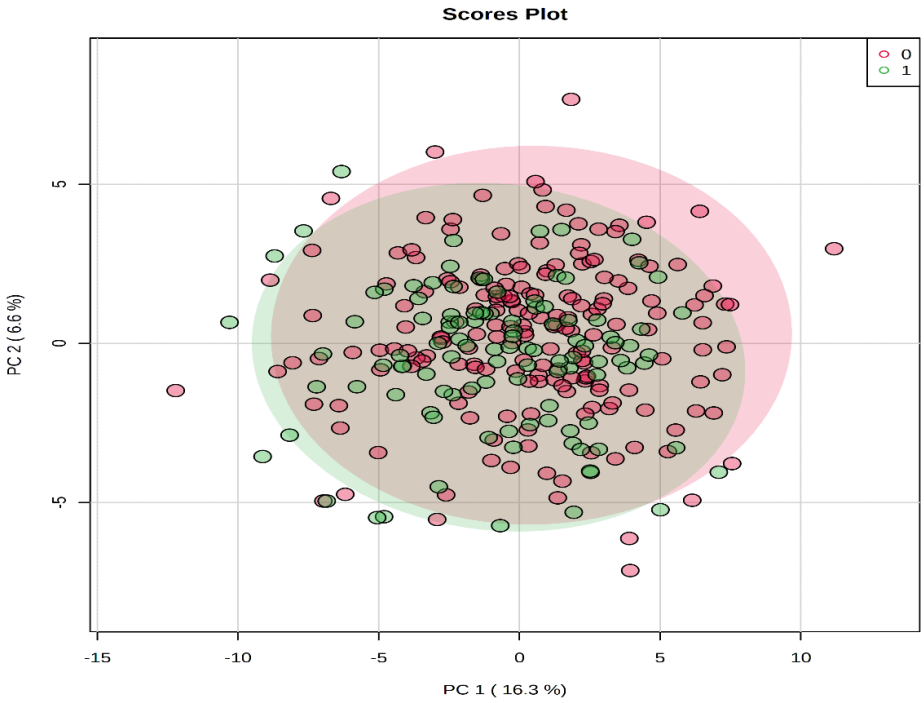


Figure 7.14. Principal component analysis plot of biomarkers for Obese (increased WHR) versus Control.

Principal component analysis (PCA) was performed to check for clusters related to obesity as defined by WHR. A WHR of ≥ 0.85 in females and ≥ 0.90 in males was used as the cut off to define obesity. There was no obvious separation into two clusters of obese and non-obese based on WHR criteria (Figure 7.13).

B) Partial least squares (PLS) discriminant analysis.

Under supervised clustering, it can be seen that there were two clusters on the PLS-DA plot with some degree of overlap. VIP scores of individual biomarkers were calculated. Similar to obesity defined by BMI ≥ 30kgm^2, growth hormone (GH), insulin growth factor binding protein (IGFBP) 1 and 2, leptin, IL-6 were important biomarkers in distinguishing obesity by an increased WHR. Both total IgE and CCL17, known serum markers of AD, had a VIP score of greater than 1 in this PLS-DA model.

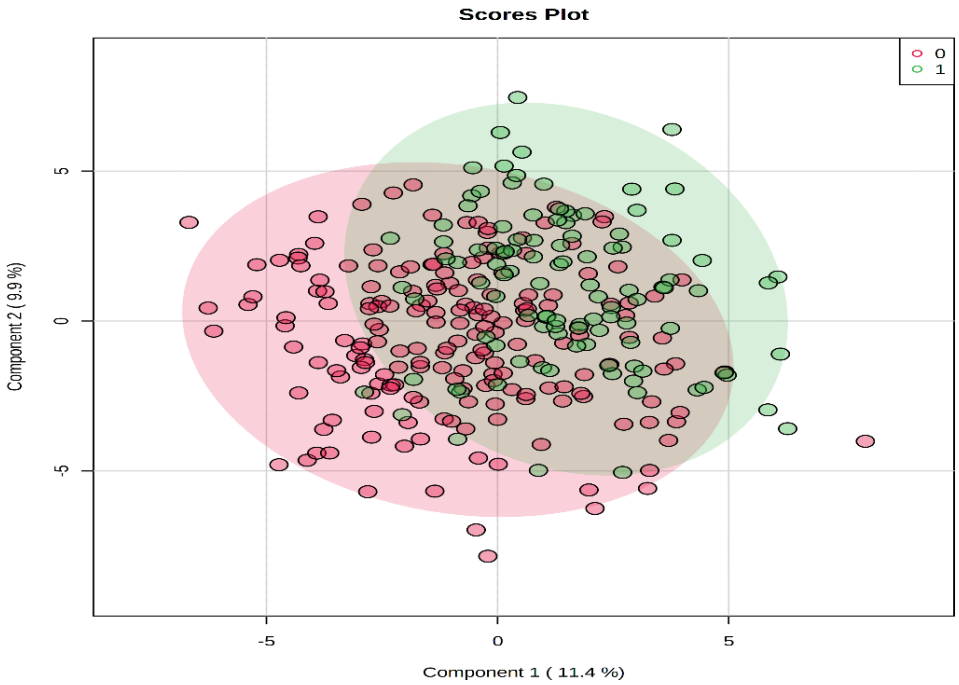


Figure 7.15. Supervised clustering PLS-DA plot: Partial least squares discriminant analysis of Obese (increased WHR) versus Control.

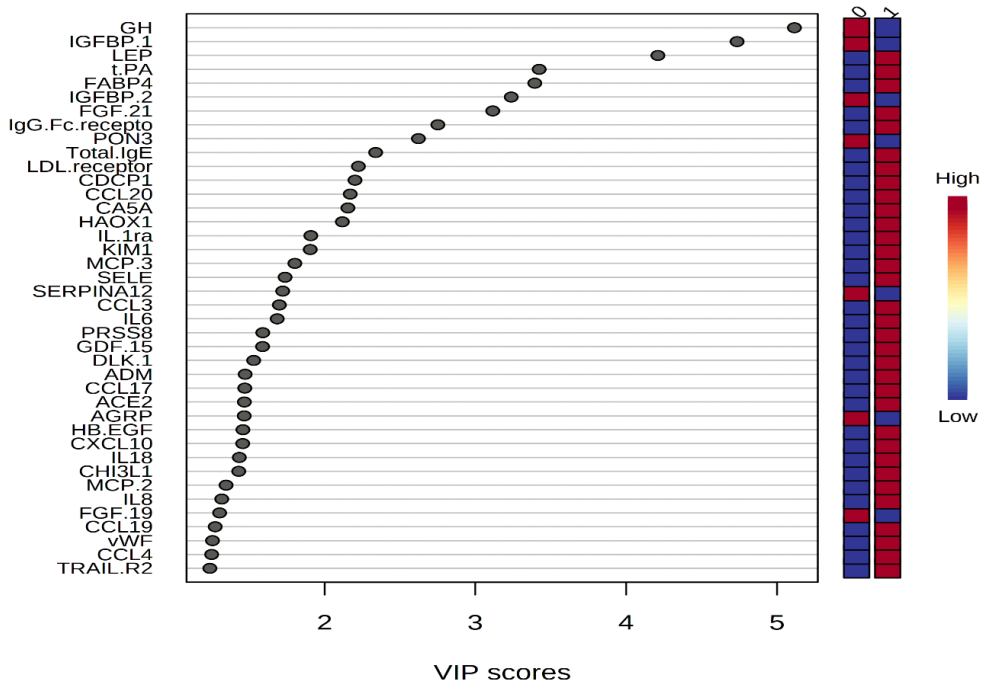
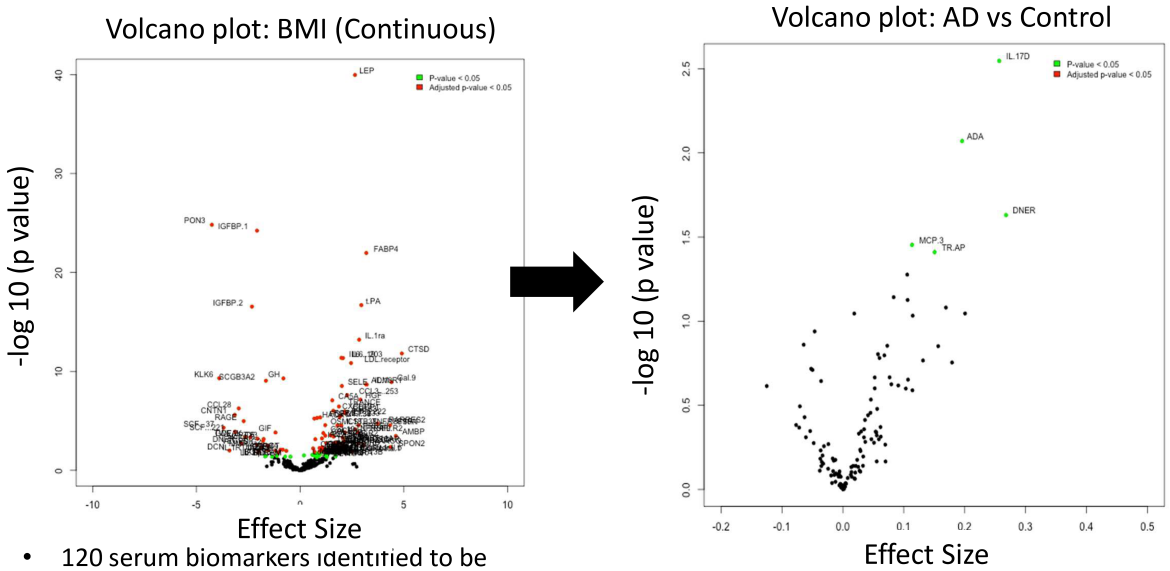


Figure 7.16. VIP scores of top 40 biomarkers of PLS-DA component 1 with VIP > 1.
The first column with heading "0" indicates control while heading "1" indicates AD. The colours (red vs blue) in the respective cells indicates whether the biomarker is elevated and lowered respectively.

7.3.6. Serum proteomics analysis on the influence of increasing adiposity on AD



- 120 serum biomarkers identified to be statistically significant (p<0.05) with increasing BMI
(adjusted by age and gender)

Analysis of 120 identified significant serum biomarkers with AD status
(adjusted by age and gender)

Figure 7.17. Two stage linear regression analysis: Volcano plots of BMI as continuous outcome and AD as binary outcome.

To analyse the influence of how adiposity might affect the underlying inflammation of AD, analysis steps decided *apriori* were taken to analyse the biomarkers. A list of significant biomarkers with increasing BMI upon linear regression adjusted for age and gender was first selected. Multiple linear regression for these selected biomarkers (adjusted for age and gender) were then performed with AD as a binary outcome to identify biomarkers that were also significant in AD participants. IL17D, ADA, DNER, MCP.3 and TR.AP were elevated among AD participants but did not reach statistical significance after multiple testing correction.

Cytokines and chemokines known to be associated with both AD inflammatory response and pathophysiology were evaluated based on multiple regression models (adjusted for age and gender) with increasing BMI and WHR (multiple testing correction with B-H procedure performed for the entire list of 267 markers) and summarized in Table 7.5 [274].

	BMI			WHR		
	Estimate	P value	Corrected P value	Estimate	P value	Corrected P value
CCL17	0.199	3.75E-01	5.44E-01	3.24	1.34E-03	1.16E-02
CCL20	0.691	2.18E-03	9.09E-03	0.00771	5.08E-02	1.74E-01
CCL28	-1.17	3.56E-07	4.13E-06	-2.74	6.54E-03	3.97E-02
CCL3	1.32	2.64E-09	4.15E-08	3.69	2.68E-04	3.41E-03
CCL4	0.851	1.40E-04	8.52E-04	0.00868	2.66E-02	1.06E-01
FGF.21	0.948	2.61E-05	1.95E-04	0.0191	9.05E-07	2.69E-05
IL.18	1.05	5.62E-06	5.36E-05	3.45	6.54E-04	6.98E-03
IL.18R1	1.34	1.13E-09	1.97E-08	3.35	9.04E-04	8.94E-03
IL.27	-0.645	4.26E-03	1.54E-02	-2.68	7.85E-03	4.57E-02
IL2.RA	0.766	9.16E-04	4.48E-03	0.00289	4.77E-01	6.63E-01
MCP.1	0.730	1.28E-03	5.68E-03	0.00922	1.98E-02	8.65E-02
MCP.4	0.562	1.54E-02	4.33E-02	0.00940	1.96E-02	8.65E-02
SELE	1.35	1.09E-09	1.97E-08	0.0164	3.36E-05	6.90E-04

* Adjusted by age and gender

Table 7.5. Significant AD-associated chemokines and cytokines with increasing adiposity (BMI and WHR).

Known AD associated chemokines such as CCL20, CCL3, CCL4, MCP.1 and MCP.4 were elevated with increasing BMI. In addition, CCL17 (TARC), whose cell of origin is specific to keratinocytes and endothelial cells, was significantly elevated with increasing WHR (Beta=0.199, p = 0.00134, corrected p = 0.0116). Serum CCL17 levels are also known to correlate with AD disease severity. Of note, CX3CL1, whose levels are also known to correlate with AD disease severity was also significantly different with increasing BMI (Beta: --0.491; corrected p = 0.0732). CCL11, another AD disease severity marker was however not significantly different with increasing BMI (Beta: -0.151; corrected p=0.695), Similar results with CX3CL1 and CCL11 were observed with increasing WHR.

However, CCL28, a chemokine that plays a role in attracting CCR10+ lymphocytes and also anti-microbial activity, was significantly reduced with increasing BMI and WHR (Beta: -1.17; corrected p <0.001 and Beta: -2.74; corrected p = 0.0397 respectively).

In addition, levels of IL18/IL18R1 as well as IL27 were significantly elevated and downregulated respectively with increasing adiposity (BMI and WHR). IL-18 inflammation, which is negatively regulated by IL27 is known to play a role in the development of Th-2 activation and subsequent relapsing dermatitis [275].

Finally, markers of endothelial activation, T cell activation and growth factors (E-selection, IL2RA, FGF21) recently reported in a pediatric AD biomarkers study were significantly elevated with increasing BMI and WHR [276].

7.4. Discussion

This study allowed the analysis of 267 individual serum biomarkers using a multiplexed platform to evaluate the serum profile of AD and obesity.

In assessing the serum proteomic profile of AD, there was no significant serum biomarker in our cohort of AD participants after multiple testing correction. Eleven serum biomarkers had p values of less than 0.05. However, all exceeded the false detection rate of 0.1 after B-H procedure. Five out of these 11 biomarkers were also statistically significant in the regression analysis with increasing BMI. Known Th-2 inflammatory pathways markers such as IL4, IL13, CCL17 and total IgE were not significantly elevated. As these AD cases were selected from the HELIOS cohort comprising participants from the general population, it is very likely that these participants were in relatively good health and any AD cases would generally be of milder severity. About 46.7% of participants with AD in the entire HELIOS cohort provided information about the severity of their disease. Only 66 out of the 149 AD participants in this proteomic analysis self-reported their disease severity and most were mild AD cases. Mild AD is often a limited skin disease and has been reported to lack the typical systemic inflammation observed in moderate to severe cases. A recent study of European American AD patients with serum proteomic analysis with similar Olink Inflammation and Cardiovascular disease (CVDII/III) panels did not identify any serum biomarkers that were dysregulated in mild AD cases [270]. Moderate and severe AD cases in that study had increases in Th1, Th2 and Th2 related inflammatory proteins. The authors of that study opined that mild disease may be a distinct clinical and molecular endotype and lacks systemic abnormalities observed in moderate to severe cases. This could be an important reason why no significant serum biomarkers could be identified. Furthermore, the differences in serum biomarkers expression

between mild AD cases and healthy control would be smaller and the planned sample size might not be statistically powered to detect the differences.

A sensitivity analysis was therefore performed by limiting the regression analysis to the 66 participants that provided disease severity information. While known serum biomarkers associated with AD such as MCP1, CX3CL1, SELE were dysregulated in AD participants, none reached statistical significance after multiple testing correction. The disease severity was self-reported and a smaller sample size might have prevented detection of any significant difference.

Both unsupervised and supervised clustering analysis were performed with principal component analysis and PLS discriminant analysis respectively to evaluate for any biomarkers that might differentiate AD and controls. There were no obvious clusters observed on both types of analysis. However, VIP scores of PLS discriminant analysis provided a ranking on the importance of each serum biomarker on distinguishing between AD and controls. Total IgE was the most important marker in the PLS-DA model distinguishing AD and controls with a VIP score of more than 6. Other known Th2, Th17 and Th22 inflammatory markers associated with AD were ranked in the top 50 biomarkers based on VIP scores.

In contrast, there are more serum proteomic biomarkers identified in obesity defined by increasing BMI or WHR. These included several known inflammatory markers of obesity and cardio-metabolic diseases such as leptin, IL-6, MCP-1, FABP4, LDL receptor and Th-1 cytokines such as Interferon-gamma and IL1-family of cytokines. These inflammatory cytokines lead to corresponding immune cells infiltration resulting in an inflammatory environment milieu that perpetuates the chronic inflammatory state in obesity [13].

However, Th-2 (CCL17), Th17 (CCL20) and known chemokines associated with AD (e.g CCL3 and CCL4) were also found to be significantly dysregulated with increasing BMI or WHR. Some of these chemokines including MCP.1, MCP.4, CCL17 and CCL20 were specific to keratinocytes in addition to leukocytes and endothelial cells [274]. Obesity is largely a disease with chronic Th-1 inflammation associated with elevated levels of leptin [13]. It has been reported that the development of leptin receptor resistance with a state of leptinemia leads to disruption of Th1 and Th2 balance. With increasing obesity and increased leptin resistance, the Th-2 immune profile becomes more prominent [277, 278].

In this study, serum total IgE was elevated in participants with increasing BMI and remained significant after multiple testing correction at FDR <0.1 (Beta = 0.519; corrected p = 0.0568). Total Ig E is a known antibody associated with allergic responses and have been one of the known markers of AD. In fact, a raised total IgE is a minor criterion of the Hanifin and Rajka diagnostic criteria of AD [29]. Several studies have evaluated the relationship of serum total IgE with obesity [279-281]. Total IgE levels were reported to be higher among obese/overweight children compared to lean children in an American general population [279]. These studies have suggested that raised total IgE levels in obese adults and children could account for an increased risk of atopic conditions such as asthma. This could potentially be one of the mechanistic links between obesity and AD.

AD associated chemokines specific to keratinocytes, CCL20 and CCL17 were elevated with increasing BMI and WHR respectively. CCL20 is an associated Th-17 marker and has been reported to be produced by keratinocytes at sites of injury or exposure to allergen, leading to migration of Langerhans cells into the epidermis [282, 283]. Despite its local action, it can be found elevated in the serum of AD patients

[284]. CCL17 or thymus and activation regulated chemokine (TARC) is a well-known and often considered to be the most reliable disease marker of AD [285, 286]. It has also been found to directly correlated with AD disease activity. Besides secretion by keratinocytes, it is also secreted by vascular endothelial cells, and work with CCL27 in mediating trafficking of lymphocytes. CCL17 was found elevated with increasing WHR but not BMI. These cytokines associated with systemic inflammation of obesity could predispose an individual to having an atopic related inflammation and subsequent development of atopic disease.

Besides exhibiting increased inflammatory cytokines that are related to what is known in AD, increasing BMI and WHR were also related to elevated levels of primary pro-inflammatory IL-18 cytokines that could play a role in the initiating events of AD. IL18 and IL18R1 were both elevated with increasing BMI and WHR. IL18 has been reported to cause skin inflammation associated with increased mast cells, Th2 cytokines, total IgE and histamine levels [275]. It is stored in macrophages and keratinocytes and is released as one of the primary pro-inflammatory cytokines that occurred after an initiating event such as mechanical trauma or skin barrier disruption. A genetic study has also supported IL18's role in AD. Single nucleotide polymorphisms (SNP) of IL18 in the exon 1 and promotor gene region was related to an AD phenotype [287]. Related to the IL-18 pathway, IL27 plays a role in balancing IL-18 activity by inducing the latter's endogenous antagonist IL18 binding protein (IL18BP). IL27 was noted to be significantly reduced with increasing BMI and WHR in this study. Measured IL18BP levels were not significantly different in the face of a significantly elevated IL18 levels. This may lead to an overall increase in IL18 activity and possibly explain the links between obesity and the increased risk of AD.

In summary, there were no significant serum proteomic markers that were found to be dysregulated in the AD cohort of this study. Participants with increasing adiposity (BMI or WHR) exhibited a characteristic inflammatory proteomic profile that is known of the chronic inflammatory state of obese individuals. However, as part of the inflammatory proteins' milieu, AD associated chemokines and cytokines were also found to be significantly elevated in participants with increasing adiposity. Some of these proteins are specific to keratinocytes origin and are also known to correlate with AD disease severity. IL18 and its associated cytokines were also dysregulated in participants with increasing adiposity, proposing that IL18 and its associated cytokines play a role in the development of allergic dermatitis as primary proinflammatory cytokines. These observations lend support to possible underlying mechanistic pathways linking obesity with AD.

8. Study 5: Mendelian randomization to assess the relationship between BMI and AD

8.1. Overview and rationale

Our understanding of AD‘s relationship with obesity is based mainly on published observational studies. A recent meta-analysis of available studies performed in 2015 reported that obese patients have up to 1.5 times the odds of having AD [11]. However, while these studies can evaluate and quantify its relationship, they are often limited by both known and unknown confounding factors such as patient or environmental variables. These observational association studies, unlike intervention trials, also cannot exclude the possibility of reverse causation [288, 289]. Therefore, it remains uncertain if obesity has a causal role in the development of AD or if the former is a consequence of having AD.

To evaluate for possible effects of an exposure upon an outcome, which would otherwise be challenging to implement in interventional studies, an approach known as Mendelian randomization could be adopted [290, 291]. This study design utilized inherited genetic variants, single nucleotide polymorphisms (SNPs) as instrumental variables that affect exposure status to investigate causal relationships of interest. Similar to the randomization process in clinical trials, these genetic variants are also randomly assorted at point of conception and therefore less affected by confounding issues such as reverse causation commonly found in observational studies.

In this study, known genetic associations of AD and obesity, published in two recently published genome-wide association study (GWAS) datasets of BMI and AD, as well as the concept of Mendelian Randomization (MR) were used to evaluate causal relationships between AD and obesity (BMI)

8.2. Methods and Analysis

A two-sample MR analysis was performed in a bi-directional manner between AD and BMI to evaluate as well as determine the direction of the relationship. This was done using summarized data on instrumental variables from published information on the genetic variants on both AD and BMI. Three key assumptions should be fulfilled in order to be valid for the instrumental variables used in MR analysis to be valid,:

1) Relevance assumption: genetic variants chosen are associated with the exposure of interest; 2) Independence assumption: genetic variants are associated with the outcome of interest with no unmeasured confounders; 3) Exclusion restriction assumption: genetic variants only exert their effects on outcome via their impact on the exposure with no other horizontal pleiotropic factors [292].

To fulfil the first assumption, genetic data from the two largest GWAS reported to date on BMI and AD were included. In addition, SNPs that might have associations with possible confounders in the relationship were excluded as part of sensitivity analysis to fulfil the independence assumption. An MR-Egger test of pleiotropy was also done to assess the exclusion restriction assumption of MR.

8.2.1. Genome-wide association studies on BMI and AD

BMI

SNPs to be used as instrumental variables for BMI were identified from the combined meta-analysis of the Genetic Investigation of ANthropometric Traits (GIANT) consortium and the UK biobank datasets. In total, these datasets analyzed genetic information of about 700,000 individuals with European ancestry [293]. 941 near-independent SNPs associated with BMI were identified at a revised genome-wide significance threshold of P <1×10−8 using an approximate conditional and joint

multiple-SNP (COJO) analysis which accounts for LD (linkage disequilibrium) between SNPs at a given locus. These 941 SNPs were used as instrumental variables for BMI in our main MR analysis. They explained about 6.0% of the variance in the cohort. However, to ensure that all SNPs included in the MR analysis were independent, further analyses were performed by excluding SNPs with a range of r^2 thresholds of > 0.1, >0.2 and >0.5 followed by distance pruning at < 500kb and SNPs with r^2 thresholds of >0.001 and >0.01 within 1000kb apart.

AD

SNPs to be used as instrumental variables for AD were identified from the EArly Genetics and Life course Epidemiology (EAGLE) Consortium dataset that analyzed genetic information of 21,000 cases and 95,000 controls from 22 cohorts of European ancestry [294]. Twenty-four independent SNPs at least 4 MB apart at genome wide significance ($P < 5 \times 10^{-8}$) for AD were included in this MR analysis. These SNPs explain about 12.3% and 2.6% of variance for the previously and newly identified SNPs respectively.

8.2.2. Steps in Mendelian randomization analysis

This MR study used a two sample MR methodology in its analysis [295]. Therefore, summary level data from two aforementioned GWAS datasets were needed.

SNPs-exposure association data were first extracted as instrumental variables from the exposure GWAS dataset. These SNPs, as instrumental variables, were deemed to be associated with the exposure at a certain pre-determined genome wide significance level. Summary level genetic data of these identified SNPs were then extracted as SNPs-outcome association data from the outcome GWAS dataset. Missing data of any identified SNPs in the outcome GWAS dataset were replaced with

proxy SNPs with linkage disequilibrium (LD) r^2 scores of a minimum of 0.6 from the 1000G Phase 1 population of European ancestry.

This was a bi-directional MR analysis. In the MR analysis of the causal relationship of increased BMI leading to AD, BMI was the exposure and AD was the outcome of interest. In the reverse direction (causal relationship of AD leading to increased BMI), AD would be the exposure and BMI, the outcome of interest.

rs11172702 was the only SNP out of the 941 identified BMI SNPs that had missing data in the AD GWAS dataset. It was represented by a proxy SNP (rs11172644; LD: r2=1) in the analysis. In contrast, ten AD SNPs (rs10199605, rs145809981, rs2227483, rs2592555, rs4809219, rs10791824, rs12188917, rs2212434, rs2918307 and rs6419573) were not represented in the BMI GWAS dataset. Proxy SNPs of LD r^2 values between 0.67 to 1.00 were used for these 10 missing SNPs. These proxy SNPs were rs2931136, rs4537545, rs2227485, rs10836538, rs6010998, rs479844, rs3091307, rs2155219, rs2164983 and rs4851575. There were no suitable proxy SNPs available for four missing SNPs (rs112111458, rs4713555, rs12730935 and rs61813875) and were therefore excluded from the MR analysis.

The effect estimates of the SNPs-exposure and SNPs-outcome were then harmonized as data were extracted from two separate GWAS datasets. The harmonization involved identifying SNPs with effect and alternate alleles that were mismatched in the two GWAS datasets. Correction would then be performed by changing the direction of their effect estimates and corresponding effect allele frequencies. Any palindromic SNPs that might invert the direction of effect in both GWAS dataset were screenedf. No evidence for such inversion were found that required further realignment.

With the extracted genetic data harmonized for both exposure and outcome, Wald ratio was calculated for each identified SNP. Wald ratio is the ratio estimate for the SNPs-outcome effect estimate divided by the SNPs-exposure effect estimate. The standard error of the Wald ratio was approximated by the delta method[296].

8.2.3. Mendelian randomization methods

Inverse variance weighted MR was the main MR estimate in this study but several methods of calculating the MR estimate were also used as part of sensitivity analysis and are described in the following paragraphs:

Inverse variance weighted MR

This method involves a random effects inverse variance weighted (IVW) meta-analysis of Wald ratios of the identified SNPs to calculate the IVW MR estimate [297]. It is based on the assumption that all SNPs are valid instrument variables and if there is underlying horizontal pleiotropy, they are balanced across the SNPs [298].

Maximum likelihood method

The MR estimate was estimated from a maximum likelihood model that assumed a linear relationship between exposure and outcome and the underlying genetic variants followed a bivariate normal distribution [213]. Standard errors for the maximum-likelihood estimates were obtained with the inverse Hessian matrix. This method is also based on the same assumption as the IVW method but performs reliably in the presence of measurement errors in SNP-exposure [299].

Weighted median-based method

This method works on the assumption that at least half of the SNPs are valid instrument variables for the MR estimate to be unbiased. The median effect of the

SNPs was evaluated and 50% of the analysis weights were based on estimates that were smaller or equal to the weighted median [300].

MR-Egger regression analysis

This MR analysis method is a form of modified IVW analysis where a non-zero intercept is allowed as part of the analysis [301]. This method does not make any assumptions with regards to the absence of horizontal pleiotropy and allows net horizontal pleiotropic effect across all SNPS to be unbalanced, or directional. However, there should not be any correlation between any horizontal pleiotropic effect present with SNP-exposure effects.

Statistical test of MR-Egger intercept was also performed as part of the MR-Egger regression analysis to evaluate if it was significantly different from zero. This would signify if the identified SNPs have any unbalanced horizontal pleiotropic effects on the outcome.

8.2.4. Sensitivity analysis to account for possible pleiotropic factors

To make the MR results more robust, further sensitivity analyses were performed to further exclude potential pleiotropic factors that may confound the MR analysis. Any genetic variants (SNPs) that have associations with known confounders in the relationship between BMI and AD were identified using the PhenoScanner V2 database [302, 303].

BMI SNPs that have significant associations with known confounding factors such as education, behavioural factors such as tobacco smoking, alcohol ingestion and physical activity, psychiatric diseases and psychological well-being (anxiety, depression) were excluded from the MR sensitivity analysis [304-308]. Similarly, AD SNPs with significant associations with known confounding factors such as education,

alcohol ingestion psychiatric diseases and psychological well-being (anxiety, depression) were excluded from the MR sensitivity analysis [304-308].

8.2.5. Sensitivity analysis with waist hip ratio as a measurement of abdominal obesity

A post-hoc sensitivity analysis was performed to determine if body fat distribution is important in determining the risk of AD based on the significant MR findings of an observed causal effect of BMI upon AD. A genome-wide association study (GWAS) of body fat distribution, as measured by waist-hip ratio adjusted for BMI was used to identify SNPs to be used as instrumental variables for this unidirectional MR sensitivity analysis study [309]. This GWAS dataset analyzed genetic information of about 694,649 individuals with European ancestry. 346 independent SNPs associated with WHR adjusted for BMI were identified at a revised genome-wide significance threshold of $P < 5 \times 10^{-9}$. Any SNPs in LD ($r2 > 0.05$) with the top associated SNP and ±5 Mb away were merged together. They explained about 3.9% of the variance of the dataset. These were subjected to the same Mendelian randomization steps and subsequent removal of possible pleiotropic factors.

8.2.6. No measurement error (NOME) assumption

Measurement errors in SNP-exposure associations would result in an attenuation of MR effect estimates due to a departure of the no measurement error (NOME) assumption. This attenuation of effect estimates derived from IVW and MR-Egger methods was estimated using F and I^2 statistics respectively in this study. Values range from 0% to 100%, with a larger value suggesting a lower attenuation of effect estimate [310, 311].

The ratio of each SNP's effect size estimate to the variance of its SNP-exposure association gives the *F* statistic for each SNP. A weighted *F* statistic is then calculated for all the SNPs used in the IVW MR analysis. Degree of attenuation of effect estimates as a result of departure of NOME assumption was estimated by:

$$\frac{\bar{F}_{GX} - 1}{\bar{F}_{GX}}$$

For the MR Egger analysis, the I^2_{GX} estimate which is the true variance of SNP-exposure associations divided by the variance of the SNP-exposure estimates calculated the amount of attenuation of the MR Egger effect estimates due to a departure of the NOME assumption.

Data clumping of SNPs and two-sample Mendelian randomization analysis were performed using the TwoSampleMR (version 0.4.26) and Mendelian Randomization (version 0.4.1) packages respectively in R statistical software (RStudio version 1.2.1335). The MR study is summarized in Figure 8.1.

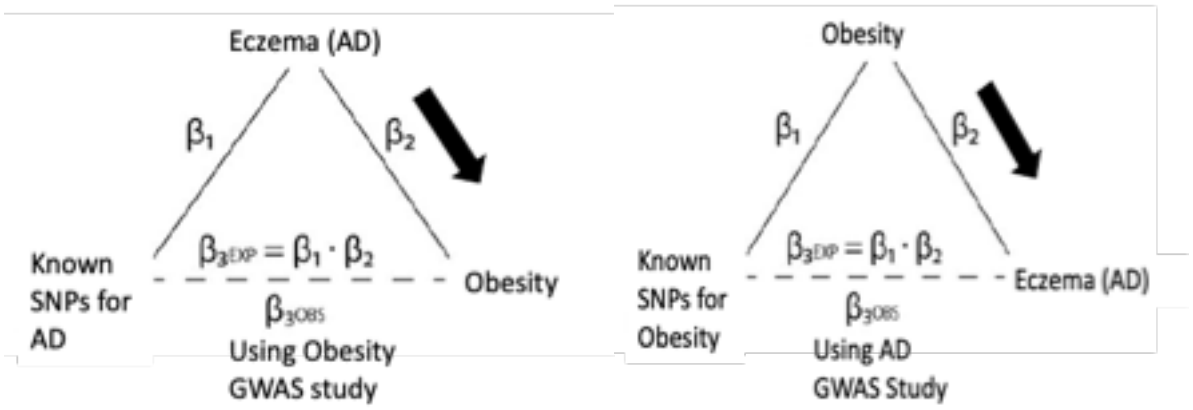


Figure 8.1. Bidirectional Mendelian Randomization analysis.

8.3. Results

8.3.1. Effect of BMI upon AD

BMI SNPs were used as instrument variables to assess the effect of obesity on the risk of AD in this study. This is illustrated in Figure 8.1.

The IVW MR estimate of BMI on AD gave an odds ratio of 1.08 (95% CI: 1.01 to 1.14; p=0.015) for having AD per unit increase in BMI (kg/m^2). Maximum-likelihood MR estimate yielded a similar odds ratio for AD of 1.08 (95% CI: 1.01 to 1.14; p=0.015) per unit increase of BMI (kg/m^2). The weighted median based method and MR-Egger regression analysis gave odd ratios of 1.07 (95% CI: 0.97 to 1.18) and 1.06 (95% CI: 0.96 to 1.16) respectively. However, neither reached statistical significance (p = 0.195 and p = 0.248 respectively). There was no significant evidence of horizontal pleiotropy on MR-Egger regression analysis, with the intercept not significantly deviated from zero (intercept: 0.000; 95% CI: -0.002 to 0.003; p=0.663).

MR analysis excluding SNPs as possible horizontal pleiotropic factors that could confound the relationship between BMI and AD was performed as a form of sensitivity analyses. The modified IVW analysis yielded a similar odds ratio for AD per unit increase in BMI (kg/m^2) of 1.12 (95% CI: 1.04 to 1.21).

This was part of a further sensitivity analysis to exclude non independent variants in high LD with BMI SNPs identified by the COJO analysis. SNPs over a range of r^2 thresholds (r^2 > 0.1, 0.2 and 0.5) followed by distance pruning at <500kb as well as SNPs with r^2 thresholds (r^2 > 0.001 and > 0.01) within 1000kb apart were systematically excluded. MR estimates obtained were statistically significant and consistent with results obtained when all 941 GWAS BMI SNPs were analyzed

8.3.2. Effect of AD upon BMI

Similarly, AD SNPs were used as instrumental variables to evaluate the effect of AD on BMI. A total of 24 independent SNPs identified from the EAGLE consortium dataset were used in this MR analysis. The IVW MR estimate for effect of AD on BMI gave an effect estimate of 0.00 unit change in BMI (kg/m^2) when AD patients were compared to controls (95% CI: -0.02 to 0.02; p=0.862). MR estimate by maximum-likelihood

method gave a similar effect estimate of 0.00 (95% CI: -0.03 to 0.02; p=0.868). The weighted median based method and MR-Egger regression analysis yielded effect estimates of -0.01 (95% CI: -0.03 to 0.01) and 0.04 (95% CI: -0.021 to 0.11) respectively. Both did not reach statistical significance (p = 0.312 and p = 0.189 respectively). There was also no evidence of significant horizontal pleiotropy on MR-Egger regression analysis given that there was no significant evidence of horizontal pleiotropy. The Egger intercept did not significantly deviate from zero (Intercept: -0.00; 95% CI: -0.01 to 0.00; p=0.152).

As part of sensitivity analysis, SNPs with associations with confounding factors were excluded as potential horizontal pleiotropic factors. The modified IVW analysis gave a similar MR estimate of 0.00 (95% CI: -0.02 to 0.02).

Effect of BMI upon Atopic Dermatitis (AD) Risk of AD per unit (kg/m2) increase in BMI based on MR (OR)		
MR Method	Beta or OR (95% CI)	P value
IVW (Random Effects)	1.08 (1.01 – 1.14)	0.015
IVW (Random Effects, modified*)	1.12 (1.04 – 1.21)	0.015
Maximum Likelihood Method	1.08 (1.01 – 1.14)	0.015
Weighted Median Method	1.07 (0.97-1.18)	0.195
MR Egger (Random Effect)	1.06 (0.96 – 1.16)	0.248
Effect of AD upon BMI Unit (kg/m^2) change in BMI among those who had AD compared to controls (β)		
IVW (Random Effects)	0.00 (-0.02 - 0.02)	0.862
IVW (Random Effects, modified*)	0.00 (-0.02 - 0.02)	0.982
Maximum Likelihood Method	0.00 (-0.03 - 0.02)	0.868
Weighted Median Method	-0.01 (-0.03 - 0.01)	0.312
MR Egger (Random Effect)	0.04 (-0.02 - 0.11)	0.189

*excluding possible pleiotropic genetic factors in analysis.

Table 8.1. Summary results of Mendelian Randomization (MR) analysis using various methods.

8.3.3. Effect of WHR upon AD

A total of 342 independent SNPs identified from the GWAS dataset of body fat distribution were used in this MR analysis [309]. Proxy SNPs of LD r^2 values between 0.89 to 0.99 were used for 2 out of the 6 missing SNPs. There were no suitable proxy SNPs available for four missing SNPs (rs139271800, rs180958337, rs2524163 and rs8080903) and were therefore excluded from the MR analysis.

The IVW MR estimate of WHR on AD gave an odds ratio of 0.927 (95% CI: 0.808 to 1.06; p=0.279) for having AD per unit increase in WHR. Maximum-likelihood MR estimate yielded a similar odds ratio for AD of 0.927 (95% CI: 0.807 to 1.06; p=0.280) per unit increase of WHR. The weighted median based method gave an odd ratio of 0.948 (95% CI: 0.774 to 1.16; p=0.607). MR-Egger regression analysis gave an odd ratio of 1.42 (95% CI: 0.998 to 2.02; p=0.052). Notably, there was significant evidence of horizontal pleiotropy on MR-Egger regression analysis, with the intercept significantly deviated from zero (intercept: -0.008; 95% CI: -0.015 to -0.002; p=0.010). Given the presence of significant horizontal pleiotropy, WHR SNPs with significant associations with aforementioned confounding factors such as education, behavioural factors such as tobacco smoking, alcohol ingestion and physical activity, psychiatric diseases and psychological well-being (anxiety, depression) were excluded from the modified WHR sensitivity analysis. MR estimates of the various methods, IVW, Maximum-likelihood and median based method remained not statistically significant. The modified IVW MR estimate of WHR on AD, after removing potential pleiotropic factors gave an odds ratio of 1.01 (95% CI: 0.868 to 1.18; p=0.860) for having AD per unit increase in WHR. However, MR-Egger regression analysis gave a significant MR estimate of odds ratio 1.61 (95% CI: 1.05 to 2.49; p=0.03). Notably, there remained significant evidence of horizontal pleiotropy on MR-Egger regression analysis, with the

intercept significantly deviated from zero (intercept: -0.009; 95% CI: -0.017 to -0.001; p=0.024).

8.3.4. No measurement error [NOME] assumption

Attenuation of MR effect estimates by IVW and MR Egger MR methods were estimated using *F* statistics and I^2_{GX} respectively.

There were almost no attenuation of effect estimates by IVW analysis, F_{GX}-1/F_{GX} were 99.6% and 93.1% for effects of BMI upon AD and AD upon BMI respectively. Similarly, the I^2_{GX} estimate for MR Egger analysis (BMI to AD) was 93.6%, suggesting minimal attenuation by any measurement errors in the SNP-BMI associations. However, the I^2_{GX} estimate for AD to BMI was 4%. Any measurement errors in the SNP AD associations attenuate the MR-Egger effect estimate to a large degree and therefore MR-Egger regression analysis may not be a robust method to evaluate the MR relationship of AD upon BMI.

There were also almost no attenuation of effect estimates by IVW analysis, F_{GX}-1/F_{GX} was 99.4% for effects of WHR upon AD. The I^2_{GX} estimate for MR Egger analysis (WHR to AD) was 95.9%, suggesting minimal attenuation by any measurement errors in the SNP-BMI associations. The respective NOME indices for the modified WHR on AD MR analysis, after removing pleiotropic factors, remained almost similar.

8.4. Discussion

This MR study provided evidence that obesity, in terms of a higher BMI, seemed to have an effect on an increased risk of having AD. There was however, no evidence otherwise to suggest AD resulted in an increase in BMI. This study was based on valid assumptions of MR and remained consistent across the various MR methods [290, 297, 312]. It had also examined the direction of effect which had remained uncertain

despite some evidence from a few prospective observational cohort studies [163, 312-315]. Possible presence of unknown confounding factors in observational studies had made establishing the directionality of the relationship between obesity and AD challenging.

Possible explanations for a role of obesity in the risk of AD have been discussed in the aforementioned studies. They examined how the disrupted barrier function, dysregulated skin microbiome and systemic inflammatory profile in obesity might be related to the risk of developing AD.

This study had utilized genetic information from to date, the two largest GWAS datasets of AD and BMI [293, 294]. Genetic variants used in this study were strong instrumental variables for MR analysis. The validity of all three key MR assumptions (relevance, independence and exclusion restriction) were evaluated and met [292]. Efforts were made to exclude possible known horizontal pleiotropic factors and there was no evidence of confounding from the MR-Egger intercept test. Further sensitivity analyses with various MR methods were performed and results remained robust across the various methods.

As part of post hoc sensitivity analysis, this study further evaluated if fat distribution is important in determining risk of AD. Genetic information from a similarly large and recent GWAS dataset of WHR were used in the analysis. While the analysis fulfilled the relevance MR assumption, there was significant evidence of horizontal pleiotropy from Egger-intercept test. MR estimates from several methods also did not yield a significant relationship between WHR and AD.

To further evaluate and fulfil the independence MR assumption, SNPs that might have associations with possible confounders in the relationship were excluded. MR-Egger

estimates yielded a significant relationship between WHR and AD. MR estimates from rest of the methods remained not significant. However, it should be noted that MR-Egger analysis allowed unbalanced horizontal pleiotropic effect and relaxed the exclusion restriction assumption of no horizontal pleiotropic factors, on the condition that the latter do not correlate with SNP-exposure. Genetically determined increase in WHR appeared to be associated with an increased risk of AD but further studies might be needed to confirm this relationship.

It is a limitation that this MR study has evaluated the relationships using only genetic variants discovered in participants of European white ancestry. There is evidence from various Asian observational studies that have similarly reported a relationship between obesity and AD [11]. However, GWAS information on participants of Asian descent is often based on studies of a small sample size and not as widely available. The strength of these genetic instruments might not be as strong and therefore they were not used in this study.

Finally, while the approach of MR provides an estimation of the effects of exposure upon outcome as well as its direction, there can be potential challenges to exclude completely confounders and horizontal pleiotropic factors when establishing the required independence and exclusion restriction assumptions of MR. Various sensitivity analyses were performed in this study to exclude potential confounders and horizontal pleiotropic factors. It is also reassuring to note that a recent MR study yielded similar results using both one-sample and two-sample MR analysis methodologies [316]. However, it is still important to interpret MR findings carefully and relate any of such estimation or evidence of causal effects to findings of other observational or longitudinal epidemiology studies.

In summary, there was evidence in this MR study that high BMI has an effect on an increased risk of AD. It provides suggestions on the approach to investigate the mechanisms linking obesity with AD and lends support to the findings discussed in the forementioned studies.

9. Integrated analysis

9.1. Overview and rationale

In-depth phenotypic profiling of a case control cohort, close to 300 study participants, provided a wealth of data to understand the mechanistic pathways between obesity and atopic dermatitis (AD). Skin microbiome and serum proteomic profile data were available in addition to meta-data and skin physiology measurements within a single cohort of participants. These had been discussed separately in the various earlier chapters and provided some insights on how obesity might influence skin physiology, skin microbiome profile and systemic inflammatory proteins/cytokines. In turn, all these might be related to AD. However, it would also be important to integrate these data from various omics platforms to see how they might be correlating and affecting each other.

It would be critical to understand how the skin microbiome and its resultant metabolic pathways or serum proteomic profile might be related to epidermal barrier function. Available functional profile of microbiome sequences on the skin and skin physiology measurements would allow evaluation on how obesity influence the functional profile of skin microbiome as well as how the resultant metabolic products of these pathways would in turn affect the epidermal barrier function in terms of trans-epidermal water loss (TEWL), skin surface moisture and skin pH (Figure 9.1a). Similarly, the effects of serum proteomic profile of obesity on skin physiology measures could be evaluated to understand how systemic inflammation related to obesity may influence skin barrier function (Figure 9.1b). Finally, understanding the relationship between skin microbiome species and serum proteomic profile in the context of obesity would also be important to evaluate the mechanisms linking skin surface communities and

systemic inflammation of obesity and how they might be related to the risk of developing of AD in obese individuals.

This chapter addresses the aforementioned gaps and provides an integrated analysis of the data that were generated in the previous studies discussed in the aforementioned chapters.

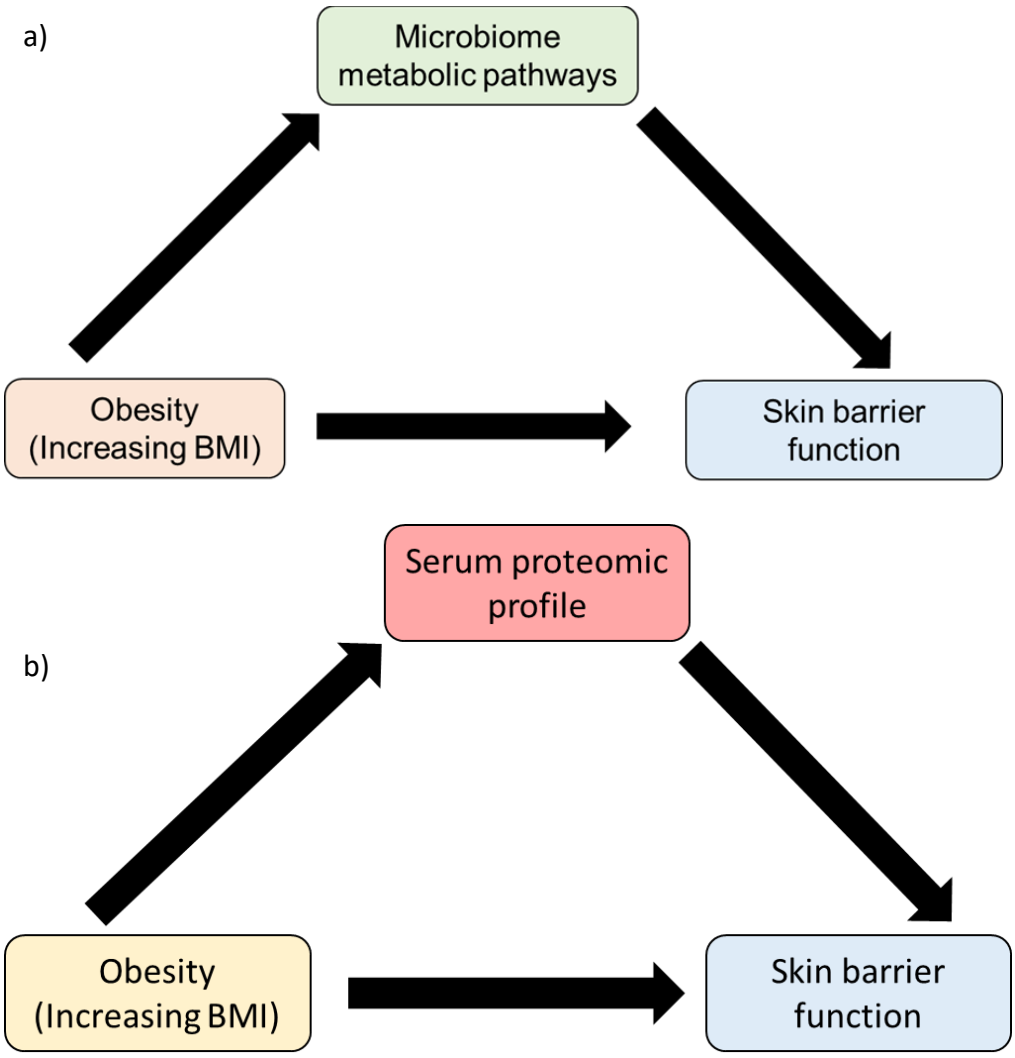


Figure 9.1. A graphical representation of how obesity might mediate its effect on skin barrier function via a) skin microbiome metabolic pathways or b) serum proteomic markers.

9.2. Methods and Analysis

9.2.1 Relationship of skin microbiome and serum proteomic profile with epidermal barrier function

To assess for relationship of the respective biomarkers (microbiome species, metabolic pathways and serum proteins) on skin barrier function, the following analysis was performed. Multiple linear regression analyses were performed with skin barrier function (represented respectively by log transformed TEWL, skin surface moisture and pH) as a continuous outcome and individual biomarkers as independent variables adjusted by age and gender. The microbiome biomarkers were defined at species level of the skin microbiome profile. As mentioned in the previous chapter, the metabolic pathways were defined by the MetaCyc database [247]. The serum proteomic biomarkers were as identified by the Olink panels. P values were subsequently subjected to multiple testing correction using the Benjamini–Hochberg (B-H) procedure at false discovery rate (FDR) threshold of 0.1 Volcano plot for each was plotted to identify any biomarkers with the most meaningful change. A list of statistically significant ($p<0.05$) biomarkers were identified and summarized. Reference was made to known biomarkers important in disease pathogenesis in AD.

9.2.2. Relationship of obesity related functional microbiome metabolic pathways and epidermal barrier function

To assess how obesity might mediate its effects on skin barrier function via skin microbiome metabolic pathways, the following analysis was performed. Multiple linear regression analyses were performed with BMI as a continuous outcome and individual metabolic pathways as independent variables adjusted by age and gender. A list of statistically significant ($p<0.05$) metabolic pathways were identified and summarized. Subsequent multiple linear regression analyses adjusted by age and gender were then

performed with skin barrier function status as the outcome and identified pathways from the previous step as independent variables. The skin barrier function status was represented by log transformed TEWL, skin surface moisture and pH. Significant pathways adjusted for multiple testing correction by B-H procedure were evaluated. Volcano plot for each measure of skin barrier function was plotted to identify any biomarkers with the most meaningful change.

9.2.3. Relationship of obesity related serum proteomics profile and epidermal barrier function

Similar analysis was performed to assess how systemic inflammation of obesity might mediate its effects on skin barrier function. Multiple linear regression analyses were performed with BMI as a continuous outcome and individual proteomics markers as independent variables adjusted by age and gender (as described in Section 7.3.4). A list of statistically significant ($p<0.05$) serum proteomics markers were identified and summarized. Subsequent multiple linear regression analyses adjusted by age and gender were then performed with skin barrier function status as the outcome and identified serum markers from the previous step as independent variables. The skin barrier function status was represented by log transformed TEWL, skin surface moisture and pH. Significant pathways adjusted for multiple testing correction by B-H procedure were evaluated. Volcano plot for each was plotted to identify any biomarkers with the most meaningful change.

9.2.4. Relationships of obesity related microbiome species and serum proteomic markers

To evaluate the relationship between skin microbiome species and serum proteomic markers in the context of obesity, the following analysis steps were performed. (Figure

9.2) Multiple linear regression analyses were performed with BMI as a continuous outcome and individual microbiome species as independent variables adjusted by age and gender. A list of statistically significant ($p<0.05$) microbiome species were then identified and summarized. Similarly, multiple linear regression analyses were performed with BMI as a continuous outcome and individual serum protein markers as independent variables adjusted by age and gender. A list of statistically significant ($p<0.05$) protein markers were then identified and summarized.

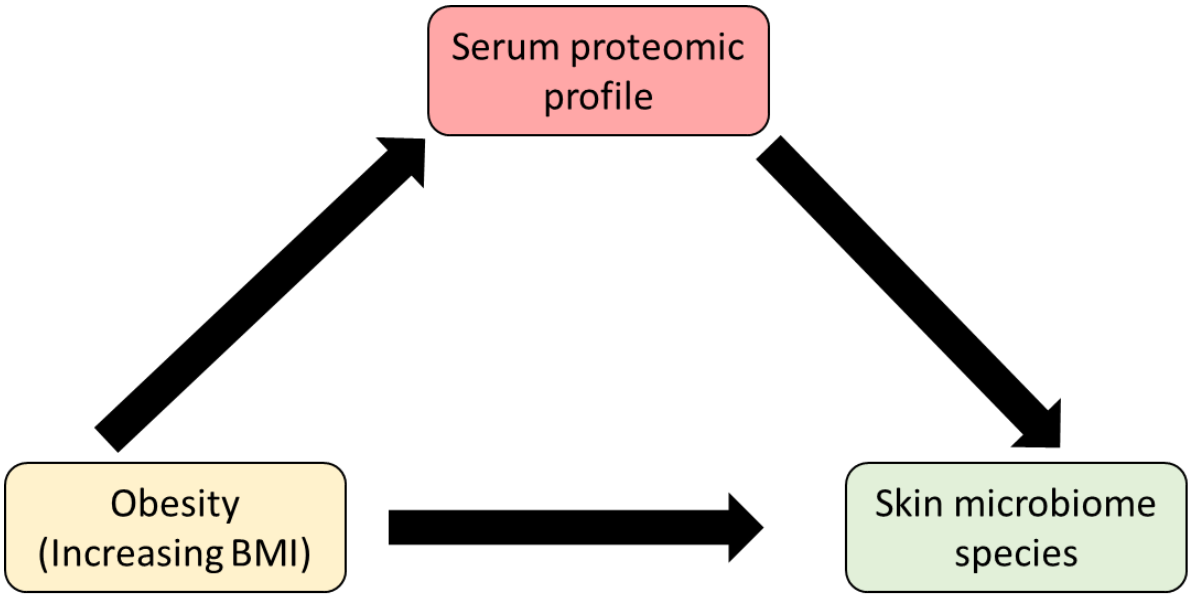


Figure 9.2. A graphical representation of how obesity might be related to the relative abundance of skin microbiome at species level and levels of specific serum proteomic markers.

Finally, the list of significant microbiome species was then analyzed against the list of significant serum protein markers to assess the relationship between skin microbiome and serum proteomic profile in obesity. This was performed with multiple linear regression adjusted for age and gender. P values were subsequently subjected to multiple testing correction using the Benjamini–Hochberg (B-H) procedure at false discovery rate (FDR) threshold of 0.1 to identify serum proteins and microbiome

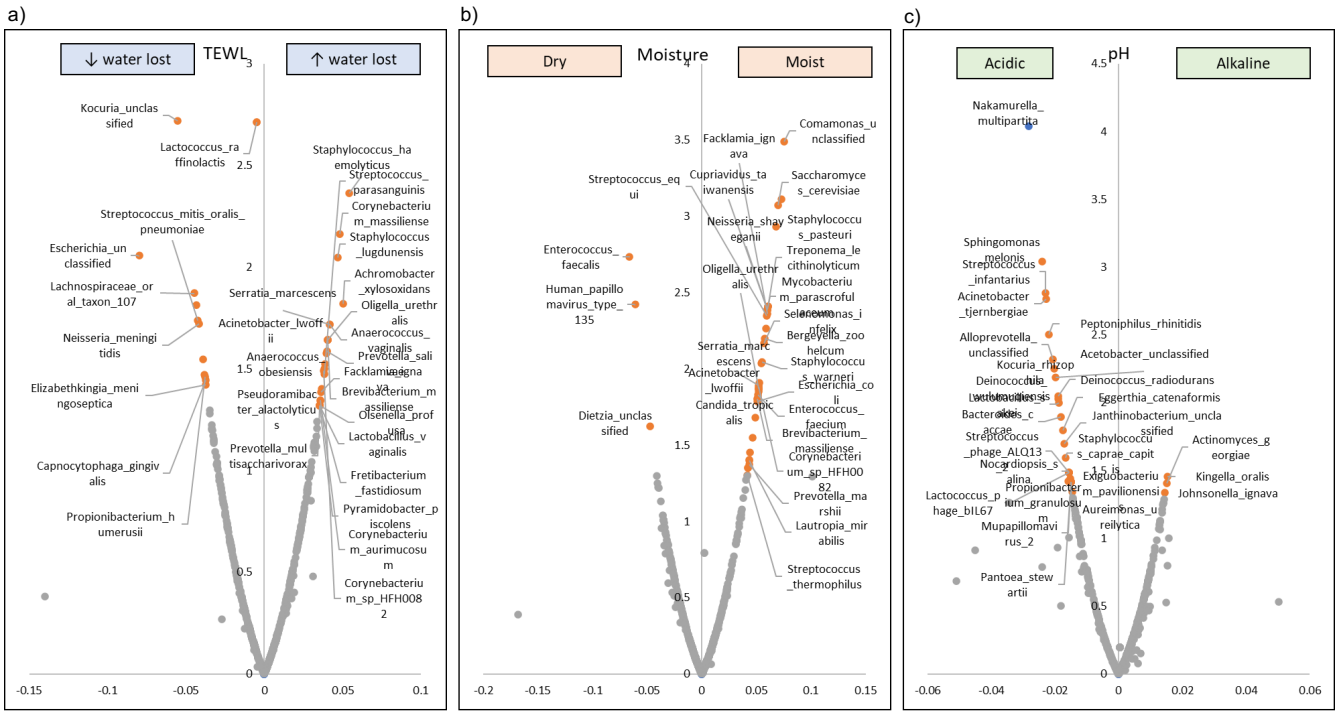
species pairs that were significant with increasing BMI. Results were visualized with a heat map (Figure 9.8) and represented as a volcano plot (Figure 9.9).

9.3. Results

9.3.1. Skin microbiome species and epidermal barrier function

Skin microbiome species

Skin microbiome species were analyzed with epidermal barrier function (log transformed TEWL, skin moisture and pH). Results are summarized in the following volcano plots (Figure 9.3). A total of 736 bacterial species were analyzed as independent variables with logTEWL, logMoist and logpH as continuous outcomes adjusted by age and gender. Forty-two species had a significant relationship with increasing TEWL after adjustment by age and gender. Of these 42 species, increasing relative abundance of 29 microbiome species was significantly associated with increasing TEWL but none remained statistically significant after multiple testing correction. Forty-one species had a significant relationship with increasing skin moisture after adjustment by age and gender. Several *Corynebacterium* species for example, *Corynebacterium massiliense, Corynebacterium aurimucosum* and *Corynebacterium sp HFH0082* were associated with increased TEWL. Three bacterial species were associated with a decreasing skin moisture with increasing relative abundance. Thirty-one microbiome species had a significant relationship with increasing skin pH adjusted by age and gender. The relative abundance of *Nakamurella multipartita* significantly decreased with increasing skin pH (Beta: -0.0282; corrected p = 0.0675)

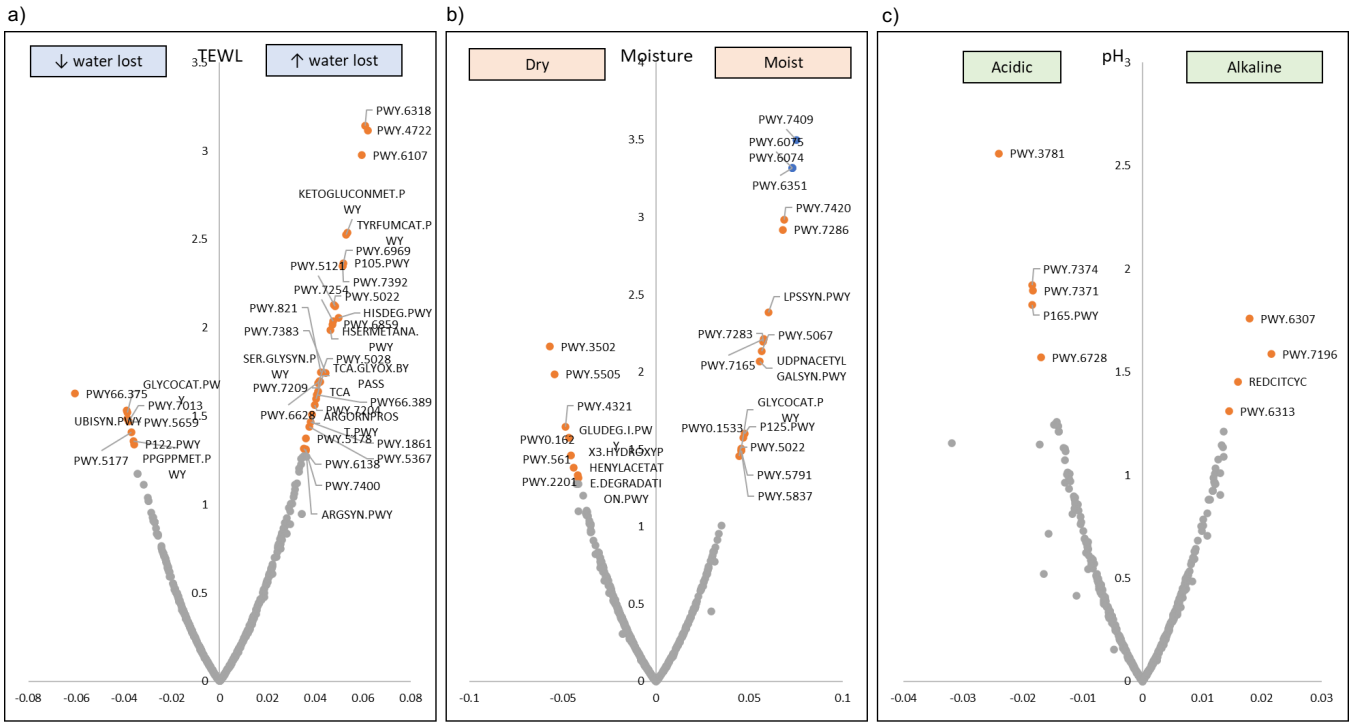


* Adjusted by age and gender

Figure 9.3. Volcano plots of skin physiology parameters with skin microbiome.
Standardized β coefficients and p values from multiple linear regression analyses of skin microbiome at species level on the log transformed skin physiology measurements (adjusted by age, gender) are plotted as x-axis and y axis of the volcano plots. Data points in blue were significant after adjusting for multiple testing by B-H procedure. Those in orange have p value <0.05 but not significant after adjusting for multiple testing.

Microbiome functional metabolic pathways

Metabolic pathways as defined by Metacyc database were analyzed with epidermal barrier function (log transformed TEWL, skin moisture and pH). Results are summarized in the following volcano plots (Figure 9.4). There was a total of 504 microbiome metabolic pathways analyzed.



* Adjusted by age and gender

Figure 9.4. Volcano plots of skin physiology parameters with microbiome functional metabolic pathways.
Standardized β coefficients and p values from multiple linear regression analyses of metabolic pathways on the log transformed skin physiology measurements (adjusted by age, gender) are plotted as x-axis and y axis of the volcano plots. Data points in blue were significant after adjusting for multiple testing by B-H procedure. Those in orange have p value <0.05 but not significant after adjusting for multiple testing.

A total of 39 metabolic pathways were significantly associated with increasing TEWL (p <0.05) after adjusting for age and gender. Notably, various metabolic pathways related to tricarboxylic acid cycle (TCA) were significantly associated with increasing TEWL. These included TCA: TCA cycle I (prokaryotic), PWY66-398: TCA cycle III (animals), PWY-6969: TCA cycle V (2-oxyglutarate:ferredoxin oxidoreductase) and TCA-GLYOX-BYPASS: superpathway of glyoxylate bypass and TCA. None remained significant after multiple testing correction.

With skin surface moisture, the following lipid related biosynthesis and metabolic pathways had a significant relationship with skin moisture: PWY-7409: phospholipid

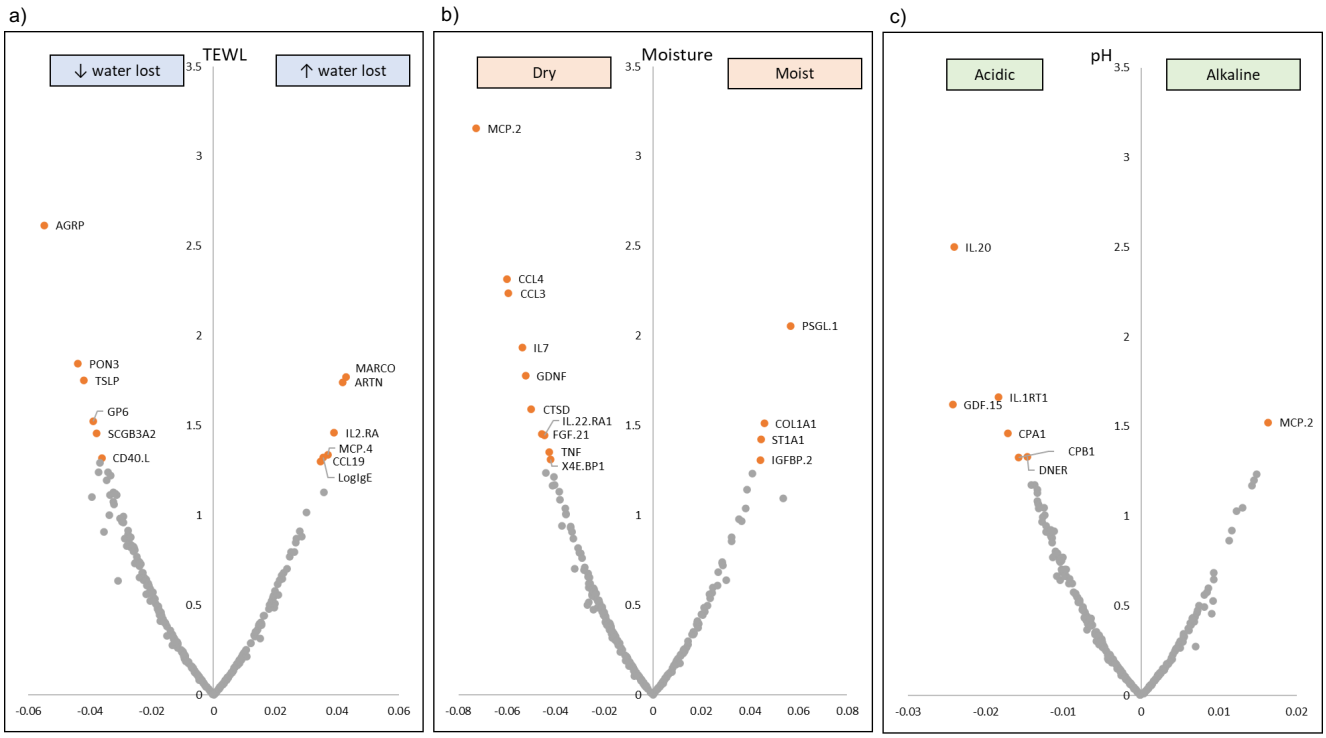
remodeling (phosphatidylethanolamine, yeast), PWY-6075: ergosterol biosynthesis I, PWY-6074: zymosterol biosynthesis, PWY-7420: monoacylglycerol metabolism (yeast) and LPSSYN-PWY: superpathway of lipopolysaccharide biosynthesis. These pathways were related with an increasing skin surface moisture but they were reduced with increasing BMI. PWY-7409: phospholipid remodelling (phosphatidylethanolamine, yeast), PWY-6075: ergosterol biosynthesis I and PWY-6074: zymosterol biosynthesis remained significant after multiple testing correction at FDR threshold <0.1.

Only nine metabolic pathways were significant with a change in skin pH after adjusting for age and gender. Some were related with degradation of amino acids or purines: PWY-6307: L-tryptophan degradation X (mammalian, via tryptamine), PWY-6313: serotonin degradation and P165-PWY: superpathway of purines degradation in plants. None remained significant after multiple testing correction.

9.3.2. Serum proteomic biomarkers and epidermal barrier function

A total of 267 unique serum proteomic biomarkers was analyzed with epidermal barrier function as a continuous outcome adjusted by age and gender. Results are illustrated in Figure 9.5.

A total of 12 serum proteomic biomarkers were significantly associated with a change in TEWL. Six of these markers (MARCO, ARTN, IL2RA, MCP4, CCL19 and Total IgE) were associated with an increasing TEWL. Notably, IL2RA, MCP4 and Total IgE are known biomarkers associated with atopic dermatitis (AD). Similarly, a total of 14 serum biomarkers were associated with a change in skin surface moisture. CCL3, CCL4, IL22RA1 and FGF21 were among the 10 biomarkers associated with a decreasing skin surface moisture. They are also known biomarkers associated with AD.



* Adjusted by age and gender

Figure 9.5. Volcano plots of skin physiology parameters with serum proteomic biomarkers.
Standardized β coefficients and p values from multiple linear regression analyses of serum biomarkers on the log transformed skin physiology measurements (adjusted by age, gender) are plotted as x-axis and y axis of the volcano plots. Data points in orange have p value <0.05 but not significant after adjusting for multiple testing by B-H procedure.

Seven serum biomarkers were associated with a change in skin pH. These include IL20, GDF15, IL1RT1, CPA1, CPB1, DNER and MCP2(CCL8).

However, none of the biomarkers reached significance after adjusting for multiple testing correction.

9.3.3. Obesity related functional microbiome metabolic pathways and their relationship with epidermal barrier function

As reported in Chapter 6, section 6.3.6, there were 103 significant metabolic pathways associated with increasing BMI (p <0.05). These significant pathways were next analyzed with epidermal barrier function as a continuous outcome adjusted by age

and gender to identify BMI significant pathways that were significant with a change in epidermal barrier function. Results are illustrated in Figure 9.6.

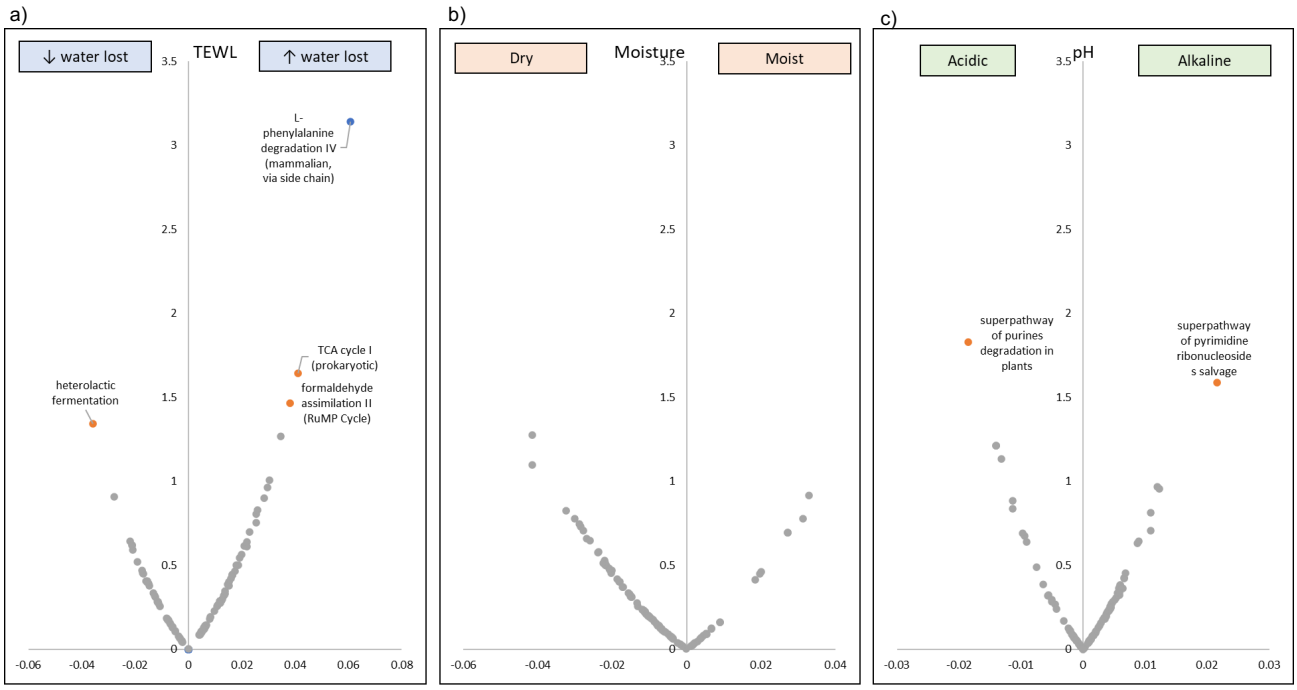


Figure 9.6. Volcano plots of skin physiology parameters with BMI significant metabolic pathways of microbiome.
Standardized β coefficients and p values from multiple linear regression analyses of metabolic pathways on the log transformed skin physiology measurements (adjusted by age, gender) are plotted as x-axis and y axis of the volcano plots. Data points in blue were significant after adjusting for multiple testing by B-H procedure. Those in orange have p value <0.05 but not significant after adjusting for multiple testing.

Four pathways were significantly associated with a change in TEWL: TCA: TCA cycle I (prokaryotic), PWY-1861: formaldehyde assimilation II (RuMP Cycle), PWY-6318: L-phenylalanine degradation IV (mammalian, via side chain) and P122-PWY: heterolactic fermentation. TCA: TCA cycle I (prokaryotic) and PWY-1861: formaldehyde assimilation II (RuMP Cycle) pathways increased with increasing BMI which in turn were associated with increasing TEWL. P122-PWY: heterolactic fermentation was reduced with increasing BMI and therefore a negative relationship with increasing TEWL would mean increasing BMI was related to an increase in water loss via this pathway. Finally, PWY-6318: L-phenylalanine degradation IV (mammalian,

via side chain) had a negative relationship with increasing BMI and therefore with increasing BMI, it was associated with a decrease in TEWL via this pathway.

There were no significant pathways associated with changes in skin surface moisture.

P165-PWY: superpathway of purines degradation in plants and PWY-7196: superpathway of pyrimidine ribonucleosides salvage were associated with a change in skin pH.

9.3.4. Obesity related serum proteomic profile and their relationship with epidermal barrier function

There was a total of 120 significant serum proteomics markers that were associated with increasing BMI ($p < 0.05$). These significant markers were next analyzed with epidermal barrier function as a continuous outcome adjusted by age and gender to identify BMI significant biomarkers that were significant with a change in epidermal barrier function. Results are illustrated in Figure 9.7.

Obesity related proteomic markers that were significantly associated with a disrupted skin barrier function were similar to the list of proteomic markers with a significant relationship with skin barrier function as reported in the earlier section 9.3.2. A total of five serum proteomic biomarkers were significantly associated with an increasing TEWL. Notably, IL2 RA, MCP4 and Total IgE are known biomarkers associated with AD. Similarly, known AD related chemokines such as CCL3, CCL4 and FGF21 were associated with a decreasing skin surface moisture.

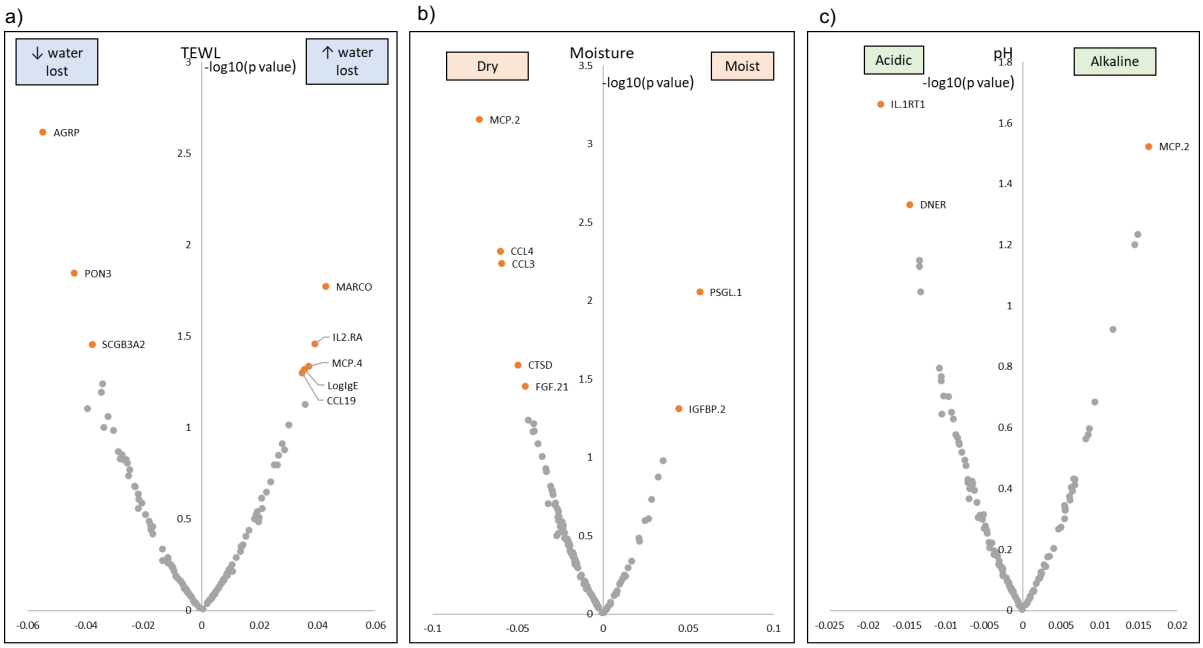


Figure 9.7. Volcano plots of skin physiology parameters with BMI significant serum proteomics biomarkers.
Standardized β coefficients and p values from multiple linear regression analyses of serum proteomics markers on the log transformed skin physiology measurements (adjusted by age, gender) are plotted as x-axis and y axis of the volcano plots. Data points in blue were significant after adjusting for multiple testing by B-H procedure. Those in orange have p value <0.05 but not significant after adjusting for multiple testing.

9.3.5. Relationships of obesity related microbiome species and serum proteomic markers

There was a total of 101 microbiome species and 120 serum proteomic biomarkers that were significantly related with increasing BMI respectively. These significant species were regressed against the significant serum proteins by multiple linear regression adjusted by age and gender. P values were then corrected by multiple testing correction via B-H procedure. The measure of significance (corrected p values) of these biomarkers are illustrated as a heatmap matrix of microbiome species and serum protein markers to visualize any significant clusters of serum biomarker-microbiome species pair. (Figure 9.8)

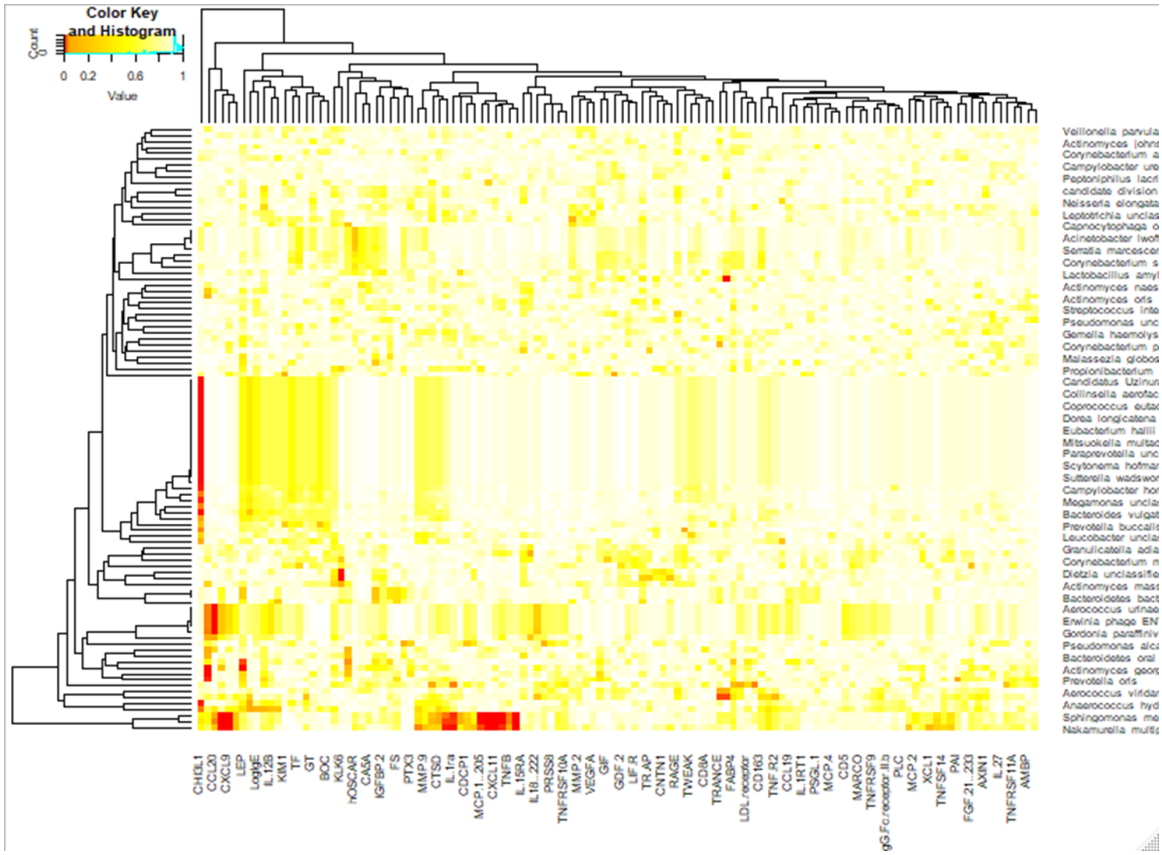


Figure 9.8. Heat map of significant skin microbiome at species level and significant serum proteomic biomarkers with increasing BMI.
Corrected p values from multiple linear regression analyses of relative abundance of microbiome species on log2 transformed levels of serum proteomic biomarkers (adjusted by age, gender) are plotted as heat map. Those in red and dark orange have a corrected p value <0.05 and <0.1.

There was a total of 81 serum biomarker-microbiome species pairs that had a significant relationship with each other after adjusting for age and gender as well as multiple testing correction by B-H procedure. Twenty serum biomarkers and 44 microbiome species contributed to these significant pairs. These serum biomarkers included known serum inflammatory markers of obesity such as leptin, IL1ra, interferon gamma and MCP1. Other serum biomarkers included CCL20, IL10 and MP3 which have previously been reported to be associated with atopic dermatitis. A volcano plot illustrates a measure of statistical significance and the magnitude of the change for these significant pairs. (Figure 9.9)

As a serum proteomic biomarker, CHI3L1, chitinase-3 like-protein-1 has been noted to be significantly associated with 27 microbiome species. These serum biomarker-microbiome species pairs were all statistically significant with increasing BMI. It has also been observed that *Acinetobacter tjernbergiae*, *Nakamurella multipartite* and *Sphingomonas melonis* were significantly related to multiple serum biomarkers. Of note, most of the serum biomarker-microbiome species pairs had a positive relationship. Only two serum biomarkers, leptin and stem cell factor (SCF) had a negative relationship with relative abundance of *Corynebacterium genitalium, Facklamia languida, Actinomyces georgiae* and *Prevotella marshii* respectively.

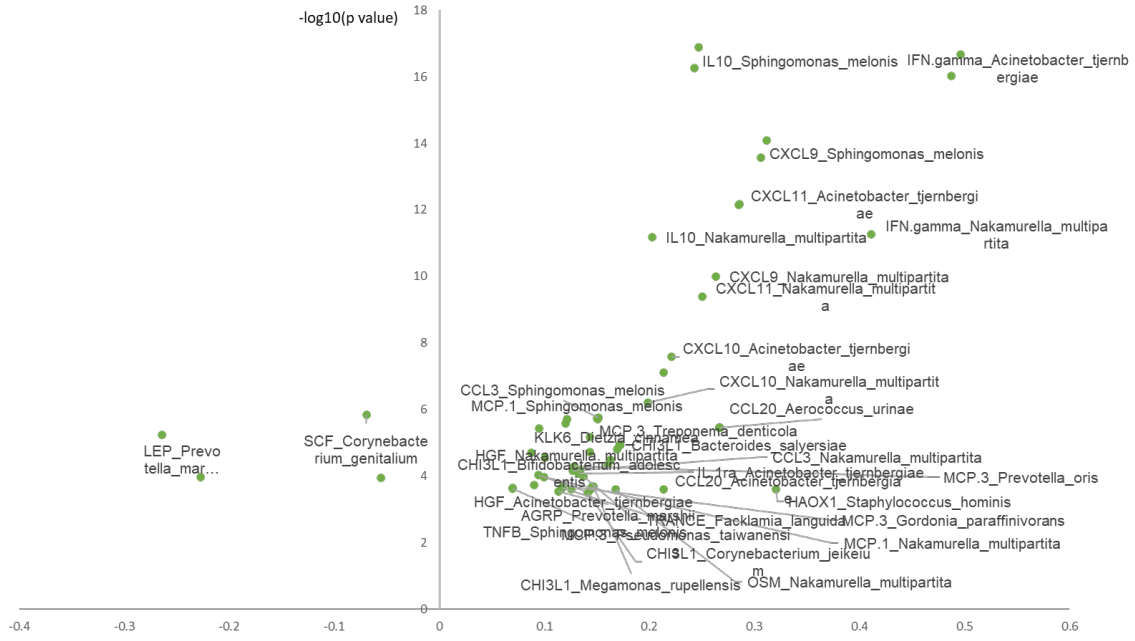


Figure 9.9. Volcano plot of significant skin microbiome at species level and significant serum proteomic biomarkers with increasing BMI.
Standardized β coefficients and p values from multiple linear regression analyses of relative abundance of microbiome species on log2 transformed levels of serum proteomic biomarkers (adjusted by age, gender) are plotted as x-axis and y axis of the volcano plots. Only significant pairs after multiple testing correction are plotted as green data points on this volcano plot.

9.4. Discussion

This chapter integrated data generated from several studies discussed in detail in the previous chapters. With the emergent of more advance technologies such as whole metagenomic sequencing and multi-array omics platforms, it provided opportunities for investigators to evaluate the phenotypes and endotypes in a high throughput and in-depth manner. However, with the amount of data generated, it could also pose challenges in terms of statistical analysis and interpretation of results. It is especially challenging to integrate microbiome data and data from other omics investigations. There have been several methods proposed in the literature on how to perform such "microbiome multi-omics network analysis" [317]. They include methods such as marginal correlation analysis, dimension reduction methods, regression-based methods and network integration. This integrated analysis chapter adopted the regression-based method that allowed adjustment for confounders (age and gender) using multiple linear regression. Each biomarker or feature was in turn specified as the independent or outcome variable for the analysis. To avoid excessive false positive, p values were adjusted with multiple testing correction by B-H procedure at a false discovery rate (FDR) threshold of 0.1. This method was easy to implement on simple statistical software tool.

To integrate data from the various studies, it was important to appreciate and hypothesize what might be underlying pathophysiological mechanisms that drive the observed relationship between obesity and AD as well as its associated disrupted barrier function. This would provide inputs on analysis steps as well as which features to be selected as independent or outcome variable at every regression stage.

It has been reported in several studies as well as in an earlier study in this thesis that obesity with increasing BMI was associated with a disrupted skin barrier function of

increased TEWL and reduction in skin surface moisture [12, 188]. Given the knowledge that obesity is a disease with systemic inflammation and it affects the overlying skin microbiome, it is therefore pertinent to investigate how skin microbiome and serum protein markers might be related to the expressed skin physiology parameters. Indeed, there were several species that were significantly associated with an increased TEWL or decreased skin surface moisture suggesting a barrier disruption. Several *Corynebacterium* species were associated with an increased TEWL and it has been noted in the earlier microbiome study chapter that the relative abundances of *Corynebacterium* species increased with increasing BMI. However, it is important to note that, while there were about 30 to 40 microbial species that were significant with the respective skin physiology measures, almost none achieve statistically significance after multiple testing correction using B-H procedures. Different commensal organisms seem to have various effects on TEWL and skin surface moisture.

For functional profiling of the overlying microbiome, it was noted that versions of metabolic pathways related to tricarboxylic acid cycle (TCA) were significantly associated with increasing TEWL. However, the TCA cycle is a very common catabolic pathway that exists in several types of organisms. It generates energy and reducing power and generates precursors for subsequent biosynthesis. A possible explanation would be this increased activity of pathways related to TCA could be a reflection of the underlying metabolic activities of the skin surface communities. It could also be intermediate products of the TCA cycle contributes to an increased TEWL.

Several lipid related biosynthesis and metabolic pathways were observed to be related to an increased with skin moisture. This might not be surprising as lipids and cholesterol metabolites can contribute to the epidermis natural moisturizing factors

which in turn contribute to the skin barrier function. It should be noted that these pathways had a negative relationship and appeared reduced with increasing BMI although there was no statistical significance.

For the analysis that evaluated the relationship of the serum proteomic markers with skin physiology measures, several known Th-2 inflammatory markers of AD such as IL2RA, Total IgE and MCP4 were associated with increasing TEWL[269]. Similarly, keratinocytes specific and AD related inflammatory markers such as CCL3, CCL4, IL22RA1 and FGF21 were associated with reduced skin surface moisture. Interestingly, general inflammatory markers such as IL1, IL6, TNF-α were not significantly associated with either a change in TEWL or skin surface moisture. This provided some suggestions that serum markers from localized inflammation might play a greater role in disrupted skin physiology compared to non-specific systemic inflammation. However, much research would still be required to evaluate how increased levels of these specific serum markers are related to the observed skin barrier function.

This chapter also worked on the hypothesis that resultant metabolic products from functional microbiome metabolic pathways might directly impact on the overlying skin physiology status. To address this hypothesis, significant functional metabolic pathways related to increasing BMI were regressed with skin physiology parameters as continuous outcome variables, adjusted by age and gender. Three metabolic pathways which are significantly associated with increasing BMI (TCA cycle I (prokaryotic), formaldehyde assimilation II (RuMP Cycle) and heterolactic fermentation) were also individually significant with increasing TEWL. While there were some suggestions that these three BMI related metabolic pathways contribute metabolic products that might affect TEWL, they were not significant after multiple

testing correction. More studies would be needed to evaluate if these observations were false positive.

Finally, analysis was performed to integrate the microbiome data and the proteomic data together. As reported, 81 serum protein-microbiome species pair were noted to have a significant relationship after adjusting for confounders and multiple testing correction. As noted on the heat map, the significant proteomic-microbiome pairs seemed to cluster among a few serum biomarkers and microbiome species. It has been noted that CHI3L1 (chitinase-3 like-protein-1) has been noted to be significantly associated with 27 microbiome species.

CHI3L1 is one of the non-enzymatic chitinase-like proteins belonging to the glycoside hydrolase family 18. Its expression is regulated by many extrinsic and intrinsic factors and is secreted by various cell types including specific white blood cells types, fibroblast and smooth muscles cells. It has been linked with various inflammatory and allergic conditions such as obesity, diabetes, arthritis and asthma [318-320]. CHI3L1 also plays an important role in Th1/Th2 balance and Th2 inflammation [321]. Studies have reported that CHI3L1 is greatly enhanced by Th2 inflammation as a result of environmental exposures such as house dust mites [322]. At the same time, it also contributes to the development of visceral obesity and asthma [323]. With this evidence suggesting its important role in Th2 inflammation as well as the observed association with changes in skin microbiome species abundance, CHI3L1 might play a pertinent role in linking obesity with the development of AD. Further studies are needed to evaluate this as a biomarker in obesity related risk of AD.

Acinetobacter tjernbergiae, Nakamurella multipartite and *Sphinomonas melonis* were found to be significantly associated with multiple serum proteins. However, their

relative abundance distributions among the samples were very skewed and therefore possibly resulted in very extreme results. Their mean relative abundance were 2.31 x 10^{-6} %, 9.43 x 10^{-4} % and 3.96 x 10^{-3} % respectively. An additional step of quality control to filter microbiome species below a certain median or mean relative abundance might be necessary to avoid such skewed distribution and extreme results [324].

In summary, although high throughout biomarkers data are available with newer omics technologies for integration with microbiome data, there are statistical challenges. This chapter illustrated some important associations of skin microbiome and serum proteomics markers with skin physiology status. It generated new data and hypotheses for further evaluation on how obesity with increasing BMI might increase the risk of AD. Interestingly, CHI3L1 was found to significantly correlated with several bacterial species. Its role in obesity and Th2 inflammation suggests possible future studies to investigate this as a possible mechanistic link between obesity and AD. This chapter highlights the feasibility of using regression-based method as an approach to combine omics-based data with microbiome data generated within the same cohort.

10. Overall Discussion

This thesis evaluated the observed association between obesity and atopic dermatitis (AD) with five specific studies to determine its relationship in our local population and possible mechanisms linking the two chronic conditions. Despite the numerous epidemiological studies reporting this association, few studies have attempted to evaluate the underlying mechanisms in a concerted manner. Current evidence and understanding of the pathophysiology of AD provided clues on the approach to working out the mechanisms of their associations. AD is a chronic skin disease with characteristic epidermal barrier dysfunction, systemic inflammation and altered skin microbiome profile with both genetic and environmental influences. While it is not possible to work through all possible pathways, this thesis investigated potential key players that could shed light on underlying mechanistic pathways. It first established the prevalence of AD and assessed its relationship with different measures of obesity in a population cohort of considerable sample size. Next, it investigated the impact of obesity on skin epidermal barrier function across the entire cohort and then subsequently evaluated the skin microbiome and serum proteomics profile in a selected matched group of AD cases and control. Finally, Mendelian randomization was performed using published genetic information to establish causal relationship and its direction between AD and obesity. This chapter summarizes the findings of this thesis and highlights their novelty and potential impact. It examines the strengths and limitations of this whole study and provides plans and directions for future studies to work on hypotheses generated from this thesis.

10.1. Summary of findings

The overall prevalence of AD in the HELIOS population cohort was 8.8% with a decreasing prevalence with increasing age strata. This closely mirrored population prevalence of adult AD of about 10% in two local cross-sectional studies despite a slightly different demographic structure [2, 3]. While it was observed that general obesity in terms of increasing BMI or obese (BMI $\geq$30 kgm^2) or increase in total body fat percentage had an increased odd of having AD, these relationships did not reach statistical significance in several statistical models. Abdominal obesity as measured by waist hip ratio and visceral fat mass measured on DXA scan were observed to have a significantly increased risk of having AD. Consistently, abdominal obesity also had a higher magnitude of risk of AD compared to general obesity in various statistical models. It is relatively known that abdominal obesity and general obesity have differential effects on cardio-metabolic diseases. The underlying differences in systemic inflammation may potentially explained the differences in AD risks observed.

The second study supported the finding of epidermal barrier dysfunction in obese individuals in terms of an increased trans-epidermal water loss (TEWL) and decreased skin surface moisture. This was consistent across various measures of obesity. Interestingly, increasing obesity was associated with an increasing acidic skin pH instead of a more alkaline one found in AD skin. A disrupted skin barrier function logically implied disturbances in the skin surface natural moisturising factors which in turn might be a consequence of skin microbiome changes and/or systemic inflammation. These two aspects were evaluated in-depth in study 3 and 4 respectively.

The microbial diversity in AD was not significantly reduced compared to controls as what would be expected. A plausible explanation would be a reduced disease severity among the AD cases in this population cohort. Similarly, there were no observed

significant differences in microbial diversity in obese individuals compared to non-obese.

However, there were characteristic microbial differences at species levels in obese compared to non-obese. Consistent with another recent study that evaluated skin microbiome profile in a population cohort with varying BMI, there were increased relative abundance of *Corynebacterium* species [15]. Corynebacterium's role in causing epidermal barrier injury and subsequent skin inflammation via mycolic acid and IL-23 pathways have previously been discussed in our studies. Integrated analysis of this cohort's skin microbiome and TEWL also revealed that Corynebacterium species were associated with an increasing TEWL. Various skin commensals such as *Staphylococcus hominis, Streptococcus dysgalactiae* and *Malassezia globosa* were increased in obese skin while abundances of *Staphylococcus aureus* and *Cutibacterium acnes* were decreased. The increased presence of some of these microbial species is beneficial for skin health while a reduced presence may promote epidermal injury and inflammation. It was noted that the super pathway of branched amino acids among the resident skin microbial species was significantly elevated in both AD and obese individuals. Its significance requires further evaluation as systemic elevated of branched amino acids in plasma has been linked with systemic inflammation of obesity. Despite the multiple pathways that appeared enhanced among the skin microbial communities with complicated interactions, it was observed that pathways of tricarboxylic cycle were observed with an increased TEWL while multiple pathways that mediate lipids or cholesterol in microbial species were associated with skin surface moisture.

In terms of serum proteomic profile, there were no significant serum biomarkers after multiple testing correction between AD and controls. However, several serum

biomarkers specific to AD inflammation were found to be significantly elevated with increasing BMI or waist-hip ratio (WHR). Some of these cytokines are keratinocytes specific and are used as AD disease severity markers in clinical practice. They include CCL17 and CCL20. This suggests that these changes were not merely part of an indiscriminate elevation of inflammatory cytokines in obese individuals. Interestingly, IL-18 related cytokines were observed to be affected with increasing obesity (both BMI and WHR). Previous evidence, as discussed in the serum proteomics chapter, supported a possible role of IL-18 related cytokines in the initiation of AD inflammation. This finding of an enhanced IL-18 pathways among obesity might underscore a possible link between obesity and its link with the development of AD. Integrated analysis between serum proteomic profile and skin microbiome species in the context of increasing BMI revealed that CHI3L1 was significantly associated with several microbiome species. It was also reported to be discriminant for AD with a VIP projection score of greater than 2.0 in partial least square discriminant analysis. CHI3L1 binds to several receptors including IL13-R2 with some impact on the IL4 and IL13 pathways [322].

Finally, with Mendelian randomization, an approach using genetic instruments, it was established that a higher BMI had an effect on an increased risk of having AD. There was no evidence otherwise to suggest AD causally resulted in an increase in BMI. Sensitivity analysis with genetic instruments of WHR suggests a similar relationship, although the analysis might be affected by presence of pleiotropic factors.

10.2. Novelty of findings

This thesis represented a concerted effort to evaluate the relationship between AD and obesity via various epidemiological methodologies and approaches from classical

epidemiology methods, molecular epidemiology as well as Mendelian randomization. It contributed data of sufficiently large sample size to estimate the disease burden of AD in terms of its prevalence in Singapore. Study 1 also highlighted the importance of abdominal obesity in addition to general obesity in its relationship with the risk of AD in an adult population cohort of median above 50 years old. It had evaluated the relationship of obesity and AD using several measures of obesity. This was also one of the few Asian epidemiology cohort studies that classified AD using the UK Working Party 2 out of 4 criteria previously proposed for assessing cardio-metabolic risks of AD in epidemiology cohort studies.

In contrast to previous studies that evaluated skin physiology status in smaller clinical cohorts with varying BMI, study 2 evaluated skin physiology in over 5,000 individuals. It was consistent with the finding that obese individuals have a drier skin with increased water loss and decreased skin surface moisture. It also highlighted that obese skin has an acidic pH instead of an alkaline pH as was expected in barrier dysfunction.

Study 3 evaluated the skin microbiome profile in obesity using whole metagenomic sequencing compared to another recent study that evaluated their microbiome profile using 16s sequencing [15]. With the whole metagenome sequences, mapping for eukaryotes and viruses as well as microbial metabolic pathways could be performed. It concurred with previous observation that Corynebacterium species were increased with BMI but additionally reported that other important commensal microbial species were also significantly affected with increasing BMI.

In terms of serum proteomic profile (study 4), besides the characteristic inflammatory profile of obesity, inflammatory cytokines specific to keratinocytes or characteristic in AD were observed to be elevated with increasing obesity. IL-18 family of cytokines,

previously hypothesized to play a role in initiation of AD, were also found to be elevated in obesity [275].

Finally, study 5 using Mendelian randomization established the relationship of BMI upon AD. This was a novel finding and by establishing the direction of the relationship, it helped to focus the approach for subsequent studies to study the mechanistic links between the two conditions.

10.3. Proposed mechanistic pathways

This thesis established that obesity has a causal relationship with the risk of developing AD. This increased risk may be related to the epidermal barrier dysfunction observed in obese individuals. While the integrated analysis has revealed that certain microbiome species, microbiome metabolic pathways and serum cytokines were related to an epidermal barrier dysfunction, there was a general lack of statistical significance after multiple testing correction. There was however clearer and significant evidence that certain aspects of inflammatory cytokines observed in obese individuals were similar to that in early AD cases.

From these observations, this study proposed that the mechanistic pathway of how obesity increased risk of AD could be as follows: the state of obesity produces a characteristic systemic inflammatory profile that in turn influence skin inflammation, the microbiome profile and skin surface metabolic pathways. These then affect the levels of skin surface metabolites and subsequently manifests as an increased TEWL, reduced skin surface moisture and an acidic skin pH. The disrupted skin barrier function together with the enhanced Th-2 and Th-17 inflammation would then contribute to the resultant AD phenotype. This risk would be in additional to other

genetic risk factors that affects underlying skin inflammation and skin barrier function directly.

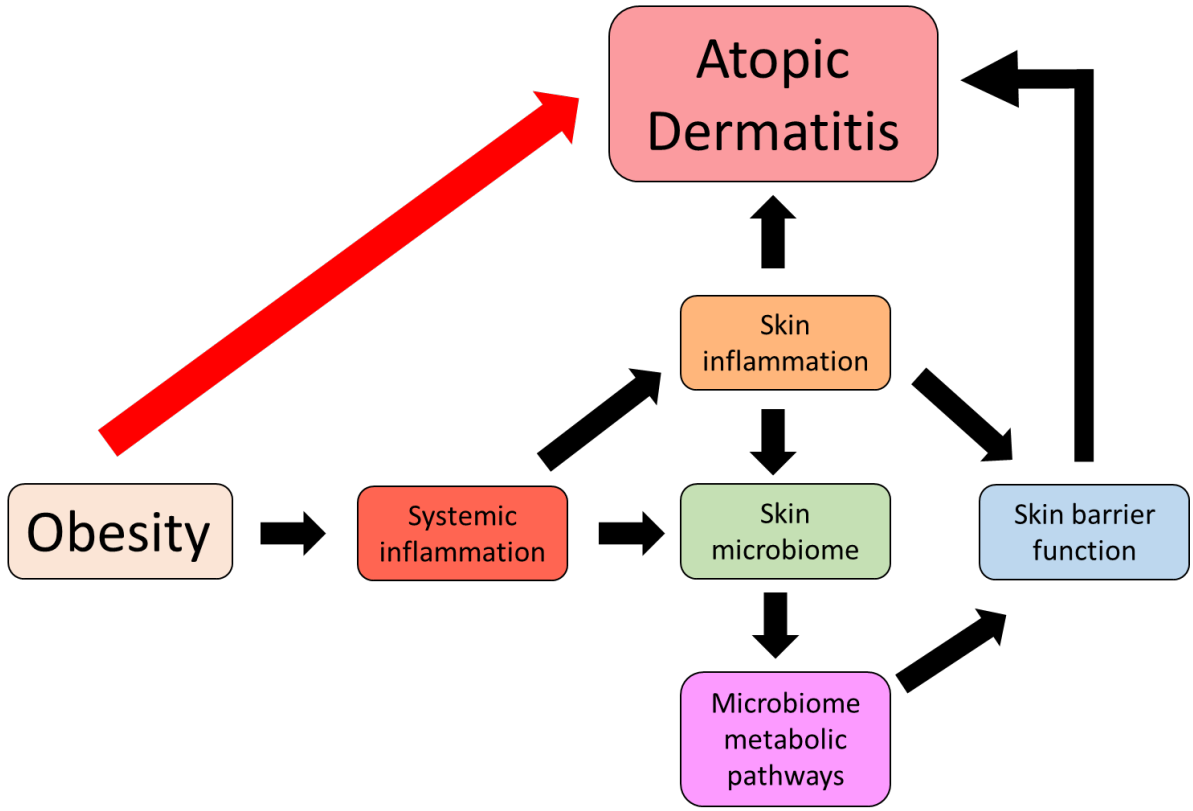


Figure 10.1. Schematic diagram illustrating hypothesized mechanistic pathways linking obesity to development of atopic dermatitis (AD).
Systemic inflammatory state of obesity leads to skin inflammation and changes in skin microbiome profile. Resultant changes in skin microbiome metabolic pathways and skin inflammation result in a disrupted skin barrier function. All these lead to an increase risk of AD which is a chronic inflammatory skin disease with characteristic disrupted epidermal barrier function. This would be in addition to known genetic factors that directly affects skin inflammation and epidermal barrier function.

10.4. Impact and implications of findings

The findings that obesity might be a modifiable disease risk factor for AD have important implications in clinical practice. While the morbidity and mortality complications of obesity are widely recognised by healthcare professionals, physicians are often less cognizant of the importance of having a normal body weight in the patients with dermatological conditions. This would therefore impact clinical management practice guidelines and advisory on the management of AD.

This study has generated observations on how the inflammatory state of obesity could impact the skin physiology status, skin microbiome compositions and systemic proteomic profile. It provided clues on possible mechanistic pathways of how AD might develop or initiate in a population or early disease cohort. It generated hypotheses and identified new microbiome or cytokines targets that future studies could focus to understand the pathophysiology of AD and its co-morbidities. This could be translated downstream to new therapeutic targets in this era of increasingly targeted therapies.

10.5. Strengths of study

This study leveraged on an ongoing large-scale population cohort where a whole breadth of meta-data and co-morbidities data are being collected across various body systems. It allowed this study to evaluate the primary association of interest with various measures of obesity while taking into account relevant confounders. The large sample size of close to 5,600 participants also provided adequate statistical power to evaluate the various hypotheses. This study is also one of the first few studies that evaluated the relationship of obesity with AD in a concerted fashion with a range of epidemiology methods, looking at how obesity might influence key players in AD's pathogenesis. By phenotyping with skin physiology measurements, skin microbiome taxonomic and functional profiles and serum proteomic profiles, this study could integrate information across these various omics platform and narrow down targets of interests for future investigations. Omics information from this study could also integrate with further information from other future omics platforms since serum samples are bio-banked as part of the cohort study. Further integrations with serum metabolomics data have already been planned. The various omics and integrated analysis have generated several interesting targets for future analysis. Finally, this study also established the causal direction between BMI and AD, supported by

sensitivity analyses. This is significant as it sets the scope and direction on current and future work to evaluate the observed links between obesity and AD. This finding has recently been published in a peer-reviewed journal [227].

10.6. Limitations of study

This study included participants of a general population cohort study. While efforts have been made to classify AD based on an established diagnostic criteria, diagnosis of AD was not confirmed by a dermatologist given the sheer number of participants involved. AD is also a heterogenous skin condition with distinct features in different ethnic groups and regions [68]. Population cohort studies on AD can therefore be subjected to risks of misclassification bias, in a non-differential manner, resulting in a bias towards the null. This can result in a reduced effect estimate and therefore requires a larger sample size with adequate statistical power to detect any differences. However, in this study, validation was performed by a dermatologist in a small subset of 50 study participants which showed good sensitivity and specificity in the AD classification. Its prevalence and characteristics of the AD cases were also aligned with other local population studies [2, 3]. Severity of AD was based on self-reporting by the participants and could be subjected to recall bias and missing data. This has prevented more in-depth analysis on severity of AD. Majority of the AD participants were likely of milder severity and this might have resulted in a lack of a distinct AD skin microbiome diversity and serum inflammatory profile. However, this has presented an opportunity to investigate AD at its early stages within a normal to early disease cohort, which is important to understanding how obesity leads to risk of developing AD.

While there was comprehensive information on skin physiology measures to skin microbiome profile and serum inflammatory markers, there is a gap in correlating skin

surface molecules with underlying systemic inflammation and overlying microbiome composition. Although adiposity is associated with cytokine disturbances in blood, it is not known whether adiposity directly influences inflammation in skin. This however provides an angle for future studies.

10.7. Plans and directions for future studies

Various targets of interest such as difference in relative abundance of common commensals microbes and identification of certain inflammatory cytokines could be explored further with basic science studies. Specifically, the impact of skin commensals microbial species such as *Corynebacterium* species, *Malassezia globosa* and *Cutibacterium acnes* on skin barrier function and inflammation could be explored further. There has been evidence that *Corynebacterium* species could lead to specific changes in skin metabolites with subsequent IL-23 inflammation [234]. Further studies could examine if the presence of these microbial species which were previously thought to be innocent, might have specific inflammatory consequences on the skin.

Additionally, further omics analysis such as metabolomics analysis could be performed in the same case-control cohort to further integrate the current findings. Obesity is a systemic metabolic disease. The serum proteomic profile in this study has revealed interesting cytokines profile that might be linked to skin inflammation. A further evaluation of systemic metabolites will add information and allow further integrated analysis with proteomic and any future skin proteomic or metabolomics analysis to understand how obesity affects skin.

Finally, future studies to evaluate the microbial disturbances in obese individuals, and how this is linked to underlying disturbances in the molecular and physiological profile

of the skin could also be performed. With an expansion of the number of obese or extreme obese cases in the future analysis cohorts, certain microbial disturbances could be better accentuated.

11. Conclusion

In conclusion, this study presented observations that obesity is a causal factor in AD by a genetic association and Mendelian randomization study and suggested that abdominal obesity may also play a role in the risk of AD. Skin epidermal barrier function was also observed to be impaired in obese individuals. Skin microbiome and serum proteomic studies also revealed characteristics changes with increasing adiposity and appeared relevant to characteristic profile of AD.

Together, observations from this study supported the hypothesis that adiposity promotes skin epidermal barrier dysfunction through activation of inflammatory cytokine pathways, and these molecular processes may be linked to the development of abnormal skin microbial colonization and worsening of atopic dermatitis.

Further studies on skin surface inflammatory and metabolic changes in correlation with underlying systemic disturbances would address the missing link in the mechanistic pathway linking obesity and impaired epidermal barrier.

A better understanding of the relationship between obesity and AD helps to improve our knowledge of the overall pathogenesis in the development of AD. Such knowledge can be translated to specific interventions in managing individuals who are at risk of AD, in particular those who might be obese. This is especially important given that the current dermatology practice typically underestimates the importance of managing obesity in AD. Further clarifications of these two conditions will underpin the importance of addressing adiposity as part of dermatological management guidelines. Overall, this will transform healthcare delivery and reduce the burden of AD.

12. References

1. Tay, Y.K., B.P. Khoo, and C.L. Goh, *The profile of atopic dermatitis in a tertiary dermatology outpatient clinic in Singapore.* Int J Dermatol, 1999. **38**(9): p. 689-92.
2. Cheok, S., et al., *Prevalence and descriptive epidemiology of atopic dermatitis and its impact on quality of life in Singapore.* Br J Dermatol, 2018. **178**(1): p. 276-277.
3. Kok, W.L., Y.W. Yew, and T.G.S. Thng, *A population-based cohort study of atopic eczema among young adult males in Singapore.* Int J Dermatol, 2018. **57**(8): p. e52-e54.
4. Tay, Y.K., et al., *The prevalence and descriptive epidemiology of atopic dermatitis in Singapore school children.* Br J Dermatol, 2002. **146**(1): p. 101-6.
5. Watson, W. and S. Kapur, *Atopic dermatitis.* Allergy Asthma Clin Immunol, 2011. **7 Suppl 1**: p. S4.
6. Dainichi, T., et al., *The epithelial immune microenvironment (EIME) in atopic dermatitis and psoriasis.* Nat Immunol, 2018. **19**(12): p. 1286-1298.
7. Paller, A., et al., *Major Comorbidities of Atopic Dermatitis: Beyond Allergic Disorders.* Am J Clin Dermatol, 2018. **19**(6): p. 821-838.
8. Asher, M.I., et al., *Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys.* Lancet, 2006. **368**(9537): p. 733-43.
9. Ng, M., et al., *Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013.* Lancet, 2014. **384**(9945): p. 766-81.
10. Lee, J.H., et al., *Association Between Obesity, Abdominal Obesity, and Adiposity and the Prevalence of Atopic Dermatitis in Young Korean Adults: the Korea National Health and Nutrition Examination Survey 2008-2010.* Allergy Asthma Immunol Res, 2016. **8**(2): p. 107-14.
11. Zhang, A. and J.I. Silverberg, *Association of atopic dermatitis with being overweight and obese: a systematic review and metaanalysis.* J Am Acad Dermatol, 2015. **72**(4): p. 606-16 e4.
12. Nino, M., et al., *The effect of obesity on skin disease and epidermal permeability barrier status in children.* Pediatr Dermatol, 2012. **29**(5): p. 567-70.
13. Gregor, M.F. and G.S. Hotamisligil, *Inflammatory mechanisms in obesity.* Annu Rev Immunol, 2011. **29**: p. 415-45.
14. Hersoug, L.G. and A. Linneberg, *The link between the epidemics of obesity and allergic diseases: does obesity induce decreased immune tolerance?* Allergy, 2007. **62**(10): p. 1205-13.
15. Brandwein, M., et al., *Beyond the gut: Skin microbiome compositional changes are associated with BMI.* Human Microbiome Journal, 2019. **13**: p. 100063.
16. Bieber, T., *Atopic dermatitis.* Ann Dermatol, 2010. **22**(2): p. 125-37.
17. Williams, H.C., *Clinical practice. Atopic dermatitis.* N Engl J Med, 2005. **352**(22): p. 2314-24.
18. Mortz, C.G., et al., *Atopic dermatitis from adolescence to adulthood in the TOACS cohort: prevalence, persistence and comorbidities.* Allergy, 2015. **70**(7): p. 836-45.

19. Williams, H., et al., *Is eczema really on the increase worldwide?* J Allergy Clin Immunol, 2008. **121**(4): p. 947-54 e15.
20. Williams, H., et al., *Worldwide variations in the prevalence of symptoms of atopic eczema in the International Study of Asthma and Allergies in Childhood.* J Allergy Clin Immunol, 1999. **103**(1 Pt 1): p. 125-38.
21. Sugiura, H., et al., *Prevalence of childhood and adolescent atopic dermatitis in a Japanese population: comparison with the disease frequency examined 20 years ago.* Acta Derm Venereol, 1998. **78**(4): p. 293-4.
22. Strachan, D.P., *Hay fever, hygiene, and household size.* BMJ, 1989. **299**(6710): p. 1259-60.
23. Disease, G.B.D., I. Injury, and C. Prevalence, *Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017.* Lancet, 2018. **392**(10159): p. 1789-1858.
24. Olsson, M., et al., *The cost of childhood atopic dermatitis in a multi-ethnic Asian population: a cost-of-illness study.* Br J Dermatol, 2020. **182**(5): p. 1245-1252.
25. Adamson, A.S., *The Economics Burden of Atopic Dermatitis.* Adv Exp Med Biol, 2017. **1027**: p. 79-92.
26. Wise, F. and M. Sulzberger, *Year Book of Dermatology and Syphilology*. 1933.
27. Bieber, T., *How to Define Atopic Dermatitis?* Dermatol Clin, 2017. **35**(3): p. 275-281.
28. Johansson, S.G., et al., *Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003.* J Allergy Clin Immunol, 2004. **113**(5): p. 832-6.
29. Hanifin, J. and G. Rajka, *Diagnostic features of atopic eczema.* Acta Derm Venereol 1980. **92**(92): p. 44-47.
30. Schultz Larsen, F., T. Diepgen, and A. Svensson, *The occurrence of atopic dermatitis in north Europe: an international questionnaire study.* J Am Acad Dermatol, 1996. **34**(5 Pt 1): p. 760-4.
31. Williams, H.C., et al., *The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation.* Br J Dermatol, 1994. **131**(3): p. 406-16.
32. Schultz Larsen, F. and J.M. Hanifin, *Secular change in the occurrence of atopic dermatitis.* Acta Derm Venereol Suppl (Stockh), 1992. **176**: p. 7-12.
33. Kang, K.F. and R.M. Tian, *Criteria for atopic dermatitis in a Chinese population.* Acta Derm Venereol Suppl (Stockh), 1989. **144**: p. 26-7.
34. Tagami, H., *Japanese Dermatology Association criteria for the diagnosis of atopic dermatitis.* J Dermatol, 1995(22): p. 966-967.
35. Rawlings, A.V. and C.R. Harding, *Moisturization and skin barrier function.* Dermatol Ther, 2004. **17 Suppl 1**: p. 43-8.
36. Kezic, S., et al., *Loss-of-function mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum.* J Invest Dermatol, 2008. **128**(8): p. 2117-9.
37. O'Regan, G.M., et al., *Filaggrin in atopic dermatitis.* J Allergy Clin Immunol, 2008. **122**(4): p. 689-693.
38. Kay, J., et al., *The prevalence of childhood atopic eczema in a general population.* J Am Acad Dermatol, 1994. **30**(1): p. 35-9.
39. Sacotte, R. and J.I. Silverberg, *Epidemiology of adult atopic dermatitis.* Clin Dermatol, 2018. **36**(5): p. 595-605.

40. Lee, H.H., et al., *A systematic review and meta-analysis of the prevalence and phenotype of adult-onset atopic dermatitis.* J Am Acad Dermatol, 2019. **80**(6): p. 1526-1532 e7.
41. Hanifin, J.M., et al., *A population-based survey of eczema prevalence in the United States.* Dermatitis, 2007. **18**(2): p. 82-91.
42. Pesce, G., et al., *Adult eczema in Italy: prevalence and associations with environmental factors.* J Eur Acad Dermatol Venereol, 2015. **29**(6): p. 1180-7.
43. Williams, H.C., et al., *Validation of the U.K. diagnostic criteria for atopic dermatitis in a population setting. U.K. Diagnostic Criteria for Atopic Dermatitis Working Party.* Br J Dermatol, 1996. **135**(1): p. 12-7.
44. Williams, H.C., et al., *A protocol for recording the sign of flexural dermatitis in children.* Br J Dermatol, 1995. **133**(6): p. 941-9.
45. Gu, H., et al., *Evaluation of diagnostic criteria for atopic dermatitis: validity of the criteria of Williams et al. in a hospital-based setting.* Br J Dermatol, 2001. **145**(3): p. 428-33.
46. Popescu, C.M., et al., *Community validation of the United Kingdom diagnostic criteria for atopic dermatitis in Romanian schoolchildren.* Br J Dermatol, 1998. **138**(3): p. 436-42.
47. Brenninkmeijer, E.E., et al., *Diagnostic criteria for atopic dermatitis: a systematic review.* Br J Dermatol, 2008. **158**(4): p. 754-65.
48. Wang, L. and L.F. Li, *Professional Committee of Dermatology, Chinese Association of Integrative Medicine. Clinical Application of the UK Working Party's Criteria for the Diagnosis of Atopic Dermatitis in the Chinese Population by Age Group. .* Chin Med J (Engl), 2016. **23**(129): p. 2829-2833.
49. Firooz, A., et al., *Validation of the diagnostic criteria for atopic dermatitis.* Arch Dermatol, 1999. **135**(5): p. 514-6.
50. Hamada, M., et al., *Prevalence of atopic dermatitis and serum IgE values in nursery school children in Ishigaki Island, Okinawa, Japan.* J Dermatol, 2005. **32**(4): p. 248-55.
51. Saeki, H., et al., *Community validation of the U.K. diagnostic criteria for atopic dermatitis in Japanese elementary schoolchildren.* J Dermatol Sci, 2007. **47**(3): p. 227-31.
52. Chalmers, D.A., et al., *Validation of the U.K. Working Party diagnostic criteria for atopic eczema in a Xhosa-speaking African population.* Br J Dermatol, 2007. **156**(1): p. 111-6.
53. Heede, N.G., et al., *Health-related quality of life in adult dermatitis patients stratified by filaggrin genotype.* Contact Dermatitis, 2017. **76**(3): p. 167-177.
54. Thyssen, J.P., et al., *The association between null mutations in the filaggrin gene and contact sensitization to nickel and other chemicals in the general population.* Br J Dermatol, 2010. **162**(6): p. 1278-85.
55. Braae Olesen, A., et al., *Development and validation of a questionnaire for diagnosing atopic dermatitis.* Acta Derm Venereol, 2001. **81**(4): p. 277-81.
56. Andersen, Y.M.F., et al., *Poor agreement in questionnaire-based diagnostic criteria for adult atopic dermatitis is a challenge when examining cardiovascular comorbidity.* Allergy, 2018. **73**(4): p. 923-931.
57. Eichenfield, L.F., et al., *Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis.* J Am Acad Dermatol, 2014. **70**(2): p. 338-51.

58. Wanitphakdeedecha, R., et al., *Validation of the diagnostic criteria for atopic dermatitis in the adult Thai population.* Asian Pac J Allergy Immunol, 2007. **25**(2-3): p. 133-8.
59. Sharma, L., *Diagnostic clinical features of atopic dermatitis.* Indian J Dermatol Venereol Leprol, 2001. **67**(1): p. 25-7.
60. Liu, P., et al., *Clinical Features of Adult/Adolescent Atopic Dermatitis and Chinese Criteria for Atopic Dermatitis.* Chin Med J (Engl), 2016. **129**(7): p. 757-62.
61. Weidinger, S. and N. Novak, *Atopic dermatitis.* Lancet, 2016. **387**(10023): p. 1109-1122.
62. Bieber, T., et al., *Clinical phenotypes and endophenotypes of atopic dermatitis: Where are we, and where should we go?* J Allergy Clin Immunol, 2017. **139**(4S): p. S58-S64.
63. Tanei, R. and K. Katsuoka, *Clinical analyses of atopic dermatitis in the aged.* J Dermatol, 2008. **35**(9): p. 562-9.
64. Kaufman, B.P., E. Guttman-Yassky, and A.F. Alexis, *Atopic dermatitis in diverse racial and ethnic groups-Variations in epidemiology, genetics, clinical presentation and treatment.* Exp Dermatol, 2018. **27**(4): p. 340-357.
65. Mei-Yen Yong, A. and Y.K. Tay, *Atopic Dermatitis: Racial and Ethnic Differences.* Dermatol Clin, 2017. **35**(3): p. 395-402.
66. Nnoruka, E.N., *Current epidemiology of atopic dermatitis in south-eastern Nigeria.* Int J Dermatol, 2004. **43**(10): p. 739-44.
67. Vachiramon, V., et al., *Atopic dermatitis in African American children: addressing unmet needs of a common disease.* Pediatr Dermatol, 2012. **29**(4): p. 395-402.
68. Yew, Y.W., J.P. Thyssen, and J.I. Silverberg, *A systematic review and meta-analysis of the regional and age-related differences in atopic dermatitis clinical characteristics.* J Am Acad Dermatol, 2019. **80**(2): p. 390-401.
69. Noda, S., et al., *The Asian atopic dermatitis phenotype combines features of atopic dermatitis and psoriasis with increased TH17 polarization.* J Allergy Clin Immunol, 2015. **136**(5): p. 1254-64.
70. Guttman-Yassky, E. and J.G. Krueger, *Atopic dermatitis and psoriasis: two different immune diseases or one spectrum?* Curr Opin Immunol, 2017. **48**: p. 68-73.
71. Nomura, T., T. Honda, and K. Kabashima, *Multipolarity of cytokine axes in the pathogenesis of atopic dermatitis in terms of age, race, species, disease stage and biomarkers.* Int Immunol, 2018. **30**(9): p. 419-428.
72. Sanyal, R.D., et al., *Atopic dermatitis in African American patients is TH2/TH22-skewed with TH1/TH17 attenuation.* Ann Allergy Asthma Immunol, 2019. **122**(1): p. 99-110 e6.
73. Rerknimitr, P., et al., *The etiopathogenesis of atopic dermatitis: barrier disruption, immunological derangement, and pruritus.* Inflamm Regen, 2017. **37**: p. 14.
74. Larsen, F.S., N.V. Holm, and K. Henningsen, *Atopic dermatitis. A genetic-epidemiologic study in a population-based twin sample.* J Am Acad Dermatol, 1986. **15**(3): p. 487-94.
75. Schultz Larsen, F., *Atopic dermatitis: a genetic-epidemiologic study in a population-based twin sample.* J Am Acad Dermatol, 1993. **28**(5 Pt 1): p. 719-23.
76. Weidinger, S., et al., *Atopic dermatitis.* Nat Rev Dis Primers, 2018. **4**(1): p. 1.

77. Pellerin, L., et al., *Defects of filaggrin-like proteins in both lesional and nonlesional atopic skin.* J Allergy Clin Immunol, 2013. **131**(4): p. 1094-102.
78. Irvine, A.D., W.H. McLean, and D.Y. Leung, *Filaggrin mutations associated with skin and allergic diseases.* N Engl J Med, 2011. **365**(14): p. 1315-27.
79. Chen, H., et al., *Wide spectrum of filaggrin-null mutations in atopic dermatitis highlights differences between Singaporean Chinese and European populations.* Br J Dermatol, 2011. **165**(1): p. 106-14.
80. Weidinger, S., et al., *Filaggrin mutations, atopic eczema, hay fever, and asthma in children.* J Allergy Clin Immunol, 2008. **121**(5): p. 1203-1209 e1.
81. Winge, M.C., et al., *Novel filaggrin mutation but no other loss-of-function variants found in Ethiopian patients with atopic dermatitis.* Br J Dermatol, 2011. **165**(5): p. 1074-80.
82. Margolis, D.J., et al., *The persistence of atopic dermatitis and filaggrin (FLG) mutations in a US longitudinal cohort.* J Allergy Clin Immunol, 2012. **130**(4): p. 912-7.
83. Henderson, J., et al., *The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study.* J Allergy Clin Immunol, 2008. **121**(4): p. 872-7 e9.
84. Novak, N. and T. Bieber, *Allergic and nonallergic forms of atopic diseases.* J Allergy Clin Immunol, 2003. **112**(2): p. 252-62.
85. Margolis, D.J., et al., *Filaggrin-2 variation is associated with more persistent atopic dermatitis in African American subjects.* J Allergy Clin Immunol, 2014. **133**(3): p. 784-9.
86. Strasser, B., et al., *Evolutionary origin and diversification of epidermal barrier proteins in amniotes.* Mol Biol Evol, 2014. **31**(12): p. 3194-205.
87. Saunders, S.P., et al., *Tmem79/Matt is the matted mouse gene and is a predisposing gene for atopic dermatitis in human subjects.* J Allergy Clin Immunol, 2013. **132**(5): p. 1121-9.
88. Deraison, C., et al., *LEKTI fragments specifically inhibit KLK5, KLK7, and KLK14 and control desquamation through a pH-dependent interaction.* Mol Biol Cell, 2007. **18**(9): p. 3607-19.
89. Weidinger, S., et al., *A genome-wide association study of atopic dermatitis identifies loci with overlapping effects on asthma and psoriasis.* Hum Mol Genet, 2013. **22**(23): p. 4841-56.
90. Paternoster, L., et al., *Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis.* Nat Genet, 2011. **44**(2): p. 187-92.
91. Lan, C.C., et al., *Distinct SPINK5 and IL-31 polymorphisms are associated with atopic eczema and non-atopic hand dermatitis in Taiwanese nursing population.* Exp Dermatol, 2011. **20**(12): p. 975-9.
92. Namkung, J.H., et al., *Association of polymorphisms in genes encoding IL-4, IL-13 and their receptors with atopic dermatitis in a Korean population.* Exp Dermatol, 2011. **20**(11): p. 915-9.
93. Hussein, Y.M., et al., *Association between genes encoding components of the IL-4/IL-4 receptor pathway and dermatitis in children.* Gene, 2014. **545**(2): p. 276-81.
94. Lee, Y.L., et al., *Association of STAT6 genetic variants with childhood atopic dermatitis in Taiwanese population.* J Dermatol Sci, 2015. **79**(3): p. 222-8.

95. Gao, P.S., et al., *Genetic variants in thymic stromal lymphopoietin are associated with atopic dermatitis and eczema herpeticum.* J Allergy Clin Immunol, 2010. **125**(6): p. 1403-1407 e4.
96. Cole, C., et al., *Filaggrin-stratified transcriptomic analysis of pediatric skin identifies mechanistic pathways in patients with atopic dermatitis.* J Allergy Clin Immunol, 2014. **134**(1): p. 82-91.
97. Seltmann, J., et al., *IL-33 impacts on the skin barrier by downregulating the expression of filaggrin.* J Allergy Clin Immunol, 2015. **135**(6): p. 1659-61 e4.
98. Flohr, C., et al., *Filaggrin loss-of-function mutations are associated with early-onset eczema, eczema severity and transepidermal water loss at 3 months of age.* Br J Dermatol, 2010. **163**(6): p. 1333-6.
99. Danby, S.G., et al., *The Effect of Water Hardness on Surfactant Deposition after Washing and Subsequent Skin Irritation in Atopic Dermatitis Patients and Healthy Control Subjects.* J Invest Dermatol, 2018. **138**(1): p. 68-77.
100. Halling-Overgaard, A.S., et al., *Skin absorption through atopic dermatitis skin: a systematic review.* Br J Dermatol, 2017. **177**(1): p. 84-106.
101. Egawa, G. and K. Kabashima, *Multifactorial skin barrier deficiency and atopic dermatitis: Essential topics to prevent the atopic march.* J Allergy Clin Immunol, 2016. **138**(2): p. 350-358 e1.
102. Steinert, P.M., et al., *Characterization of a class of cationic proteins that specifically interact with intermediate filaments.* Proc Natl Acad Sci U S A, 1981. **78**(7): p. 4097-101.
103. Leyvraz, C., et al., *The epidermal barrier function is dependent on the serine protease CAP1/Prss8.* J Cell Biol, 2005. **170**(3): p. 487-96.
104. Hoste, E., et al., *Caspase-14 is required for filaggrin degradation to natural moisturizing factors in the skin.* J Invest Dermatol, 2011. **131**(11): p. 2233-41.
105. Kirschner, N., et al., *Tight junctions form a barrier in human epidermis.* Eur J Cell Biol, 2010. **89**(11): p. 839-42.
106. Brattsand, M., et al., *A proteolytic cascade of kallikreins in the stratum corneum.* J Invest Dermatol, 2005. **124**(1): p. 198-203.
107. Kezic, S., et al., *Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency.* J Allergy Clin Immunol, 2012. **129**(4): p. 1031-9 e1.
108. Briot, A., et al., *Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome.* J Exp Med, 2009. **206**(5): p. 1135-47.
109. Oyoshi, M.K., et al., *Mechanical injury polarizes skin dendritic cells to elicit a T(H)2 response by inducing cutaneous thymic stromal lymphopoietin expression.* J Allergy Clin Immunol, 2010. **126**(5): p. 976-84, 984 e1-5.
110. Yoon, J., et al., *IL-23 induced in keratinocytes by endogenous TLR4 ligands polarizes dendritic cells to drive IL-22 responses to skin immunization.* J Exp Med, 2016. **213**(10): p. 2147-66.
111. Onoue, A., et al., *Induction of eosinophil- and Th2-attracting epidermal chemokines and cutaneous late-phase reaction in tape-stripped skin.* Exp Dermatol, 2009. **18**(12): p. 1036-43.
112. Kim, J.E., et al., *Molecular Mechanisms of Cutaneous Inflammatory Disorder: Atopic Dermatitis.* Int J Mol Sci, 2016. **17**(8).

113. Fernandez, M.I., et al., *The human cytokine TSLP triggers a cell-autonomous dendritic cell migration in confined environments.* Blood, 2011. **118**(14): p. 3862-9.
114. Ebner, S., et al., *Thymic stromal lymphopoietin converts human epidermal Langerhans cells into antigen-presenting cells that induce proallergic T cells.* J Allergy Clin Immunol, 2007. **119**(4): p. 982-90.
115. Howell, M.D., et al., *Cytokine modulation of atopic dermatitis filaggrin skin expression.* J Allergy Clin Immunol, 2007. **120**(1): p. 150-5.
116. Kim, B.E., et al., *Loricrin and involucrin expression is down-regulated by Th2 cytokines through STAT-6.* Clin Immunol, 2008. **126**(3): p. 332-7.
117. Amano, W., et al., *The Janus kinase inhibitor JTE-052 improves skin barrier function through suppressing signal transducer and activator of transcription 3 signaling.* J Allergy Clin Immunol, 2015. **136**(3): p. 667-677 e7.
118. Gittler, J.K., et al., *Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis.* J Allergy Clin Immunol, 2012. **130**(6): p. 1344-54.
119. Toda, M., et al., *Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions.* J Allergy Clin Immunol, 2003. **111**(4): p. 875-81.
120. Koga, C., et al., *Possible pathogenic role of Th17 cells for atopic dermatitis.* J Invest Dermatol, 2008. **128**(11): p. 2625-2630.
121. Bautista, D.M., S.R. Wilson, and M.A. Hoon, *Why we scratch an itch: the molecules, cells and circuits of itch.* Nat Neurosci, 2014. **17**(2): p. 175-82.
122. Ishiuji, Y., et al., *Distinct patterns of brain activity evoked by histamine-induced itch reveal an association with itch intensity and disease severity in atopic dermatitis.* Br J Dermatol, 2009. **161**(5): p. 1072-80.
123. Tominaga, M., et al., *Psoralen-ultraviolet A therapy alters epidermal Sema3A and NGF levels and modulates epidermal innervation in atopic dermatitis.* J Dermatol Sci, 2009. **55**(1): p. 40-6.
124. Mollanazar, N.K., P.K. Smith, and G. Yosipovitch, *Mediators of Chronic Pruritus in Atopic Dermatitis: Getting the Itch Out?* Clin Rev Allergy Immunol, 2016. **51**(3): p. 263-292.
125. Wilson, S.R., et al., *The epithelial cell-derived atopic dermatitis cytokine TSLP activates neurons to induce itch.* Cell, 2013. **155**(2): p. 285-95.
126. Oetjen, L.K., et al., *Sensory Neurons Co-opt Classical Immune Signaling Pathways to Mediate Chronic Itch.* Cell, 2017. **171**(1): p. 217-228 e13.
127. Kato, A., et al., *Distribution of IL-31 and its receptor expressing cells in skin of atopic dermatitis.* J Dermatol Sci, 2014. **74**(3): p. 229-35.
128. Kong, H.H., et al., *Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis.* Genome Res, 2012. **22**(5): p. 850-9.
129. Jin, S., et al., *DAMP molecules S100A9 and S100A8 activated by IL-17A and house-dust mites are increased in atopic dermatitis.* Exp Dermatol, 2014. **23**(12): p. 938-41.
130. Glaser, R., et al., *The antimicrobial protein psoriasin (S100A7) is upregulated in atopic dermatitis and after experimental skin barrier disruption.* J Invest Dermatol, 2009. **129**(3): p. 641-9.
131. Islander, U., et al., *Superantigenic Staphylococcus aureus stimulates production of interleukin-17 from memory but not naive T cells.* Infect Immun, 2010. **78**(1): p. 381-6.

132. Hepburn, L., et al., *The complex biology and contribution of Staphylococcus aureus in atopic dermatitis, current and future therapies.* Br J Dermatol, 2017. **177**(1): p. 63-71.
133. Takeuchi, O. and S. Akira, *Pattern recognition receptors and inflammation.* Cell, 2010. **140**(6): p. 805-20.
134. Brunner, P.M., et al., *Increasing Comorbidities Suggest that Atopic Dermatitis Is a Systemic Disorder.* J Invest Dermatol, 2017. **137**(1): p. 18-25.
135. Schneider, L., et al., *Study of the Atopic March: Development of Atopic Comorbidities.* Pediatr Dermatol, 2016. **33**(4): p. 388-98.
136. Leung, D.Y., *New insights into atopic dermatitis: role of skin barrier and immune dysregulation.* Allergol Int, 2013. **62**(2): p. 151-61.
137. Kubo, A., K. Nagao, and M. Amagai, *Epidermal barrier dysfunction and cutaneous sensitization in atopic diseases.* J Clin Invest, 2012. **122**(2): p. 440-7.
138. Guttman-Yassky, E., K.E. Nograles, and J.G. Krueger, *Contrasting pathogenesis of atopic dermatitis and psoriasis--part I: clinical and pathologic concepts.* J Allergy Clin Immunol, 2011. **127**(5): p. 1110-8.
139. Beygi, S., V. Lajevardi, and R. Abedini, *C-reactive protein in psoriasis: a review of the literature.* J Eur Acad Dermatol Venereol, 2014. **28**(6): p. 700-11.
140. Leung, D.Y. and E. Guttman-Yassky, *Deciphering the complexities of atopic dermatitis: shifting paradigms in treatment approaches.* J Allergy Clin Immunol, 2014. **134**(4): p. 769-79.
141. Schmitt, J., et al., *Atopic dermatitis is associated with an increased risk for rheumatoid arthritis and inflammatory bowel disease, and a decreased risk for type 1 diabetes.* J Allergy Clin Immunol, 2016. **137**(1): p. 130-136.
142. Wu, L.C., et al., *Autoimmune disease comorbidities in patients with atopic dermatitis: a nationwide case-control study in Taiwan.* Pediatr Allergy Immunol, 2014. **25**(6): p. 586-92.
143. Legendre, L., et al., *Risk of lymphoma in patients with atopic dermatitis and the role of topical treatment: A systematic review and meta-analysis.* J Am Acad Dermatol, 2015. **72**(6): p. 992-1002.
144. Tennis, P., J.M. Gelfand, and K.J. Rothman, *Evaluation of cancer risk related to atopic dermatitis and use of topical calcineurin inhibitors.* Br J Dermatol, 2011. **165**(3): p. 465-73.
145. Chockalingam, R., C. Downing, and S.K. Tyring, *Cutaneous Squamous Cell Carcinomas in Organ Transplant Recipients.* J Clin Med, 2015. **4**(6): p. 1229-39.
146. Josephs, D.H., et al., *Epidemiological associations of allergy, IgE and cancer.* Clin Exp Allergy, 2013. **43**(10): p. 1110-23.
147. Linabery, A.M., et al., *The association between atopy and childhood/adolescent leukemia: a meta-analysis.* Am J Epidemiol, 2010. **171**(7): p. 749-64.
148. Deckert, S., C. Kopkow, and J. Schmitt, *Nonallergic comorbidities of atopic eczema: an overview of systematic reviews.* Allergy, 2014. **69**(1): p. 37-45.
149. Fu, S.L., et al., *Immunoglobulin E antibodies from pancreatic cancer patients mediate antibody-dependent cell-mediated cytotoxicity against pancreatic cancer cells.* Clin Exp Immunol, 2008. **153**(3): p. 401-9.
150. Chrostowska-Plak, D., A. Reich, and J.C. Szepietowski, *Relationship between itch and psychological status of patients with atopic dermatitis.* J Eur Acad Dermatol Venereol, 2013. **27**(2): p. e239-42.

151. Yarlagadda, A., E. Alfson, and A.H. Clayton, *The blood brain barrier and the role of cytokines in neuropsychiatry.* Psychiatry (Edgmont), 2009. **6**(11): p. 18-22.
152. Silverberg, J.I., *Association between adult atopic dermatitis, cardiovascular disease, and increased heart attacks in three population-based studies.* Allergy, 2015. **70**(10): p. 1300-8.
153. Hjuler, K.F., et al., *Increased Prevalence of Coronary Artery Disease in Severe Psoriasis and Severe Atopic Dermatitis.* Am J Med, 2015. **128**(12): p. 1325-34 e2.
154. Silverberg, J.I. and P. Greenland, *Eczema and cardiovascular risk factors in 2 US adult population studies.* J Allergy Clin Immunol, 2015. **135**(3): p. 721-8 e6.
155. Su, V.Y., et al., *Atopic dermatitis and risk of ischemic stroke: a nationwide population-based study.* Ann Med, 2014. **46**(2): p. 84-9.
156. Andersen, Y.M.F., et al., *Risk of myocardial infarction, ischemic stroke, and cardiovascular death in patients with atopic dermatitis.* J Allergy Clin Immunol, 2016. **138**(1): p. 310-312 e3.
157. Drucker, A.M., et al., *Atopic dermatitis is not independently associated with nonfatal myocardial infarction or stroke among US women.* Allergy, 2016. **71**(10): p. 1496-500.
158. Augustin, M., et al., *Epidemiology and Comorbidity in Children with Psoriasis and Atopic Eczema.* Dermatology, 2015. **231**(1): p. 35-40.
159. Radtke, M.A., et al., *Prevalence and comorbidities in adults with psoriasis compared to atopic eczema.* J Eur Acad Dermatol Venereol, 2017. **31**(1): p. 151-157.
160. Ronmark, E.P., et al., *Different risk factor patterns for adult asthma, rhinitis and eczema: results from West Sweden Asthma Study.* Clin Transl Allergy, 2016. **6**: p. 28.
161. Sybilski, A.J., et al., *Obesity--a risk factor for asthma, but not for atopic dermatitis, allergic rhinitis and sensitization.* Public Health Nutr, 2015. **18**(3): p. 530-6.
162. Silverberg, J.I., et al., *Central obesity and high blood pressure in pediatric patients with atopic dermatitis.* JAMA Dermatol, 2015. **151**(2): p. 144-52.
163. Silverberg, J.I., et al., *Association between obesity and atopic dermatitis in childhood: a case-control study.* J Allergy Clin Immunol, 2011. **127**(5): p. 1180-6 e1.
164. Mitchell, E.A., et al., *The association between BMI, vigorous physical activity and television viewing and the risk of symptoms of asthma, rhinoconjunctivitis and eczema in children and adolescents: ISAAC Phase Three.* Clin Exp Allergy, 2013. **43**(1): p. 73-84.
165. Silverberg, J.I. and E.L. Simpson, *Association between obesity and eczema prevalence, severity and poorer health in US adolescents.* Dermatitis, 2014. **25**(4): p. 172-81.
166. Kok, W.L., Y.W. Yew, and T.G. Thng, *Comorbidities Associated with Severity of Atopic Dermatitis in Young Adult Males: A National Cohort Study.* Acta Derm Venereol, 2019. **99**(7): p. 652-656.
167. *Global status report on noncommunicable diseases 2014.* 2014 [cited 2019 01 Aug]; Available from: http://apps.who.int/iris/bitstream/10665/148114/1/9789241564854_eng.pdf?ua=1.

168. Stevens, G.A., et al., *National, regional, and global trends in adult overweight and obesity prevalences.* Popul Health Metr, 2012. **10**(1): p. 22.
169. Workgroup., M.O. *HPB-MOH Clinical Practice Guidelines on Obesity 1/2016*. 2016 [cited 2017 15 Aug]; Available from: https://www.hpb.gov.sg/docs/default-source/pdf/obesity-cpg_main_for-online-30-aug.pdf?sfvrsn=2288eb72_0
170. Consultation, W.H.O.E., *Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies.* Lancet, 2004. **363**(9403): p. 157-63.
171. Shoelson, S.E., J. Lee, and A.B. Goldfine, *Inflammation and insulin resistance.* J Clin Invest, 2006. **116**(7): p. 1793-801.
172. Berg, A.H. and P.E. Scherer, *Adipose tissue, inflammation, and cardiovascular disease.* Circ Res, 2005. **96**(9): p. 939-49.
173. Solinas, G. and M. Karin, *JNK1 and IKKbeta: molecular links between obesity and metabolic dysfunction.* FASEB J, 2010. **24**(8): p. 2596-611.
174. Nakamura, T., et al., *Double-stranded RNA-dependent protein kinase links pathogen sensing with stress and metabolic homeostasis.* Cell, 2010. **140**(3): p. 338-48.
175. Song, M.J., et al., *Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes.* Biochem Biophys Res Commun, 2006. **346**(3): p. 739-45.
176. Harman-Boehm, I., et al., *Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity.* J Clin Endocrinol Metab, 2007. **92**(6): p. 2240-7.
177. Chen, Y., et al., *Variations in DNA elucidate molecular networks that cause disease.* Nature, 2008. **452**(7186): p. 429-35.
178. Danso, M.O., et al., *TNF-alpha and Th2 cytokines induce atopic dermatitis-like features on epidermal differentiation proteins and stratum corneum lipids in human skin equivalents.* J Invest Dermatol, 2014. **134**(7): p. 1941-1950.
179. Lam, Q.L. and L. Lu, *Role of leptin in immunity.* Cell Mol Immunol, 2007. **4**(1): p. 1-13.
180. Masaki, T., et al., *Adiponectin protects LPS-induced liver injury through modulation of TNF-alpha in KK-Ay obese mice.* Hepatology, 2004. **40**(1): p. 177-84.
181. Nagel, G., et al., *Associations of adipokines with asthma, rhinoconjunctivitis, and eczema in German schoolchildren.* Pediatr Allergy Immunol, 2009. **20**(1): p. 81-8.
182. Zeyda, M., et al., *Severe obesity increases adipose tissue expression of interleukin-33 and its receptor ST2, both predominantly detectable in endothelial cells of human adipose tissue.* Int J Obes (Lond), 2013. **37**(5): p. 658-65.
183. de Oliveira, M.F.A., A. Talvani, and E. Rocha-Vieira, *IL-33 in obesity: where do we go from here?* Inflamm Res, 2019. **68**(3): p. 185-194.
184. Murray, C.S., et al., *Body mass index in young children and allergic disease: gender differences in a longitudinal study.* Clin Exp Allergy, 2011. **41**(1): p. 78-85.
185. Ali, Z. and C.S. Ulrik, *Obesity and asthma: a coincidence or a causal relationship? A systematic review.* Respir Med, 2013. **107**(9): p. 1287-300.
186. Kopelman, P.G., *Hormones and obesity.* Baillieres Clin Endocrinol Metab, 1994. **8**(3): p. 549-75.

187. Shah, M.G. and H.I. Maibach, *Estrogen and skin. An overview.* Am J Clin Dermatol, 2001. **2**(3): p. 143-50.
188. Loffler, H., J.U. Aramaki, and I. Effendy, *The influence of body mass index on skin susceptibility to sodium lauryl sulphate.* Skin Res Technol, 2002. **8**(1): p. 19-22.
189. Guida, B., et al., *The impact of obesity on skin disease and epidermal permeability barrier status.* J Eur Acad Dermatol Venereol, 2010. **24**(2): p. 191-5.
190. Monteiro Rodrigues, L.M., et al., *Excessive Weight Favours Skin Physiology - Up to a Point: Another Expression of the Obesity Paradox.* Skin Pharmacol Physiol, 2017. **30**(2): p. 94-101.
191. Saadeh, D., et al., *High body mass index and allergies in schoolchildren: the French six cities study.* BMJ Open Respir Res, 2014. **1**(1): p. e000054.
192. Paller, A.S., et al., *The microbiome in patients with atopic dermatitis.* J Allergy Clin Immunol, 2019. **143**(1): p. 26-35.
193. Wolf, K.J. and R.G. Lorenz, *Gut Microbiota and Obesity.* Curr Obes Rep, 2012. **1**(1): p. 1-8.
194. Chng, K.R., et al., *Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare.* Nat Microbiol, 2016. **1**(9): p. 16106.
195. Edwards, R.K., et al., *Gravidas with class III obesity: evaluating the abdominal skin microbiota above and below the panniculus (.).* J Matern Fetal Neonatal Med, 2016. **29**(20): p. 3312-6.
196. Gilbert, J.A., et al., *Current understanding of the human microbiome.* Nat Med, 2018. **24**(4): p. 392-400.
197. Ley, R.E., et al., *Obesity alters gut microbial ecology.* Proc Natl Acad Sci U S A, 2005. **102**(31): p. 11070-5.
198. Sanmiguel, C., A. Gupta, and E.A. Mayer, *Gut Microbiome and Obesity: A Plausible Explanation for Obesity.* Curr Obes Rep, 2015. **4**(2): p. 250-61.
199. Turnbaugh, P.J., et al., *A core gut microbiome in obese and lean twins.* Nature, 2009. **457**(7228): p. 480-4.
200. Lee, S.Y., et al., *Microbiome in the Gut-Skin Axis in Atopic Dermatitis.* Allergy Asthma Immunol Res, 2018. **10**(4): p. 354-362.
201. Marrs, T. and C. Flohr, *The role of skin and gut microbiota in the development of atopic eczema.* Br J Dermatol, 2016. **175 Suppl 2**: p. 13-18.
202. Gensollen, T. and R.S. Blumberg, *Correlation between early-life regulation of the immune system by microbiota and allergy development.* J Allergy Clin Immunol, 2017. **139**(4): p. 1084-1091.
203. Belkaid, Y. and T.W. Hand, *Role of the microbiota in immunity and inflammation.* Cell, 2014. **157**(1): p. 121-41.
204. Furusawa, Y., et al., *Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells.* Nature, 2013. **504**(7480): p. 446-50.
205. Tanaka, M. and J. Nakayama, *Development of the gut microbiota in infancy and its impact on health in later life.* Allergol Int, 2017. **66**(4): p. 515-522.
206. Zuccotti, G., et al., *Probiotics for prevention of atopic diseases in infants: systematic review and meta-analysis.* Allergy, 2015. **70**(11): p. 1356-71.
207. Salem, I., et al., *The Gut Microbiome as a Major Regulator of the Gut-Skin Axis.* Front Microbiol, 2018. **9**: p. 1459.

208. Schwarz, A., A. Bruhs, and T. Schwarz, *The Short-Chain Fatty Acid Sodium Butyrate Functions as a Regulator of the Skin Immune System.* J Invest Dermatol, 2017. **137**(4): p. 855-864.
209. Tremblay, K., et al., *Association study between the CX3CR1 gene and asthma.* Genes Immun, 2006. **7**(8): p. 632-9.
210. Sirois-Gagnon, D., et al., *Association of common polymorphisms in the fractalkine receptor (CX3CR1) with obesity.* Obesity (Silver Spring), 2011. **19**(1): p. 222-7.
211. Hill, A.B., *The Environment and Disease: Association or Causation?* Proc R Soc Med, 1965. **58**: p. 295-300.
212. Haycock, P.C., et al., *Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies.* Am J Clin Nutr, 2016. **103**(4): p. 965-78.
213. Pierce, B.L. and S. Burgess, *Efficient design for Mendelian randomization studies: subsample and 2-sample instrumental variable estimators.* Am J Epidemiol, 2013. **178**(7): p. 1177-84.
214. Dehghan, M. and A.T. Merchant, *Is bioelectrical impedance accurate for use in large epidemiological studies?* Nutr J, 2008. **7**: p. 26.
215. Brownbill, R.A. and J.Z. Ilich, *Measuring body composition in overweight individuals by dual energy x-ray absorptiometry.* BMC Med Imaging, 2005. **5**(1): p. 1.
216. Micklesfield, L.K., et al., *Dual-energy X-ray performs as well as clinical computed tomography for the measurement of visceral fat.* Obesity (Silver Spring), 2012. **20**(5): p. 1109-14.
217. Neeland, I.J., et al., *Comparison of visceral fat mass measurement by dual-X-ray absorptiometry and magnetic resonance imaging in a multiethnic cohort: the Dallas Heart Study.* Nutr Diabetes, 2016. **6**(7): p. e221.
218. Ministry of Health, S. *National Health Survey 2010*. 2011 [cited 2017 15 Aug]; Available from: https://www.moh.gov.sg/resources-statistics/reports/national-health-survey-2010.
219. Pepe, M.S., et al., *Limitations of the odds ratio in gauging the performance of a diagnostic, prognostic, or screening marker.* Am J Epidemiol, 2004. **159**(9): p. 882-90.
220. Statistics, S.D.o. *Census of Population 2010*. 2011 [cited 2021 01 Apr]; Available from: https://www.singstat.gov.sg/-/media/files/publications/cop2010/census_2010_release1/cop2010sr1.pdf.
221. Board, S.H.P. *National Population Health Survey 2019*. 2019 [cited 2021 01 April]; Available from: https://www.hpb.gov.sg/docs/default-source/default-document-library/national-population-health-survey-2019.pdf.
222. Barbarot, S., et al., *Epidemiology of atopic dermatitis in adults: Results from an international survey.* Allergy, 2018. **73**(6): p. 1284-1293.
223. Czarnowicki, T., et al., *Atopic dermatitis endotypes and implications for targeted therapeutics.* J Allergy Clin Immunol, 2019. **143**(1): p. 1-11.
224. Price, G.M., et al., *Weight, shape, and mortality risk in older persons: elevated waist-hip ratio, not high body mass index, is associated with a greater risk of death.* Am J Clin Nutr, 2006. **84**(2): p. 449-60.
225. Morkedal, B., P.R. Romundstad, and L.J. Vatten, *Informativeness of indices of blood pressure, obesity and serum lipids in relation to ischaemic heart disease mortality: the HUNT-II study.* Eur J Epidemiol, 2011. **26**(6): p. 457-61.

226. Wardle, J., et al., *Stress and adiposity: a meta-analysis of longitudinal studies.* Obesity (Silver Spring), 2011. **19**(4): p. 771-8.
227. Yew, Y.W., et al., *Investigating causal relationships between Body Mass Index and risk of atopic dermatitis: a Mendelian randomization analysis.* Sci Rep, 2020. **10**(1): p. 15279.
228. Mori, S., et al., *Characterization of skin function associated with obesity and specific correlation to local/systemic parameters in American women.* Lipids Health Dis, 2017. **16**(1): p. 214.
229. Crewe, C., Y.A. An, and P.E. Scherer, *The ominous triad of adipose tissue dysfunction: inflammation, fibrosis, and impaired angiogenesis.* J Clin Invest, 2017. **127**(1): p. 74-82.
230. Byrd, A.L., et al., *Staphylococcus aureus and Staphylococcus epidermidis strain diversity underlying pediatric atopic dermatitis.* Sci Transl Med, 2017. **9**(397).
231. Shi, B., et al., *The skin microbiome is different in pediatric versus adult atopic dermatitis.* J Allergy Clin Immunol, 2016. **138**(4): p. 1233-1236.
232. Seite, S., et al., *Microbiome of affected and unaffected skin of patients with atopic dermatitis before and after emollient treatment.* J Drugs Dermatol, 2014. **13**(11): p. 1365-72.
233. Naik, S., et al., *Commensal-dendritic-cell interaction specifies a unique protective skin immune signature.* Nature, 2015. **520**(7545): p. 104-8.
234. Ridaura, V.K., et al., *Contextual control of skin immunity and inflammation by Corynebacterium.* J Exp Med, 2018. **215**(3): p. 785-799.
235. Moestrup, K.S., et al., *Dietary Control of Skin Lipid Composition and Microbiome.* J Invest Dermatol, 2018. **138**(5): p. 1225-1228.
236. Hill, M.O., *Diversity and Evenness: A Unifying Notation and Its Consequences.* Ecology, 1973. **54**(2): p. 427-432.
237. Kong, H.H., et al., *Performing Skin Microbiome Research: A Method to the Madness.* J Invest Dermatol, 2017. **137**(3): p. 561-568.
238. Kumar, R., et al., *Getting started with microbiome analysis: sample acquisition to bioinformatics.* Curr Protoc Hum Genet, 2014. **82**: p. 18 8 1-29.
239. Costello, E.K., et al., *Bacterial community variation in human body habitats across space and time.* Science, 2009. **326**(5960): p. 1694-7.
240. Grice, E.A., et al., *Topographical and temporal diversity of the human skin microbiome.* Science, 2009. **324**(5931): p. 1190-2.
241. Andrews, S. *FastQC: A Quality Control Tool for High Throughput Sequence Data [Online]*. 2010; Available from: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.
242. Bolger, A.M., M. Lohse, and B. Usadel, *Trimmomatic: a flexible trimmer for Illumina sequence data.* Bioinformatics, 2014. **30**(15): p. 2114-20.
243. Mclver, L., *Kneaddata software [Online].* 2015.
244. Truong, D.T., et al., *MetaPhlAn2 for enhanced metagenomic taxonomic profiling.* Nat Methods, 2015. **12**(10): p. 902-3.
245. McIntyre, A.B.R., et al., *Comprehensive benchmarking and ensemble approaches for metagenomic classifiers.* Genome Biol, 2017. **18**(1): p. 182.
246. Franzosa, E.A., et al., *Species-level functional profiling of metagenomes and metatranscriptomes.* Nat Methods, 2018. **15**(11): p. 962-968.
247. Caspi, R., et al., *The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of Pathway/Genome Databases.* Nucleic Acids Res, 2014. **42**(Database issue): p. D459-71.

248. Lande, R., *Statistics and Partitioning of Species Diversity, and Similarity among Multiple Communities.* Oikos, 1996. **76**(1): p. 5-13.
249. Greenacre, M.J. and R. Primicerio, *Multivariate analysis of ecological data Chapter 5.*, in *Multivariate analysis of ecological data*, B.F.n. BBVA., Editor. 2013. p. 1-10.
250. Segata, N., et al., *Metagenomic biomarker discovery and explanation.* Genome Biol, 2011. **12**(6): p. R60.
251. Cole, J.R., et al., *The Ribosomal Database Project: improved alignments and new tools for rRNA analysis.* Nucleic Acids Res, 2009. **37**(Database issue): p. D141-5.
252. Otto, M., *Staphylococcus colonization of the skin and antimicrobial peptides.* Expert Rev Dermatol, 2010. **5**(2): p. 183-195.
253. Stacy, A. and Y. Belkaid, *Microbial guardians of skin health.* Science, 2019. **363**(6424): p. 227-228.
254. Dong, Y., C.P. Speer, and K. Glaser, *Beyond sepsis: Staphylococcus epidermidis is an underestimated but significant contributor to neonatal morbidity.* Virulence, 2018. **9**(1): p. 621-633.
255. Uckay, I., et al., *Foreign body infections due to Staphylococcus epidermidis.* Ann Med, 2009. **41**(2): p. 109-19.
256. Hon, K.L., et al., *Exploring Staphylococcus epidermidis in atopic eczema: friend or foe?* Clin Exp Dermatol, 2016. **41**(6): p. 659-63.
257. Cau, L., et al., *Staphylococcus epidermidis protease EcpA can be a deleterious component of the skin microbiome in atopic dermatitis.* J Allergy Clin Immunol, 2020.
258. Sparber, F., et al., *The Skin Commensal Yeast Malassezia Triggers a Type 17 Response that Coordinates Anti-fungal Immunity and Exacerbates Skin Inflammation.* Cell Host Microbe, 2019. **25**(3): p. 389-403 e6.
259. Nowicka, D. and U. Nawrot, *Contribution of Malassezia spp. to the development of atopic dermatitis.* Mycoses, 2019. **62**(7): p. 588-596.
260. Li, H., et al., *Skin Commensal Malassezia globosa Secreted Protease Attenuates Staphylococcus aureus Biofilm Formation.* J Invest Dermatol, 2018. **138**(5): p. 1137-1145.
261. Barnard, E. and H. Li, *Shaping of cutaneous function by encounters with commensals.* J Physiol, 2017. **595**(2): p. 437-450.
262. Williams, M.R., et al., *Quorum sensing between bacterial species on the skin protects against epidermal injury in atopic dermatitis.* Sci Transl Med, 2019. **11**(490).
263. Shu, M., et al., *Fermentation of Propionibacterium acnes, a commensal bacterium in the human skin microbiome, as skin probiotics against methicillin-resistant Staphylococcus aureus.* PLoS One, 2013. **8**(2): p. e55380.
264. McLaughlin, J., et al., *Propionibacterium acnes and Acne Vulgaris: New Insights from the Integration of Population Genetic, Multi-Omic, Biochemical and Host-Microbe Studies.* Microorganisms, 2019. **7**(5).
265. Monirujjaman, M. and A. Ferdouse, *Metabolic and Physiological Roles of Branched-Chain Amino Acids.* Advances in Molecular Biology, 2014. **2014**: p. 1-6.
266. Thijs, J.L., et al., *Serum biomarker profiles suggest that atopic dermatitis is a systemic disease.* J Allergy Clin Immunol, 2018. **141**(4): p. 1523-1526.

267. Lundberg, M., et al., *Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood.* Nucleic Acids Res, 2011. **39**(15): p. e102.
268. He, H., et al., *Increased cardiovascular and atherosclerosis markers in blood of older patients with atopic dermatitis.* Ann Allergy Asthma Immunol, 2020. **124**(1): p. 70-78.
269. Brunner, P.M., et al., *The atopic dermatitis blood signature is characterized by increases in inflammatory and cardiovascular risk proteins.* Sci Rep, 2017. **7**(1): p. 8707.
270. He, H., et al., *Mild atopic dermatitis lacks systemic inflammation and shows reduced nonlesional skin abnormalities.* J Allergy Clin Immunol, 2020.
271. Assarsson, E., et al., *Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability.* PLoS One, 2014. **9**(4): p. e95192.
272. Brereton, R.G. and G.R. Lloyd, *Partial least squares discriminant analysis: taking the magic away.* Journal of Chemometrics, 2014. **28**(4): p. 213-225.
273. Chong, I.-G. and C.-H. Jun, *Performance of some variable selection methods when multicollinearity is present.* Chemometrics and Intelligent Laboratory Systems, 2005. **78**(1-2): p. 103-112.
274. Homey, B., et al., *Cytokines and chemokines orchestrate atopic skin inflammation.* J Allergy Clin Immunol, 2006. **118**(1): p. 178-89.
275. Konishi, H., et al., *IL-18 contributes to the spontaneous development of atopic dermatitis-like inflammatory skin lesion independently of IgE/stat6 under specific pathogen-free conditions.* Proc Natl Acad Sci U S A, 2002. **99**(17): p. 11340-5.
276. Brunner, P.M., et al., *The blood proteomic signature of early-onset pediatric atopic dermatitis shows systemic inflammation and is distinct from adult long-standing disease.* J Am Acad Dermatol, 2019. **81**(2): p. 510-519.
277. Youssef, D.M., et al., *The influence of leptin on Th1/Th2 balance in obese children with asthma.* J Bras Pneumol, 2013. **39**(5): p. 562-8.
278. Brito Díaz, B., et al., *An Overview of Leptin and the Th1/Th2 Balance.* Open Journal of Immunology, 2014. **04**(02): p. 42-50.
279. Visness, C.M., et al., *Association of obesity with IgE levels and allergy symptoms in children and adolescents: results from the National Health and Nutrition Examination Survey 2005-2006.* J Allergy Clin Immunol, 2009. **123**(5): p. 1163-9, 1169 e1-4.
280. Fitzpatrick, S., R. Joks, and J.I. Silverberg, *Obesity is associated with increased asthma severity and exacerbations, and increased serum immunoglobulin E in inner-city adults.* Clin Exp Allergy, 2012. **42**(5): p. 747-59.
281. Garozzo, M.T., et al., *RELATION OF BODY MASS INDEX WITH IgE LEVELS, ALLERGIC SENSITIZATION AND LUNG FUNCTION IN ASTHMATIC CHILDREN: OUR EXPERIENCE AND REVISION OF LITERATURE.* J Biol Regul Homeost Agents, 2015. **29**(2 Suppl 1): p. 89-95.
282. Guttman-Yassky, E., J.G. Krueger, and M.G. Lebwohl, *Systemic immune mechanisms in atopic dermatitis and psoriasis with implications for treatment.* Exp Dermatol, 2018. **27**(4): p. 409-417.
283. Vanbervliet, B., et al., *Sequential involvement of CCR2 and CCR6 ligands for immature dendritic cell recruitment: possible role at inflamed epithelial surfaces.* Eur J Immunol, 2002. **32**(1): p. 231-42.

284. Nakayama, T., et al., *Inducible expression of a CC chemokine liver- and activation-regulated chemokine (LARC)/macrophage inflammatory protein (MIP)-3 alpha/CCL20 by epidermal keratinocytes and its role in atopic dermatitis.* Int Immunol, 2001. **13**(1): p. 95-103.
285. Hijnen, D., et al., *Serum thymus and activation-regulated chemokine (TARC) and cutaneous T cell- attracting chemokine (CTACK) levels in allergic diseases: TARC and CTACK are disease-specific markers for atopic dermatitis.* J Allergy Clin Immunol, 2004. **113**(2): p. 334-40.
286. Kakinuma, T., et al., *Thymus and activation-regulated chemokine in atopic dermatitis: Serum thymus and activation-regulated chemokine level is closely related with disease activity.* J Allergy Clin Immunol, 2001. **107**(3): p. 535-41.
287. Novak, N., et al., *Single nucleotide polymorphisms of the IL18 gene are associated with atopic eczema.* J Allergy Clin Immunol, 2005. **115**(4): p. 828-33.
288. Violante, R., et al., *Obesity risk factors in the ISAAC (International Study of Asthma and Allergies in Childhood) in Mexico City.* Rev Alerg Mex, 2005. **52**(4): p. 141-5.
289. Sidoroff, V., et al., *Overweight does not increase asthma risk but may decrease allergy risk at school age after infantile bronchiolitis.* Acta Paediatr, 2012. **101**(1): p. 43-7.
290. Smith, G.D. and S. Ebrahim, *'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease?* Int J Epidemiol, 2003. **32**(1): p. 1-22.
291. Lawlor, D.A., et al., *Mendelian randomization: using genes as instruments for making causal inferences in epidemiology.* Stat Med, 2008. **27**(8): p. 1133-63.
292. Davies, N.M., M.V. Holmes, and G. Davey Smith, *Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians.* BMJ, 2018. **362**: p. k601.
293. Yengo, L., et al., *Meta-analysis of genome-wide association studies for height and body mass index in approximately 700000 individuals of European ancestry.* Hum Mol Genet, 2018. **27**(20): p. 3641-3649.
294. Paternoster, L., et al., *Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis.* Nat Genet, 2015. **47**(12): p. 1449-1456.
295. Hartwig, F.P., et al., *Two-sample Mendelian randomization: avoiding the downsides of a powerful, widely applicable but potentially fallible technique.* Int J Epidemiol, 2016. **45**(6): p. 1717-1726.
296. Thomas, D.C., D.A. Lawlor, and J.R. Thompson, *Re: Estimation of bias in nongenetic observational studies using "Mendelian triangulation" by Bautista et al.* Ann Epidemiol, 2007. **17**(7): p. 511-3.
297. Didelez, V. and N. Sheehan, *Mendelian randomization as an instrumental variable approach to causal inference.* Stat Methods Med Res, 2007. **16**(4): p. 309-30.
298. Burgess, S., A. Butterworth, and S.G. Thompson, *Mendelian randomization analysis with multiple genetic variants using summarized data.* Genet Epidemiol, 2013. **37**(7): p. 658-65.
299. Hemani, G., et al., *The MR-Base platform supports systematic causal inference across the human phenome.* Elife, 2018. **7**.

300. Bowden, J., et al., *Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator.* Genet Epidemiol, 2016. **40**(4): p. 304-14.
301. Bowden, J., G. Davey Smith, and S. Burgess, *Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression.* Int J Epidemiol, 2015. **44**(2): p. 512-25.
302. Staley, J.R., et al., *PhenoScanner: a database of human genotype-phenotype associations.* Bioinformatics, 2016. **32**(20): p. 3207-3209.
303. Kamat, M.A., et al., *PhenoScanner V2: an expanded tool for searching human genotype-phenotype associations.* Bioinformatics, 2019. **35**(22): p. 4851-4853.
304. Shaw, T.E., et al., *Eczema prevalence in the United States: data from the 2003 National Survey of Children's Health.* J Invest Dermatol, 2011. **131**(1): p. 67-73.
305. Kantor, R., et al., *Association of atopic dermatitis with smoking: A systematic review and meta-analysis.* J Am Acad Dermatol, 2016. **75**(6): p. 1119-1125 e1.
306. Halling-Overgaard, A.S., et al., *Atopic dermatitis and alcohol use - a meta-analysis and systematic review.* J Eur Acad Dermatol Venereol, 2018. **32**(8): p. 1238-1245.
307. Silverberg, J.I., et al., *Atopic Dermatitis Is Associated with Less Physical Activity in US Adults.* J Invest Dermatol, 2016. **136**(8): p. 1714-1716.
308. Ronnstad, A.T.M., et al., *Association of atopic dermatitis with depression, anxiety, and suicidal ideation in children and adults: A systematic review and meta-analysis.* J Am Acad Dermatol, 2018. **79**(3): p. 448-456 e30.
309. Pulit, S.L., et al., *Meta-analysis of genome-wide association studies for body fat distribution in 694 649 individuals of European ancestry.* Hum Mol Genet, 2019. **28**(1): p. 166-174.
310. Bowden, J., et al., *A framework for the investigation of pleiotropy in two-sample summary data Mendelian randomization.* Stat Med, 2017. **36**(11): p. 1783-1802.
311. Bowden, J., et al., *Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic.* Int J Epidemiol, 2016. **45**(6): p. 1961-1974.
312. Burgess, S. and S.G. Thompson, *Interpreting findings from Mendelian randomization using the MR-Egger method.* Eur J Epidemiol, 2017. **32**(5): p. 377-389.
313. O'Donovan, S.M., et al., *Neonatal adiposity increases the risk of atopic dermatitis during the first year of life.* J Allergy Clin Immunol, 2016. **137**(1): p. 108-117.
314. Lei, Y., H. Yang, and L. Zhen, *Obesity is a risk factor for allergic rhinitis in children of Wuhan (China).* Asia Pac Allergy, 2016. **6**(2): p. 101-4.
315. Ali, Z., et al., *Is atopic dermatitis associated with obesity? A systematic review of observational studies.* J Eur Acad Dermatol Venereol, 2018. **32**(8): p. 1246-1255.
316. Budu-Aggrey, A., et al., *Assessment of a causal relationship between body mass index and atopic dermatitis.* J Allergy Clin Immunol, 2021. **147**(1): p. 400-403.
317. Jiang, D., et al., *Microbiome Multi-Omics Network Analysis: Statistical Considerations, Limitations, and Opportunities.* Front Genet, 2019. **10**: p. 995.
318. Rathcke, C.N., J.S. Johansen, and H. Vestergaard, *YKL-40, a biomarker of inflammation, is elevated in patients with type 2 diabetes and is related to insulin resistance.* Inflamm Res, 2006. **55**(2): p. 53-9.

319. Johansen, J.S., et al., *Serum YKL-40 concentrations in patients with rheumatoid arthritis: relation to disease activity.* Rheumatology (Oxford), 1999. **38**(7): p. 618-26.
320. Chupp, G.L., et al., *A chitinase-like protein in the lung and circulation of patients with severe asthma.* N Engl J Med, 2007. **357**(20): p. 2016-27.
321. Kim, D.H., et al., *Regulation of chitinase-3-like-1 in T cell elicits Th1 and cytotoxic responses to inhibit lung metastasis.* Nat Commun, 2018. **9**(1): p. 503.
322. Lee, C.G., et al., *Role of breast regression protein 39 (BRP-39)/chitinase 3-like-1 in Th2 and IL-13-induced tissue responses and apoptosis.* J Exp Med, 2009. **206**(5): p. 1149-66.
323. Ahangari, F., et al., *Chitinase 3-like-1 regulates both visceral fat accumulation and asthma-like Th2 inflammation.* Am J Respir Crit Care Med, 2015. **191**(7): p. 746-57.
324. Gloor, G.B. and G. Reid, *Compositional analysis: a valid approach to analyze microbiome high-throughput sequencing data.* Can J Microbiol, 2016. **62**(8): p. 692-703.

Annex A: Supplementary Tables and Figures

Characteristics	HELIOS (n=5560)	NPHS 2019 (n=6254)	Census 2010* (n=2304492)	p value**
Age (years, mean)	50.4	18-79	30-84	
Gender (%)				
Female	59.7	54.8	51.1	<0.001
Ethnic Groups (%)				
Chinese	75.2	74.1	76.9	0.488
Malay	8.5	12.6	11.2	<0.001
Indian	15.2	10.4	8.6	<0.001
Others	1.2	2.9	3.3	<0.001
Education level (%)				
Primary School or no education	5.3		37.0	<0.001
O levels or ITE	23.7		19.9	<0.001
A levels or diploma	24.2		21.4	<0.001
Undergraduate/Graduate	45.9		21.5	<0.001
Household income ($)/month (%)				
<2000	13.1		11.9	0.0058
2000 - 3999	16.0		18.6	<0.001
4000 - 5999	19.3		16.4	<0.001
6000 - 9999	23.9		20.9	<0.001
>= 10000	27.7		21.9	<0.001
Alcohol Consumption (%)	20.3	10.9		<0.001ǂ
Current smokers (%)	8.1	10.6		<0.001 ǂ

* Singapore Census 2010 adults aged 30 to 84 years

** based on 2 sample z test

ǂ comparison between HELIOS and NPHS (National Population Health Survey 2019)

Table S.1. Comparison of characteristics of study participants with other national cohorts

Characteristics	Moderate/Severe AD (n=109)	Mild AD (n=121)	Total (n=230)	P value
Age (years, mean)	48.9	51.5	51.2	***<0.001****
Gender (%)				
Female	66.1	62.0	63.9	0.521
Ethnic Groups (n, %)				0.528
Chinese	77.1	83.5	80.4	
Malay	5.5	5.8	5.7	
Indian	16.5	9.9	13.0	
Others	0.9	0.8	0.9	
Education level (n, %)				***0.041****
Primary School or no education	2.8	0.8	1.7	
O levels or ITE	23.9	11.6	23.7	
A levels or diploma	21.1	20.7	20.9	
Undergraduate/Graduate	52.3	66.9	45.9	
Household income ($)/month (n, %)				0.377
<2000	14.7	8.3	11.1	
2000 - 3999	20.0	13.9	13.5	
4000 - 5999	10.5	13.9	16.3	
6000 - 9999	22.1	24.1	20.3	
>= 10000	32.6	39.8	23.5	
Alcohol Consumption (%)	22.9	21.5	22.2	0.792
Current smokers (%)	13.8	8.3	10.9	0.181
Asthma (%)	20.2	28.1	24.3	0.163
Allergic Rhinitis (%)	11.9	24.8	18.7	***0.012****
Atopy (%)	26.6	46.3	8.9	***0.002****
Diabetes Mellitus (%)	7.3	9.1	8.3	0.630
Hypertension(%)	18.3	17.4	17.8	0.844
Hyperlipidemia(%)	32.1	26.1	28.9	0.320

ITE: Institute of Technical Education

Table S.2. Characteristics of study participants with mild and moderate to severe AD

	Relative odds of moderate to severe vs mild AD with obesity						
	Model 1			Model 2		Model 3	
	OR	95% CI	P value	OR	P value	OR	P value
Obesity (BMI ≥ 30.0)	1.40	0.52-3.18	0.418	1.41	0.456	1.43	0.434
BMI			**0.043***		0.056		0.059
Lean	1.00			1.00		1.00	
Overweight	2.15	1.15-4.02		2.27		2.25	
Obese	1.89	0.80-4.46		1.90		1.93	
BMI (kg/m^2)	1.03	0.97-1.10	0.293	1.01	0.463	1.03	0.401
Waist-Hip ratio (WHR)	39.63	0.74-2114.87	0.070	137.73	***0.032****	96.25	0.051
Obesity by WHR Female ≥0.85, Male ≥0.90	1.24	1.01-1.51	0.251	1.65	0.127	1.65	0.130
Percentage body fat (%)- Bioimpedance	1.00	0.96-1.05	0.849	1.01	0.676	1.01	0.641
Total Fat Percentage (%)- DXA	1.00	0.95-1.06	0.901	1.00	0.907	1.00	0.899
Total Fat Mass (kg)-DXA	1.01	0.98-1.05	0.482	1.02	0.404	1.02	0.436
Visceral Fat Mass (kg)-DXA	1.43	0.52-3.98	0.483	1.77	0.321	1.72	0.353

Model 1: adjusted for age, gender, ethnicity
Model 2: adjusted for Model 1 + education level, household income
Model 3: adjusted for Model 2 + current smoking, drinking alcohol

Table S.3. Relationship of AD severity (Moderate/Severe vs Mild) with different measures of obesity in various logistic regression models.

	Log (TEWL) (n=5442)		Log (Moisture) (n=5424)		Log (PH) (n=5113)	
	Beta	P value	Beta	P value	Beta	P value
BMI						
Lean						
Overweight	0.001	0.872	-0.021	0.057	-0.008	**0.013**
Obese	0.043	***0.001***	-0.066	***<0.001***	-0.009	0.054
BMI (kg/m^2)	0.002	***0.006***	-0.005	***<0.001***	-0.001	***0.001***
Waist-Hip ratio (WHR)	0.157	***0.008***	-0.347	***<0.001***	-0.049	**0.017**
High WHR (Male ≥ 0.90) (Female ≥ 0.80)	0.019	***0.023***	-0.037	***<0.001***	-0.006	**0.049**
Total Fat Mass (kg)	0.000	0.597	-0.004	***<0.001***	-0.000	0.220
Visceral Fat Mass (kg)	-0.004	0.798	-0.108	***<0.001***	-0.011	0.056

*Adjusted for age, gender and ethnicity

Extreme outliers defined as data values outside 3rd quartile + 3 times interquartile range and 1st quartile – 3 times interquartile range

Table S.4. Sensitivity Analysis on relationship of various obesity measures on skin barrier function (removing extreme outliers).

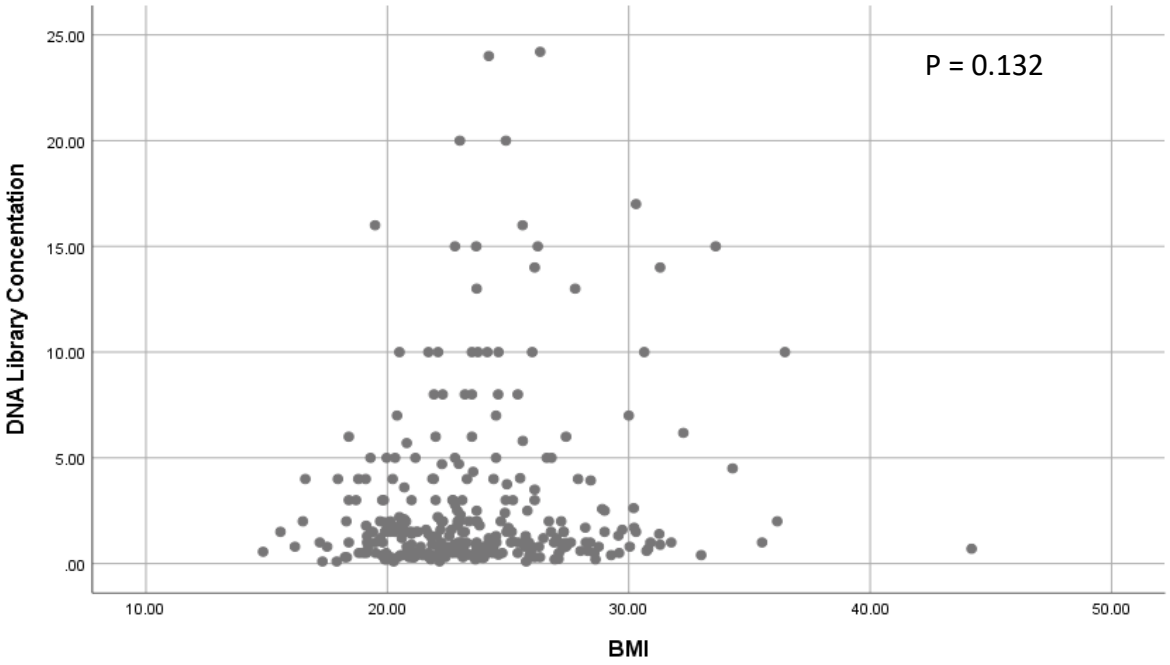


Figure S.1. Relationship of Skin tapes DNA library concentration with body mass index(BMI)

Scatterplot of skin tapes DNA library concentration with increasing body mass index (BMI). P value was 0.132 after adjusting for age and gender. Each data point represents an individual participant sample.

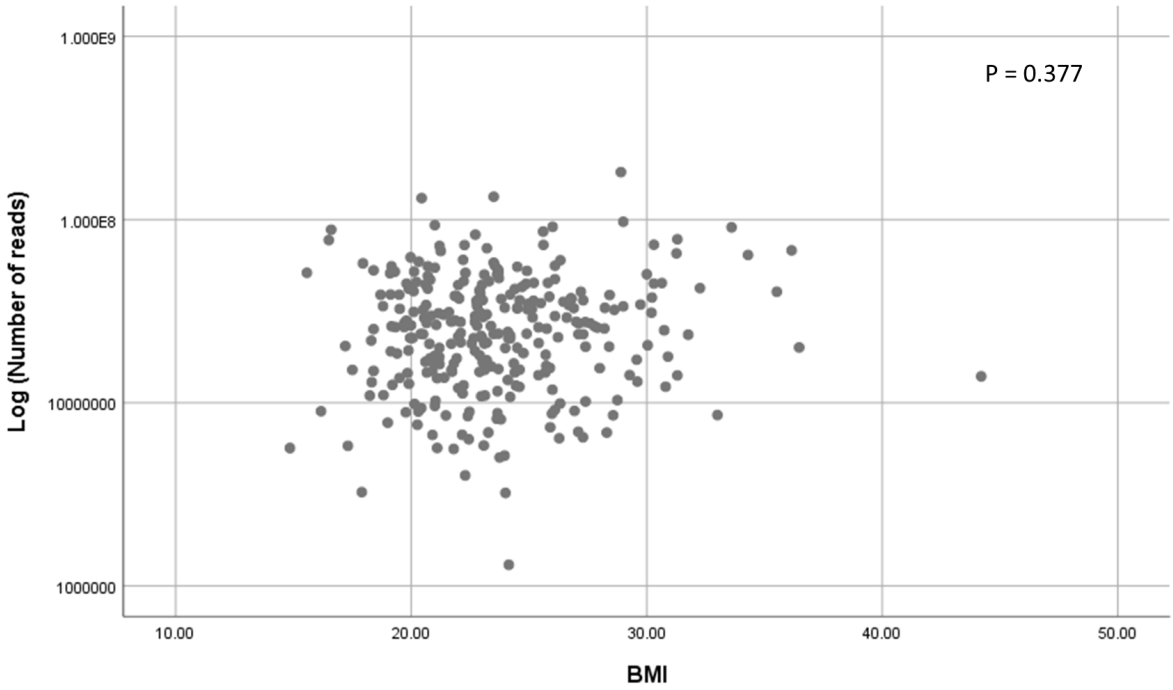


Figure S.2. Relationship of number of reads with body mass index(BMI)

Scatterplot of log(number of reads) with increasing body mass index (BMI). P value was 0.377, after adjusting for age and gender. Each data point represents an individual participant sample.

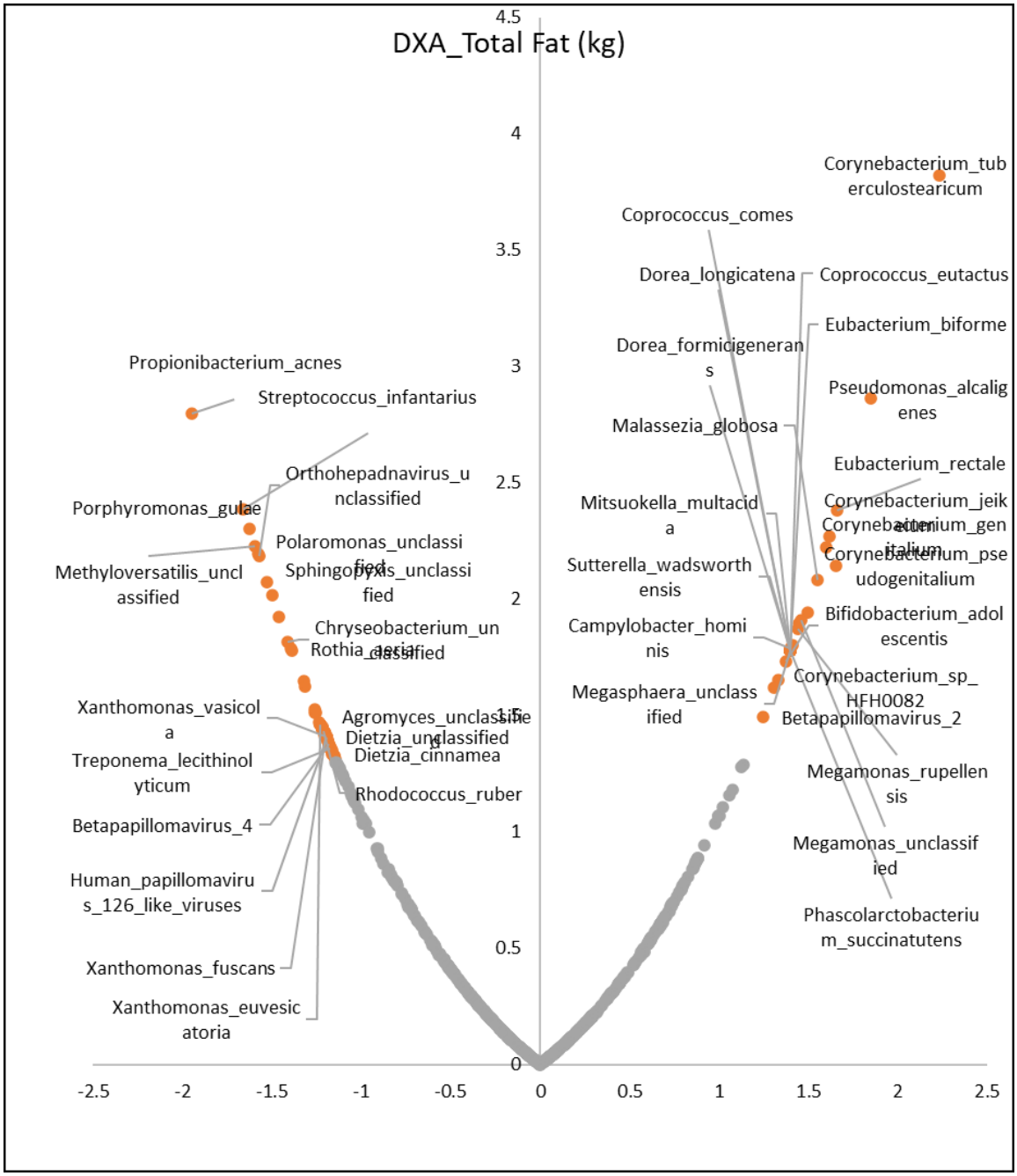


Figure S.3. Volcano plot of Total Fat (DXA, kg) against skin microbiome species.

Standardized β coefficients and p values from multiple linear regression analyses of all identified microbiome species with increasing total fat (DXA, kg) adjusted by age and, gender) are plotted as x-axis and y axis of the volcano plots. Data points in blue were significant after adjusting for multiple testing by B-H procedure. Data points in orange have p value <0.05 but not significant after adjusting for multiple testing.

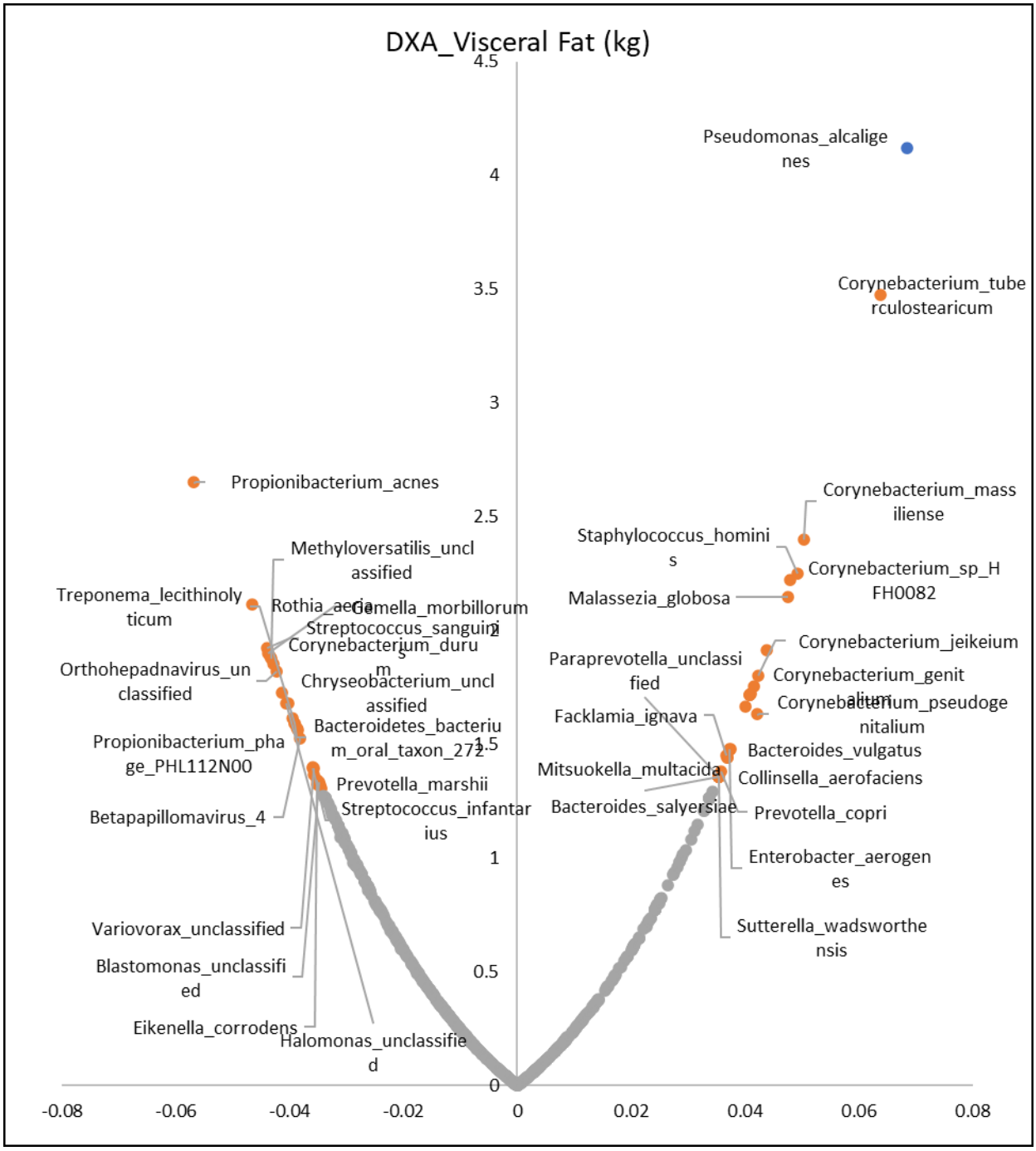


Figure S.4. Volcano plot of Visceral Fat (DXA, kg) against skin microbiome species.

Standardized β coefficients and p values from multiple linear regression analyses of all identified microbiome species with increasing visceral fat (DXA, kg) adjusted by age and, gender) are plotted as x-axis and y axis of the volcano plots. Data points in blue were significant after adjusting for multiple testing by B-H procedure. Data points in orange have p value <0.05 but not significant after adjusting for multiple testing.

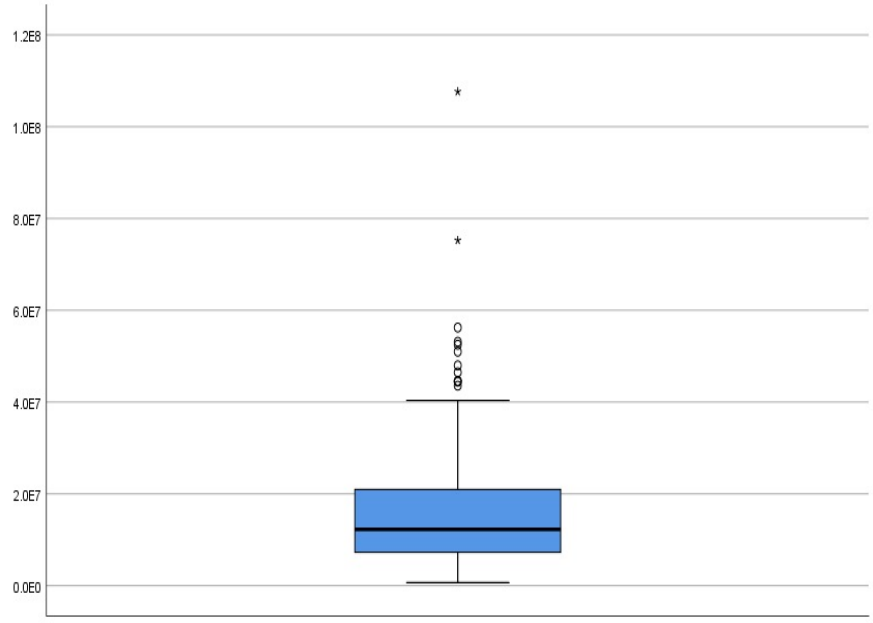


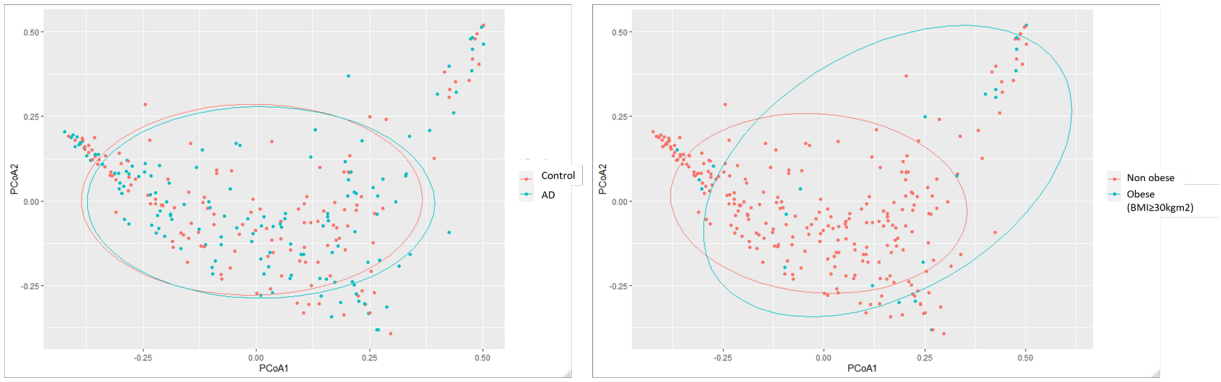
Figure S.5. Boxplot illustrate the distribution of the pre-mapping reads for the 294 samples

Boxplot illustrate the distribution of the reads for the 294 samples and as noted, there were 12 samples which were outliers. Outliers defined as samples with reads greater than 3 times interquartile range above 75th percentile.

AD	Estimate	P value
Shannon	0.044	0.746
Inverse Simpson	3.74	0.283
Species	-0.002	0.517
Evenness	0.637	0.329

Obese (BMI)	Estimate	P value
Shannon	-0.312	0.237
Inverse Simpson	3.48	0.567
Species	-0.007	0.253
Evenness	-1.41	0.228

Alpha diversity measures



Beta diversity measures

FigureS.6. Alpha and Beta diversity measures of AD versus Controls and Obese vs non-Obese after removing 12 outliers in terms of pre-mapping reads

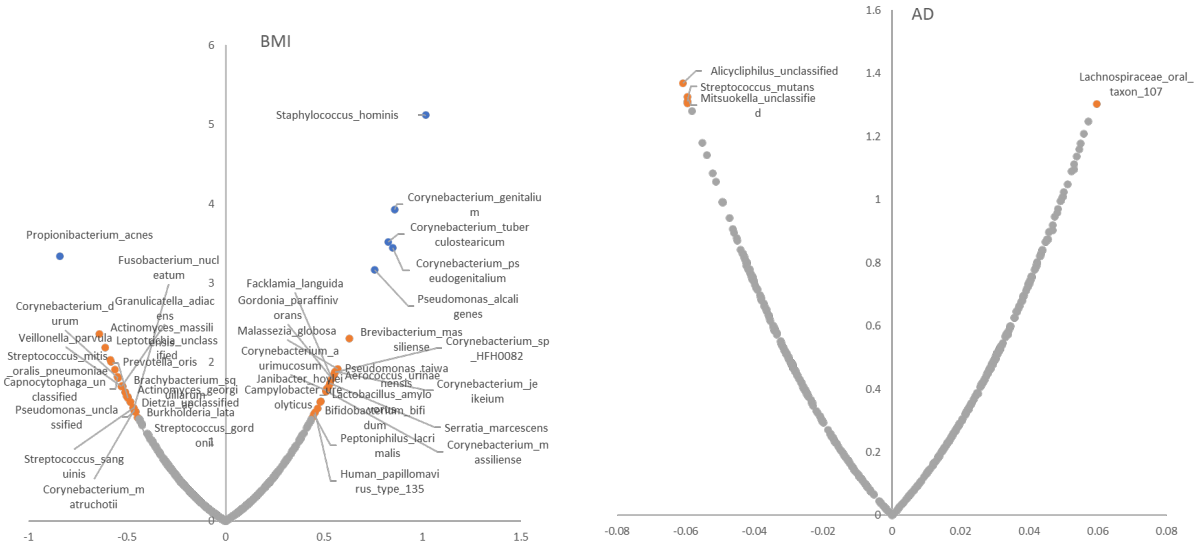
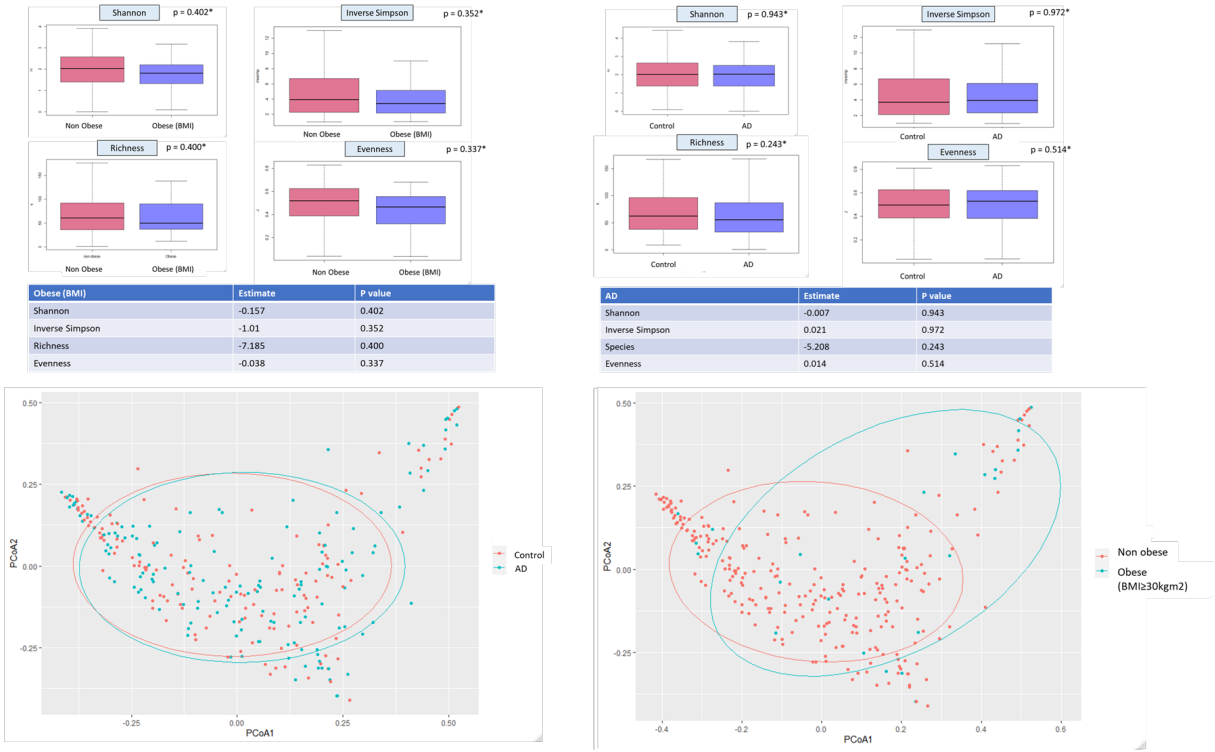


Figure S.7 Microbiome analysis at species level after removing 12 outliers in terms of pre-mapping reads



Obese (BMI)	Estimate	P value
Shannon	-0.157	0.402
Inverse Simpson	-1.01	0.352
Richness	-7.185	0.400
Evenness	-0.038	0.337

AD	Estimate	P value
Shannon	-0.007	0.943
Inverse Simpson	0.021	0.972
Species	-5.208	0.243
Evenness	0.014	0.514

Figure S.8 Alpha and Beta diversity measures of AD versus Controls and Obese vs non-Obese after down reading 12 outliers to median number of pre-mapping reads.

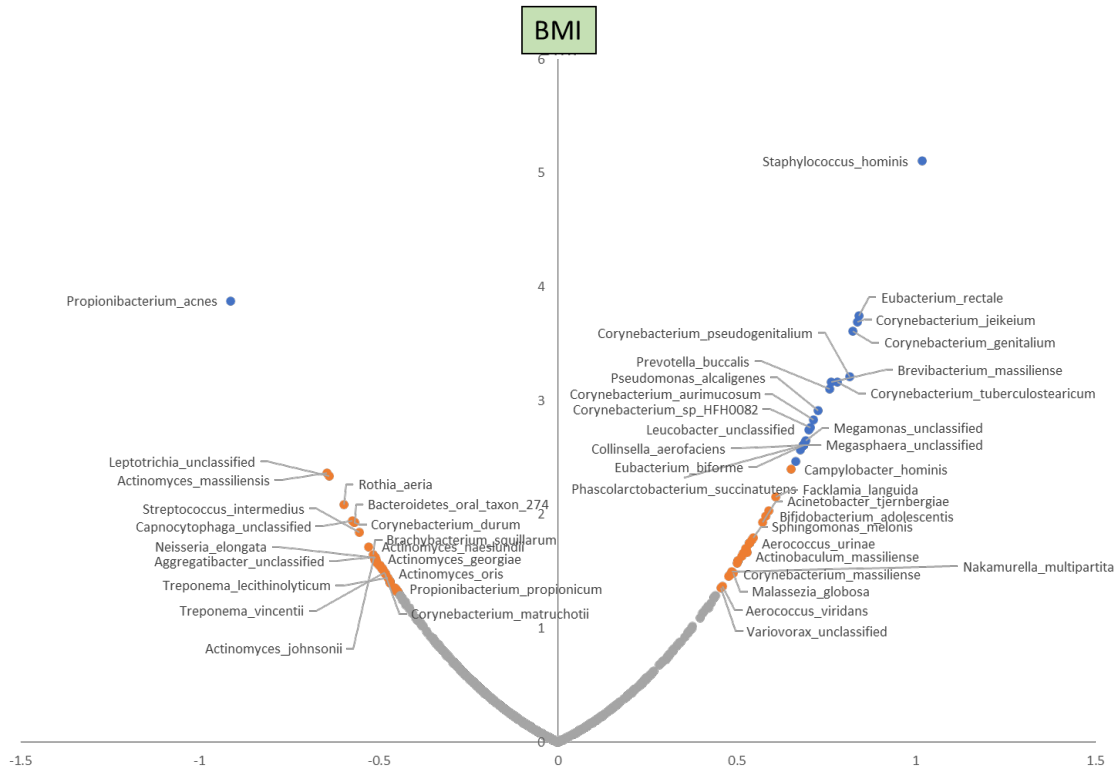


Figure S.9 Microbiome analysis at species level against BMI after down reading 12 outliers to median number of pre-mapping reads.

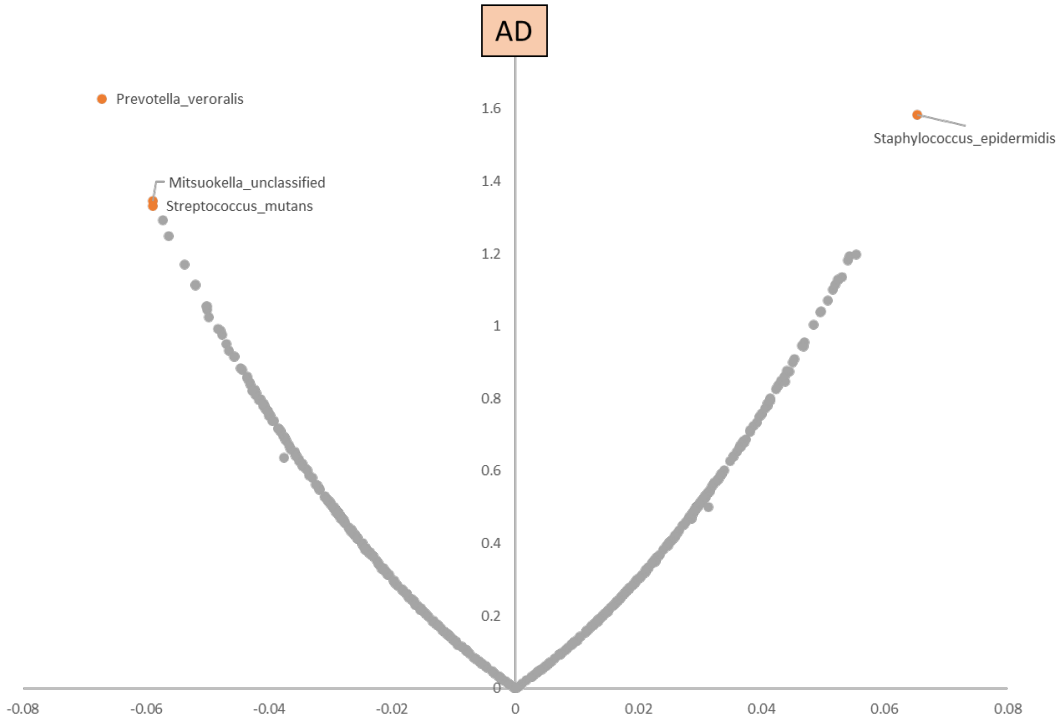


Figure S.10 Microbiome analysis at species level against AD after down reading 12 outliers to median number of pre-mapping reads.

Annex B: Skin Domain questions in HELIOS Study

SK1	Do you currently have a skin condition?	• No • Yes	
SK1A	What is the severity of your skin condition?	• Not at all • Mild • Moderate • Severe • Extremely severe	Only if answered 'Yes' for question SK1
SK2	In the last year, have you suffered from a dry skin in general?	• No • Yes	
SK3	Have you ever had an ITCHY skin condition in the last year - by itchy we mean having an unpleasant sensation that leads to the desire to scratch or rub the skin?	• No • Yes	
SK3A	How old were you when this itchy skin condition began?	• Under 2 years • 2 to 5 years • 5 to 10 years • Over 10 years	Only if answered 'Yes' for question SK3
SK3B	Has this skin condition ever affected the skin creases - by skin creases we mean fronts of elbows, behind the knees, fronts of ankles, around the neck, or around the eyes?	• No • Yes •	
SK3C	Have you had this itchy skin condition in the LAST WEEK?	• No • Yes	
SK3D	On a scale of 0 to 10, how itchy has your skin been for the LAST WEEK? (0 being no itch and 10 being the most itchy you ever felt)	• Enter number 0 to 10 _____	Only if answered 'Yes' for question SK2A
SK3E	On a scale of 0 to 10, how has your skin affected your sleep for the LAST WEEK? (0 being no problem and 10 being unable to sleep at all)	• Enter number 0 to 10 _____	

SK4	Have you ever had Eczema (intermittent itchy, red, flaky or oozy rashes)?	• No • Yes	
SK4A	Which parts of the body have you had the eczema in the past?	1. Whole body - involving the trunk, limbs and face 2. Mainly the limbs, (includes the neck) 3. Mainly the face and scalp 4. Mainly the hands and feet only 5. More than one area of involvement – a combination of 2,3, and 4 6. Don't know	Only if answered 'Yes' for question SK4
SK4	Have you ever had any of the following skin conditions?	• Acne vulgaris requiring >1 month of oral medications • Psoriasis • Vitiligo • Viral warts • Scabies • Fungal infection affecting feet, body or groin • Chronic Urticaria "hives" lasting more than 6 weeks • Recurrent bacterial infection of the skin (Impetigo, abscess, cellulitis) • Chronic ulcers or wounds that are difficult to heal • Skin cancers (including basal cell carcinoma, squamous cell carcinoma of skin, melanoma) • Alopecia areata (Patchy hair loss for more than 1 month) • None of the above	
Additional questions for diagnosing atopic dermatitis			
R1	Has a doctor ever told you that you have or had asthma?	• No • Yes	
R2	Has a doctor ever told you that you have or had allergic rhinitis?	• No • Yes	

Annex C: MetaCyc pathway IDs and names

Pathway ID	Pathway names
1CMET2-PWY	N10-formyl-tetrahydrofolate biosynthesis
3-HYDROXYPHENYLACETATE-DEGRADATION-PWY	4-hydroxyphenylacetate degradation
4-HYDROXYMANDELATE-DEGRADATION-PWY	4-hydroxymandelate degradation
AEROBACTINSYN-PWY	aerobactin biosynthesis
ALLANTOINDEG-PWY	superpathway of allantoin degradation in yeast
ANAEROFRUCAT-PWY	homolactic fermentation
ANAGLYCOLYSIS-PWY	glycolysis III (from glucose)
ARG+POLYAMINE-SYN	superpathway of arginine and polyamine biosynthesis
ARGDEG-PWY	superpathway of L-arginine, putrescine, and 4-aminobutanoate degradation
ARGININE-SYN4-PWY	L-ornithine de novo biosynthesis
ARGORNPROST-PWY	arginine, ornithine and proline interconversion
ARGSYN-PWY	L-arginine biosynthesis I (via L-ornithine)
ARGSYNBSUB-PWY	L-arginine biosynthesis II (acetyl cycle)
ARO-PWY	chorismate biosynthesis I
ASPASN-PWY	superpathway of L-aspartate and L-asparagine biosynthesis
AST-PWY	L-arginine degradation II (AST pathway)
BIOTIN-BIOSYNTHESIS-PWY	biotin biosynthesis I
BRANCHED-CHAIN-AA-SYN-PWY	superpathway of branched amino acid biosynthesis
CALVIN-PWY	Calvin-Benson-Bassham cycle
CATECHOL-ORTHO-CLEAVAGE-PWY	catechol degradation to β-ketoadipate
CENTFERM-PWY	pyruvate fermentation to butanoate
CHLOROPHYLL-SYN	chlorophyllide a biosynthesis I (aerobic, light-dependent)
CITRULBIO-PWY	L-citrulline biosynthesis
COA-PWY-1	coenzyme A biosynthesis II (mammalian)
COA-PWY	coenzyme A biosynthesis I
COBALSYN-PWY	adenosylcobalamin salvage from cobinamide I
COLANSYN-PWY	colanic acid building blocks biosynthesis
COMPLETE-ARO-PWY	superpathway of aromatic amino acid biosynthesis
CRNFORCAT-PWY	creatinine degradation I
DAPLYSINESYN-PWY	L-lysine biosynthesis I
DENITRIFICATION-PWY	nitrate reduction I (denitrification)
DENOVOPURINE2-PWY	superpathway of purine nucleotides de novo biosynthesis II
DHGLUCONATE-PYR-CAT-PWY	glucose degradation (oxidative)
DTDPRHAMSYN-PWY	dTDP-L-rhamnose biosynthesis I
ECASYN-PWY	enterobacterial common antigen biosynthesis
ENTBACSYN-PWY	enterobactin biosynthesis
FAO-PWY	fatty acid β-oxidation I

FASYN-ELONG-PWY	fatty acid elongation -- saturated
FASYN-INITIAL-PWY	superpathway of fatty acid biosynthesis initiation (E. coli)
FERMENTATION-PWY	mixed acid fermentation
FOLSYN-PWY	superpathway of tetrahydrofolate biosynthesis and salvage
FUC-RHAMCAT-PWY	superpathway of fucose and rhamnose degradation
FUCCAT-PWY	fucose degradation
GALACT-GLUCUROCAT-PWY	superpathway of hexuronide and hexuronate degradation
GALACTARDEG-PWY	D-galactarate degradation I
GALACTUROCAT-PWY	D-galacturonate degradation I
GALLATE-DEGRADATION-I-PWY	gallate degradation II
GLCMANNANAUT-PWY	superpathway of N-acetylglucosamine, N-acetylmannosamine and N-acetylneuraminate degradation
GLUCARDEG-PWY	D-glucarate degradation I
GLUCARGALACTSUPER-PWY	superpathway of D-glucarate and D-galactarate degradation
GLUCONEO-PWY	gluconeogenesis I
GLUCOSE1PMETAB-PWY	glucose and glucose-1-phosphate degradation
GLUCUROCAT-PWY	superpathway of β-D-glucuronide and D-glucuronate degradation
GLUDEG-I-PWY	GABA shunt
GLUTORN-PWY	L-ornithine biosynthesis
GLYCOCAT-PWY	glycogen degradation I (bacterial)
GLYCOGENSYNTH-PWY	glycogen biosynthesis I (from ADP-D-Glucose)
GLYCOL-GLYOXDEG-PWY	superpathway of glycol metabolism and degradation
GLYCOLYSIS-E-D	superpathway of glycolysis and Entner-Doudoroff
GLYCOLYSIS-TCA-GLYOX-BYPASS	superpathway of glycolysis, pyruvate dehydrogenase, TCA, and glyoxylate bypass
GLYCOLYSIS	glycolysis I (from glucose 6-phosphate)
GLYOXYLATE-BYPASS	glyoxylate cycle
GOLPDLCAT-PWY	superpathway of glycerol degradation to 1,3-propanediol
HCAMHPDEG-PWY	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation to 2-oxopent-4-enoate
HEME-BIOSYNTHESIS-II	heme biosynthesis I (aerobic)
HEMESYN2-PWY	heme biosynthesis II (anaerobic)
HEXITOLDEGSUPER-PWY	superpathway of hexitol degradation (bacteria)
HISDEG-PWY	L-histidine degradation I
HISTSYN-PWY	L-histidine biosynthesis
HOMOSER-METSYN-PWY	L-methionine biosynthesis I
HSERMETANA-PWY	L-methionine biosynthesis III
ILEUDEG-PWY	L-isoleucine degradation I
ILEUSYN-PWY	L-isoleucine biosynthesis I (from threonine)
KDO-NAGLIPASYN-PWY	superpathway of (Kdo)2-lipid A biosynthesis
KETOGLUCONMET-PWY	ketogluconate metabolism
LACTOSECAT-PWY	lactose and galactose degradation I
LEU-DEG2-PWY	L-leucine degradation I
LIPASYN-PWY	phospholipases
LPSSYN-PWY	superpathway of lipopolysaccharide biosynthesis

LYSINE-AMINOAD-PWY	L-lysine biosynthesis IV
LYSINE-DEG1-PWY	L-lysine degradation XI (mammalian)
MET-SAM-PWY	superpathway of S-adenosyl-L-methionine biosynthesis
METHGLYUT-PWY	superpathway of methylglyoxal degradation
METHYLGALLATE-DEGRADATION-PWY	methylgallate degradation
METSYN-PWY	L-homoserine and L-methionine biosynthesis
NAD-BIOSYNTHESIS-II	NAD salvage pathway II
NADSYN-PWY	NAD biosynthesis II (from tryptophan)
NAGLIPASYN-PWY	lipid IVA biosynthesis
NONMEVIPP-PWY	methylerythritol phosphate pathway I
NONOXIPENT-PWY	pentose phosphate pathway (non-oxidative branch)
OANTIGEN-PWY	O-antigen building blocks biosynthesis (E. coli)
ORNARGDEG-PWY	superpathway of L-arginine and L-ornithine degradation
ORNDEG-PWY	superpathway of ornithine degradation
P101-PWY	ectoine biosynthesis
P105-PWY	TCA cycle IV (2-oxoglutarate decarboxylase)
P108-PWY	pyruvate fermentation to propanoate I
P122-PWY	heterolactic fermentation
P124-PWY	Bifidobacterium shunt
P125-PWY	superpathway of (R,R)-butanediol biosynthesis
P161-PWY	acetylene degradation
P162-PWY	L-glutamate degradation V (via hydroxyglutarate)
P163-PWY	L-lysine fermentation to acetate and butanoate
P164-PWY	purine nucleobases degradation I (anaerobic)
P165-PWY	superpathway of purines degradation in plants
P184-PWY	protocatechuate degradation I (meta-cleavage pathway)
P185-PWY	formaldehyde assimilation III (dihydroxyacetone cycle)
P221-PWY	octane oxidation
P23-PWY	reductive TCA cycle I
P261-PWY	coenzyme M biosynthesis I
P4-PWY	superpathway of L-lysine, L-threonine and L-methionine biosynthesis I
P42-PWY	incomplete reductive TCA cycle
P441-PWY	superpathway of N-acetylneuraminate degradation
P461-PWY	hexitol fermentation to lactate, formate, ethanol and acetate
P621-PWY	nylon-6 oligomer degradation
PANTO-PWY	phosphopantothenate biosynthesis I
PANTOSYN-PWY	pantothenate and coenzyme A biosynthesis I
PENTOSE-P-PWY	pentose phosphate pathway
PEPTIDOGLYCANSYN-PWY	peptidoglycan biosynthesis I (meso-diaminopimelate containing)
PHOSLIPSYN-PWY	superpathway of phospholipid biosynthesis I (bacteria)
PHOTOALL-PWY	oxygenic photosynthesis
POLYAMINSYN3-PWY	superpathway of polyamine biosynthesis II
POLYAMSYN-PWY	superpathway of polyamine biosynthesis I

POLYISOPRENSYN-PWY	polyisoprenoid biosynthesis (E. coli)
PPGPPMET-PWY	ppGpp biosynthesis
PROTOCATECHUATE-ORTHO-CLEAVAGE-PWY	protocatechuate degradation II (ortho-cleavage pathway)
PRPP-PWY	superpathway of histidine, purine, and pyrimidine biosynthesis
PWY-101	photosynthesis light reactions
PWY-1042	glycolysis IV (plant cytosol)
PWY-1269	CMP-3-deoxy-D-manno-octulosonate biosynthesis I
PWY-1361	benzoyl-CoA degradation I (aerobic)
PWY-1541	superpathway of taurine degradation
PWY-1622	formaldehyde assimilation I (serine pathway)
PWY-181	photorespiration
PWY-1861	formaldehyde assimilation II (RuMP Cycle)
PWY-1882	superpathway of C1 compounds oxidation to CO2
PWY-2201	folate transformations I
PWY-2221	Entner-Doudoroff pathway III (semi-phosphorylative)
PWY-241	C4 photosynthetic carbon assimilation cycle, NADP-ME type
PWY-2723	trehalose degradation V
PWY-2941	L-lysine biosynthesis II
PWY-2942	L-lysine biosynthesis III
PWY-3001	superpathway of L-isoleucine biosynthesis I
PWY-3502	superpathway of NAD biosynthesis in eukaryotes
PWY-3661	glycine betaine degradation I
PWY-3781	aerobic respiration I (cytochrome c)
PWY-3801	sucrose degradation II (sucrose synthase)
PWY-3841	folate transformations II
PWY-3941	β-alanine biosynthesis II
PWY-4041	γ-glutamyl cycle
PWY-4221	pantothenate and coenzyme A biosynthesis II (plants)
PWY-4242	pantothenate and coenzyme A biosynthesis III
PWY-4321	L-glutamate degradation IV
PWY-4361	S-methyl-5-thio-α-D-ribose 1-phosphate degradation
PWY-4702	phytate degradation I
PWY-4722	creatinine degradation II
PWY-4981	L-proline biosynthesis II (from arginine)
PWY-4984	urea cycle
PWY-5004	superpathway of L-citrulline metabolism
PWY-5005	biotin biosynthesis II
PWY-5022	4-aminobutanoate degradation V
PWY-5028	L-histidine degradation II
PWY-5030	L-histidine degradation III
PWY-5067	glycogen biosynthesis II (from UDP-D-Glucose)
PWY-5079	L-phenylalanine degradation III
PWY-5080	very long chain fatty acid biosynthesis I
PWY-5083	NAD/NADH phosphorylation and dephosphorylation

PWY-5088	L-glutamate degradation VIII (to propanoate)
PWY-5097	L-lysine biosynthesis VI
PWY-5100	pyruvate fermentation to acetate and lactate II
PWY-5101	L-isoleucine biosynthesis II
PWY-5103	L-isoleucine biosynthesis III
PWY-5104	L-isoleucine biosynthesis IV
PWY-5109	2-methylbutanoate biosynthesis
PWY-5121	superpathway of geranylgeranyl diphosphate biosynthesis II (via MEP)
PWY-5129	sphingolipid biosynthesis (plants)
PWY-5136	fatty acid β-oxidation II (peroxisome)
PWY-5138	unsaturated, even numbered fatty acid β-oxidation
PWY-5154	L-arginine biosynthesis III (via N-acetyl-L-citrulline)
PWY-5156	superpathway of fatty acid biosynthesis II (plant)
PWY-5173	superpathway of acetyl-CoA biosynthesis
PWY-5177	glutaryl-CoA degradation
PWY-5178	toluene degradation IV (aerobic) (via catechol)
PWY-5179	toluene degradation V (aerobic) (via toluene-cis-diol)
PWY-5180	toluene degradation I (aerobic) (via o-cresol)
PWY-5181	toluene degradation III (aerobic) (via p-cresol)
PWY-5182	toluene degradation II (aerobic) (via 4-methylcatechol)
PWY-5183	superpathway of aerobic toluene degradation
PWY-5188	tetrapyrrole biosynthesis I (from glutamate)
PWY-5189	tetrapyrrole biosynthesis II (from glycine)
PWY-5198	factor 420 biosynthesis
PWY-5265	peptidoglycan biosynthesis II (staphylococci)
PWY-5273	p-cumate degradation
PWY-5304	superpathway of sulfur oxidation (Acidianus ambivalens)
PWY-5328	superpathway of L-methionine salvage and degradation
PWY-5345	superpathway of L-methionine biosynthesis (by sulfhydrylation)
PWY-5347	superpathway of L-methionine biosynthesis (transsulfuration)
PWY-5367	petroselinate biosynthesis
PWY-5384	sucrose degradation IV (sucrose phosphorylase)
PWY-5415	catechol degradation I (meta-cleavage pathway)
PWY-5417	catechol degradation III (ortho-cleavage pathway)
PWY-5419	catechol degradation to 2-oxopent-4-enoate II
PWY-5420	catechol degradation II (meta-cleavage pathway)
PWY-5430	meta cleavage pathway of aromatic compounds
PWY-5431	aromatic compounds degradation via β-ketoadipate
PWY-5464	superpathway of cytosolic glycolysis (plants), pyruvate dehydrogenase and TCA cycle
PWY-5484	glycolysis II (from fructose 6-phosphate)
PWY-5505	L-glutamate and L-glutamine biosynthesis
PWY-5509	adenosylcobalamin biosynthesis from cobyrinate a,c-diamide I
PWY-5531	chlorophyllide a biosynthesis II (anaerobic)

PWY-561	superpathway of glyoxylate cycle and fatty acid degradation
PWY-5647	2-nitrobenzoate degradation I
PWY-5651	L-tryptophan degradation to 2-amino-3-carboxymuconate semialdehyde
PWY-5654	2-amino-3-carboxymuconate semialdehyde degradation to 2-oxopentenoate
PWY-5655	L-tryptophan degradation IX
PWY-5656	mannosylglycerate biosynthesis I
PWY-5659	GDP-mannose biosynthesis
PWY-5667	CDP-diacylglycerol biosynthesis I
PWY-5675	nitrate reduction V (assimilatory)
PWY-5676	acetyl-CoA fermentation to butanoate II
PWY-5686	UMP biosynthesis
PWY-5690	TCA cycle II (plants and fungi)
PWY-5695	urate biosynthesis/inosine 5'-phosphate degradation
PWY-5705	allantoin degradation to glyoxylate III
PWY-5723	Rubisco shunt
PWY-5741	ethylmalonyl-CoA pathway
PWY-5747	2-methylcitrate cycle II
PWY-5791	1,4-dihydroxy-2-naphthoate biosynthesis II (plants)
PWY-5837	1,4-dihydroxy-2-naphthoate biosynthesis I
PWY-5838	superpathway of menaquinol-8 biosynthesis I
PWY-5840	superpathway of menaquinol-7 biosynthesis
PWY-5845	superpathway of menaquinol-9 biosynthesis
PWY-5850	superpathway of menaquinol-6 biosynthesis I
PWY-5855	ubiquinol-7 biosynthesis (prokaryotic)
PWY-5856	ubiquinol-9 biosynthesis (prokaryotic)
PWY-5857	ubiquinol-10 biosynthesis (prokaryotic)
PWY-5860	superpathway of demethylmenaquinol-6 biosynthesis I
PWY-5861	superpathway of demethylmenaquinol-8 biosynthesis
PWY-5862	superpathway of demethylmenaquinol-9 biosynthesis
PWY-5863	superpathway of phylloquinol biosynthesis
PWY-5870	ubiquinol-8 biosynthesis (eukaryotic)
PWY-5871	ubiquinol-9 biosynthesis (eukaryotic)
PWY-5872	ubiquinol-10 biosynthesis (eukaryotic)
PWY-5873	ubiquinol-7 biosynthesis (eukaryotic)
PWY-5896	superpathway of menaquinol-10 biosynthesis
PWY-5897	superpathway of menaquinol-11 biosynthesis
PWY-5898	superpathway of menaquinol-12 biosynthesis
PWY-5899	superpathway of menaquinol-13 biosynthesis
PWY-5910	superpathway of geranylgeranyldiphosphate biosynthesis I (via mevalonate)
PWY-5913	TCA cycle VI (obligate autotrophs)
PWY-5918	superpathay of heme biosynthesis from glutamate
PWY-5920	superpathway of heme biosynthesis from glycine
PWY-5941	glycogen degradation II (eukaryotic)

PWY-5971	palmitate biosynthesis II (bacteria and plants)
PWY-5973	cis-vaccenate biosynthesis
PWY-5989	stearate biosynthesis II (bacteria and plants)
PWY-5994	palmitate biosynthesis I (animals and fungi)
PWY-6074	zymosterol biosynthesis
PWY-6075	ergosterol biosynthesis I
PWY-6107	chlorosalicylate degradation
PWY-6113	superpathway of mycolate biosynthesis
PWY-6121	5-aminoimidazole ribonucleotide biosynthesis I
PWY-6122	5-aminoimidazole ribonucleotide biosynthesis II
PWY-6123	inosine-5'-phosphate biosynthesis I
PWY-6124	inosine-5'-phosphate biosynthesis II
PWY-6125	superpathway of guanosine nucleotides de novo biosynthesis II
PWY-6126	superpathway of adenosine nucleotides de novo biosynthesis II
PWY-6138	CMP-N-acetylneuraminate biosynthesis I (eukaryotes)
PWY-6147	6-hydroxymethyl-dihydropterin diphosphate biosynthesis I
PWY-6151	S-adenosyl-L-methionine cycle I
PWY-6163	chorismate biosynthesis from 3-dehydroquinate
PWY-6168	flavin biosynthesis III (fungi)
PWY-6182	superpathway of salicylate degradation
PWY-6185	4-methylcatechol degradation (ortho cleavage)
PWY-6210	2-aminophenol degradation
PWY-621	sucrose degradation III (sucrose invertase)
PWY-622	starch biosynthesis
PWY-6263	superpathway of menaquinol-8 biosynthesis II
PWY-6270	isoprene biosynthesis I
PWY-6277	superpathway of 5-aminoimidazole ribonucleotide biosynthesis
PWY-6282	palmitoleate biosynthesis I (from (5Z)-dodec-5-enoate)
PWY-6284	superpathway of unsaturated fatty acids biosynthesis (E. coli)
PWY-6285	superpathway of fatty acids biosynthesis (E. coli)
PWY-6305	putrescine biosynthesis IV
PWY-6307	L-tryptophan degradation X (mammalian, via tryptamine)
PWY-6309	L-tryptophan degradation XI (mammalian, via kynurenine)
PWY-6313	serotonin degradation
PWY-6317	galactose degradation I (Leloir pathway)
PWY-6318	L-phenylalanine degradation IV (mammalian, via side chain)
PWY-6338	superpathway of vanillin and vanillate degradation
PWY-6342	noradrenaline and adrenaline degradation
PWY-6351	D-myo-inositol (1,4,5)-trisphosphate biosynthesis
PWY-6353	purine nucleotides degradation II (aerobic)
PWY-6383	mono-trans, poly-cis decaprenyl phosphate biosynthesis
PWY-6385	peptidoglycan biosynthesis III (mycobacteria)
PWY-6386	UDP-N-acetylmuramoyl-pentapeptide biosynthesis II (lysine-containing)

PWY-6387	UDP-N-acetylmuramoyl-pentapeptide biosynthesis I (meso-diaminopimelate containing)
PWY-6396	superpathway of 2,3-butanediol biosynthesis
PWY-6397	mycolyl-arabinogalactan-peptidoglycan complex biosynthesis
PWY-6404	superpathway of mycolyl-arabinogalactan-peptidoglycan complex biosynthesis
PWY-6435	4-hydroxybenzoate biosynthesis V
PWY-6471	peptidoglycan biosynthesis IV (Enterococcus faecium)
PWY-6486	D-galacturonate degradation II
PWY-6507	4-deoxy-L-threo-hex-4-enopyranuronate degradation
PWY-6519	8-amino-7-oxononanoate biosynthesis I
PWY-6527	stachyose degradation
PWY-6531	mannitol cycle
PWY-6545	pyrimidine deoxyribonucleotides de novo biosynthesis III
PWY-6549	L-glutamine biosynthesis III
PWY-6562	norspermidine biosynthesis
PWY-6588	pyruvate fermentation to acetone
PWY-6590	superpathway of Clostridium acetobutylicum acidogenic fermentation
PWY-6595	superpathway of guanosine nucleotides degradation (plants)
PWY-6598	sciadonate biosynthesis
PWY-6606	guanosine nucleotides degradation II
PWY-6608	guanosine nucleotides degradation III
PWY-6609	adenine and adenosine salvage III
PWY-6612	superpathway of tetrahydrofolate biosynthesis
PWY-6628	superpathway of L-phenylalanine biosynthesis
PWY-6629	superpathway of L-tryptophan biosynthesis
PWY-6630	superpathway of L-tyrosine biosynthesis
PWY-6641	superpathway of sulfolactate degradation
PWY-6660	2-heptyl-3-hydroxy-4(1H)-quinolone biosynthesis
PWY-6662	superpathway of quinolone and alkylquinolone biosynthesis
PWY-6690	cinnamate and 3-hydroxycinnamate degradation to 2-oxopent-4-enoate
PWY-6700	queuosine biosynthesis
PWY-6703	preQ0 biosynthesis
PWY-6708	ubiquinol-8 biosynthesis (prokaryotic)
PWY-6728	methylaspartate cycle
PWY-6731	starch degradation III
PWY-6737	starch degradation V
PWY-6748	nitrate reduction VII (denitrification)
PWY-6749	CMP-legionaminate biosynthesis I
PWY-6760	xylose degradation III
PWY-6785	hydrogen production VIII
PWY-6803	phosphatidylcholine acyl editing
PWY-6823	molybdenum cofactor biosynthesis
PWY-6837	fatty acid beta-oxidation V (unsaturated, odd number, di-isomerase-dependent)

PWY-6857	retinol biosynthesis
PWY-6859	all-trans-farnesol biosynthesis
PWY-6876	isopropanol biosynthesis
PWY-6891	thiazole biosynthesis II (Bacillus)
PWY-6892	thiazole biosynthesis I (E. coli)
PWY-6895	superpathway of thiamin diphosphate biosynthesis II
PWY-6897	thiamin salvage II
PWY-6901	superpathway of glucose and xylose degradation
PWY-6936	seleno-amino acid biosynthesis
PWY-6945	cholesterol degradation to androstenedione I (cholesterol oxidase)
PWY-6948	sitosterol degradation to androstenedione
PWY-6969	TCA cycle V (2-oxoglutarate:ferredoxin oxidoreductase)
PWY-7003	glycerol degradation to butanol
PWY-7007	methyl ketone biosynthesis
PWY-7013	L-1,2-propanediol degradation
PWY-7031	protein N-glycosylation (bacterial)
PWY-7039	phosphatidate metabolism, as a signaling molecule
PWY-7090	UDP-2,3-diacetamido-2,3-dideoxy-α-D-mannuronate biosynthesis
PWY-7094	fatty acid salvage
PWY-7097	vanillin and vanillate degradation I
PWY-7111	pyruvate fermentation to isobutanol (engineered)
PWY-7115	C4 photosynthetic carbon assimilation cycle, NAD-ME type
PWY-7117	C4 photosynthetic carbon assimilation cycle, PEPCK type
PWY-7118	chitin degradation to ethanol
PWY-7124	ethylene biosynthesis V (engineered)
PWY-7159	chlorophyllide a biosynthesis III (aerobic, light independent)
PWY-7165	L-ascorbate biosynthesis VI (engineered pathway)
PWY-7184	pyrimidine deoxyribonucleotides de novo biosynthesis I
PWY-7187	pyrimidine deoxyribonucleotides de novo biosynthesis II
PWY-7196	superpathway of pyrimidine ribonucleosides salvage
PWY-7197	pyrimidine deoxyribonucleotide phosphorylation
PWY-7198	pyrimidine deoxyribonucleotides de novo biosynthesis IV
PWY-7199	pyrimidine deoxyribonucleosides salvage
PWY-7200	superpathway of pyrimidine deoxyribonucleoside salvage
PWY-7204	pyridoxal 5'-phosphate salvage II (plants)
PWY-7208	superpathway of pyrimidine nucleobases salvage
PWY-7209	superpathway of pyrimidine ribonucleosides degradation
PWY-7210	pyrimidine deoxyribonucleotides biosynthesis from CTP
PWY-7211	superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis
PWY-7218	photosynthetic 3-hydroxybutanoate biosynthesis (engineered)
PWY-7219	adenosine ribonucleotides de novo biosynthesis
PWY-7220	adenosine deoxyribonucleotides de novo biosynthesis II
PWY-7221	guanosine ribonucleotides de novo biosynthesis

PWY-7222	guanosine deoxyribonucleotides de novo biosynthesis II
PWY-7228	superpathway of guanosine nucleotides de novo biosynthesis I
PWY-7229	superpathway of adenosine nucleotides de novo biosynthesis I
PWY-722	nicotinate degradation I
PWY-7234	inosine-5'-phosphate biosynthesis III
PWY-7235	superpathway of ubiquinol-6 biosynthesis (eukaryotic)
PWY-7237	myo-, chiro- and scillo-inositol degradation
PWY-7242	D-fructuronate degradation
PWY-7245	superpathway NAD/NADP - NADH/NADPH interconversion (yeast)
PWY-724	superpathway of L-lysine, L-threonine and L-methionine biosynthesis II
PWY-7254	TCA cycle VII (acetate-producers)
PWY-7268	NAD/NADP-NADH/NADPH cytosolic interconversion (yeast)
PWY-7269	NAD/NADP-NADH/NADPH mitochondrial interconversion (yeast)
PWY-7270	L-methionine salvage cycle II (plants)
PWY-7279	aerobic respiration II (cytochrome c) (yeast)
PWY-7282	4-amino-2-methyl-5-phosphomethylpyrimidine biosynthesis (yeast)
PWY-7283	wybutosine biosynthesis
PWY-7286	7-(3-amino-3-carboxypropyl)-wyosine biosynthesis
PWY-7288	fatty acid β-oxidation (peroxisome, yeast)
PWY-7315	dTDP-N-acetylthomosamine biosynthesis
PWY-7323	superpathway of GDP-mannose-derived O-antigen building blocks biosynthesis
PWY-7328	superpathway of UDP-glucose-derived O-antigen building blocks biosynthesis
PWY-7337	10-cis-heptadecenoyl-CoA degradation (yeast)
PWY-7338	10-trans-heptadecenoyl-CoA degradation (reductase-dependent, yeast)
PWY-7345	superpathway of anaerobic sucrose degradation
PWY-7357	thiamin formation from pyrithiamine and oxythiamine (yeast)
PWY-7371	1,4-dihydroxy-6-naphthoate biosynthesis II
PWY-7374	1,4-dihydroxy-6-naphthoate biosynthesis I
PWY-7383	anaerobic energy metabolism (invertebrates, cytosol)
PWY-7384	anaerobic energy metabolism (invertebrates, mitochondrial)
PWY-7385	1,3-propanediol biosynthesis (engineered)
PWY-7388	octanoyl-[acyl-carrier protein] biosynthesis (mitochondria, yeast)
PWY-7389	superpathway of anaerobic energy metabolism (invertebrates)
PWY-7391	isoprene biosynthesis II (engineered)
PWY-7392	taxadiene biosynthesis (engineered)
PWY-7400	L-arginine biosynthesis IV (archaebacteria)
PWY-7409	phospholipid remodeling (phosphatidylethanolamine, yeast)
PWY-7411	superpathway of phosphatidate biosynthesis (yeast)
PWY-7420	monoacylglycerol metabolism (yeast)
PWY-7431	aromatic biogenic amine degradation (bacteria)
PWY-7446	sulfoglycolysis

PWY-7456	mannan degradation
PWY-7527	L-methionine salvage cycle III
PWY-7528	L-methionine salvage cycle I (bacteria and plants)
PWY-7539	6-hydroxymethyl-dihydropterin diphosphate biosynthesis III (Chlamydia)
PWY-7560	methylerythritol phosphate pathway II
PWY-7616	methanol oxidation to carbon dioxide
PWY-7619	juniperonate biosynthesis
PWY-7663	gondoate biosynthesis (anaerobic)
PWY-7664	oleate biosynthesis IV (anaerobic)
PWY-821	superpathway of sulfur amino acid biosynthesis (Saccharomyces cerevisiae)
PWY-841	superpathway of purine nucleotides de novo biosynthesis I
PWY-922	mevalonate pathway I
PWY0-1061	superpathway of L-alanine biosynthesis
PWY0-1241	ADP-L-glycero-β-D-manno-heptose biosynthesis
PWY0-1261	anhydromuropeptides recycling
PWY0-1277	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation
PWY0-1296	purine ribonucleosides degradation
PWY0-1297	superpathway of purine deoxyribonucleosides degradation
PWY0-1298	superpathway of pyrimidine deoxyribonucleosides degradation
PWY0-1319	CDP-diacylglycerol biosynthesis II
PWY0-1338	polymyxin resistance
PWY0-1415	superpathway of heme biosynthesis from uroporphyrinogen-III
PWY0-1479	tRNA processing
PWY0-1533	methylphosphonate degradation I
PWY0-1586	peptidoglycan maturation (meso-diaminopimelate containing)
PWY0-162	superpathway of pyrimidine ribonucleotides de novo biosynthesis
PWY0-166	superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis (E. coli)
PWY0-42	2-methylcitrate cycle I
PWY0-781	aspartate superpathway
PWY0-845	superpathway of pyridoxal 5'-phosphate biosynthesis and salvage
PWY0-862	(5Z)-dodec-5-enoate biosynthesis
PWY0-881	superpathway of fatty acid biosynthesis I (E. coli)
PWY1F-823	leucopelargonidin and leucocyanidin biosynthesis
PWY1G-0	mycothiol biosynthesis
PWY3DJ-35471	L-ascorbate biosynthesis IV
PWY3O-19	ubiquinol-6 biosynthesis from 4-hydroxybenzoate (eukaryotic)
PWY3O-355	stearate biosynthesis III (fungi)
PWY490-3	nitrate reduction VI (assimilatory)
PWY4FS-7	phosphatidylglycerol biosynthesis I (plastidic)
PWY4FS-8	phosphatidylglycerol biosynthesis II (non-plastidic)
PWY4LZ-257	superpathway of fermentation (Chlamydomonas reinhardtii)

PWY5F9-12	biphenyl degradation
PWY66-201	nicotine degradation IV
PWY66-367	ketogenesis
PWY66-375	leukotriene biosynthesis
PWY66-388	fatty acid α-oxidation III
PWY66-389	phytol degradation
PWY66-391	fatty acid β-oxidation VI (peroxisome)
PWY66-398	TCA cycle III (animals)
PWY66-399	gluconeogenesis III
PWY66-400	glycolysis VI (metazoan)
PWY66-409	superpathway of purine nucleotide salvage
PWY66-422	D-galactose degradation V (Leloir pathway)
PWY6666-2	dopamine degradation
PWYG-321	mycolate biosynthesis
PYRIDNUCSAL-PWY	NAD salvage pathway I
PYRIDNUCSYN-PWY	NAD biosynthesis I (from aspartate)
PYRIDOXSYN-PWY	pyridoxal 5'-phosphate biosynthesis I
REDCITCYC	TCA cycle VIII (helicobacter)
RHAMCAT-PWY	L-rhamnose degradation I
RIBOSYN2-PWY	flavin biosynthesis I (bacteria and plants)
RUMP-PWY	formaldehyde oxidation I
SALVADEHYPOX-PWY	adenosine nucleotides degradation II
SER-GLYSYN-PWY	superpathway of L-serine and glycine biosynthesis I
SO4ASSIM-PWY	sulfate reduction I (assimilatory)
SPHINGOLIPID-SYN-PWY	sphingolipid biosynthesis (yeast)
SULFATE-CYS-PWY	superpathway of sulfate assimilation and cysteine biosynthesis
TCA-GLYOX-BYPASS	superpathway of glyoxylate bypass and TCA
TCA	TCA cycle I (prokaryotic)
TEICHOICACID-PWY	teichoic acid (poly-glycerol) biosynthesis
THISYN-PWY	superpathway of thiamin diphosphate biosynthesis I
THISYNARA-PWY	superpathway of thiamin diphosphate biosynthesis III (eukaryotes)
THRESYN-PWY	superpathway of L-threonine biosynthesis
TRIGLSYN-PWY	diacylglycerol and triacylglycerol biosynthesis
TRNA-CHARGING-PWY	tRNA charging
TRPSYN-PWY	L-tryptophan biosynthesis
TYRFUMCAT-PWY	L-tyrosine degradation I
UBISYN-PWY	superpathway of ubiquinol-8 biosynthesis (prokaryotic)
UDPNACETYLGALSYN-PWY	UDP-N-acetyl-D-glucosamine biosynthesis II
UDPNAGSYN-PWY	UDP-N-acetyl-D-glucosamine biosynthesis I
URSIN-PWY	ureide biosynthesis
VALDEG-PWY	L-valine degradation I
VALSYN-PWY	L-valine biosynthesis

Annex D: Olink panels and proteomic targets

Olink Inflammation panel
Adenosine Deaminase (ADA)
Artemin (ARTN)
Axin-1 (AXIN1)
Beta-nerve growth factor (Beta-NGF)
Caspase 8 (CASP-8)
C-C motif chemokine 4 (CCL4)
C-C motif chemokine 19 (CCL19)
C-C motif chemokine 20 (CCL20)
C-C motif chemokine 23 (CCL23)
C-C motif chemokine 25 (CCL25)
C-C motif chemokine 28 (CCL28)
CD40L receptor (CD40)
CUB domain-containing protein 1 (CDCP1)
C-X-C motif chemokine 1 (CXCL1)
C-X-C motif chemokine 5 (CXCL5)
C-X-C motif chemokine 6 (CXCL6)
C-X-C motif chemokine 9 (CXCL9)
C-X-C motif chemokine 10 (CXCL10)
C-X-C motif chemokine 11 (CXCL11)
Cystatin D (CST5)
Delta and Notch-like epidermal growth factor- related recep (DNER)
Eotaxin-1 (CCL11)
Eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1)
Fibroblast growth factor 5 (FGF-5)
Fibroblast growth factor 19 (FGF-19)
Fibroblast growth factor 21 (FGF-21)
Fibroblast growth factor 23 (FGF-23)
Fms-related tyrosine kinase 3 ligand (Flt3L)
Fractalkine (CX3CL1)
Glial cell line-derived neurotrophic factor (GDNF)
Hepatocyte growth factor (HGF)
Interferon gamma (IFN-gamma)
Interleukin-1 alpha (IL-1 alpha)
Interleukin-2 (IL-2)
Interleukin-2 receptor subunit beta (IL-2RB)
Interleukin-4 (IL-4)
Interleukin-5 (IL-5)
Interleukin-6 (IL-6)

Interleukin-7 (IL-7)
Interleukin-8 (IL-8)
Interleukin-10 (IL-10)
Interleukin-10 receptor subunit alpha (IL-10RA)
Interleukin-10 receptor subunit beta (IL-10RB)
Interleukin-12 subunit beta (IL-12B)
Interleukin-13 (IL-13)
Interleukin-15 receptor subunit alpha (IL-15RA)
Interleukin-17A (IL-17A)
Interleukin-17C (IL-17C)
Interleukin-18 (IL-18)
Interleukin-18 receptor 1 (IL-18R1)
Interleukin-20 (IL-20)
Interleukin-20 receptor subunit alpha (IL-20RA)
Interleukin-22 receptor subunit alpha-1 (IL-22 RA1)
Interleukin-24 (IL-24)
Interleukin-33 (IL-33)
Latency-associated peptide transforming growth factor beta 1 (LAP TGF-beta-1)
Leukemia inhibitory factor (LIF)
Leukemia inhibitory factor receptor (LIF-R)
Macrophage colony-stimulating factor 1 (CSF-1)
Macrophage inflammatory protein 1-alpha (CCL3)
Matrix metalloproteinase-1 (MMP-1)
Matrix metalloproteinase-10 (MMP-10)
Monocyte chemotactic protein 1 (MCP-1)
Monocyte chemotactic protein 2 (MCP-2)
Monocyte chemotactic protein 3 (MCP-3)
Monocyte chemotactic protein 4 (MCP-4)
Natural killer cell receptor 2B4 (CD244)
Neurotrophin-3 (NT-3)
Neurturin (NRTN)
Oncostatin-M (OSM)
Osteoprotegerin (OPG)
Programmed cell death 1 ligand 1 (PD-L1)
Protein S100-A12 (EN-RAGE)
Signaling lymphocytic activation molecule (SLAMF1)
SIR2-like protein 2 (SIRT2)
STAM-binding protein (STAMPB)
Stem cell factor (SCF)
Sulfotransferase 1A1 (ST1A1)
T-cell surface glycoprotein CD5 (CD5)
T-cell surface glycoprotein CD6 isoform (CD6)

T-cell surface glycoprotein CD8 alpha chain (CD8A)
Thymic stromal lymphopoietin (TSLP)
TNF-beta (TNFB)
TNF-related activation-induced cytokine (TRANCE)
TNF-related apoptosis-inducing ligand (TRAIL)
Transforming growth factor alpha (TGF-alpha)
Tumor necrosis factor (Ligand) superfamily, member 12 (TWEAK)
Tumor necrosis factor (TNF)
Tumor necrosis factor ligand superfamily member 14 (TNFSF14)
Tumor necrosis factor receptor superfamily member 9 (TNFRSF9)
Urokinase-type plasminogen activator (uPA)
Vascular endothelial growth factor A (VEGF-A)
Olink Cadiovascular Disease II Panel
2,4-dienoyl-CoA reductase, mitochondrial (DECR1)
A disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAM-TS13)
ADM (ADM)
Agouti-related protein (AGRP)
Alpha-L-iduronidase (IDUA)
Angiopoietin-1 (ANGPT1)
Angiopoietin-1 receptor (TIE2)
Angiotensin-converting enzyme 2 (ACE2)
Bone morphogenetic protein 6 (BMP-6)
Brother of CDO (BOC)
Carbonic anhydrase 5A, mitochondrial (CA5A)
Carcinoembryonic antigenrelated cell adhesion molecule 8 (CEACAM8)
Cathepsin L1 (CTSL1)
C-C motif chemokine 17 (CCL17)
C-C motif chemokine 3 (CCL3)
CD40 ligand (CD40-L)
Chymotrypsin C (CTRC)
C-X-C motif chemokine 1 (CXCL1)
Decorin (DCN)
Dickkopf-related protein 1 (Dkk-1)
Fatty acid-binding protein, intestinal (FABP2)
Fibroblast growth factor 21 (FGF-21)
Fibroblast growth factor 23 (FGF-23)
Follistatin (FS)
Galectin-9 (Gal-9)
Gastric intrinsic factor (GIF)
Gastrotropin (GT)
Growth hormone (GH)
Growth/differentiation factor 2 (GDF-2)

Heat shock 27 kDa protein (HSP 27)
Heme oxygenase 1 (HO-1)
Hydroxyacid oxidase 1 (HAOX1)
Interleukin-1 receptor antagonist protein (IL-1ra)
Interleukin-1 receptor-like 2 (IL1RL2)
Interleukin-17D (IL-17D)
Interleukin-18 (IL-18)
Interleukin-27 (IL-27)
Interleukin-4 receptor subunit alpha (IL-4RA)
Interleukin-6 (IL-6)
Kidney injury molecule 1 (KIM1)
Lactoylglutathione lyase (GLO1)
Lectin-like oxidized LDL receptor 1 (LOX-1)
Leptin (LEP)
Lipoprotein lipase (LPL)
Low affinity immunoglobulin gamma Fc region receptor II-b (IgG Fc receptor II-b)
Lymphotactin (XCL1)
Macrophage receptor MARCO (MARCO)
Matrix metalloproteinase-12 (MMP12)
Matrix metalloproteinase-7 (MMP7)
Melusin (ITGB1BP2)
Natriuretic peptides B (BNP)
NF-kappa-B essential modulator (NEMO)
Osteoclast-associated immunoglobulin- like receptor (hOSCAR)
Pappalysin-1 (PAPPA)
Pentraxin-related protein PTX3 (PTX3)
Placenta growth factor (PGF)
Platelet-derived growth factor subunit B (PDGF subunit B)
Poly [ADP-ribose] polymerase 1 (PARP-1)
Polymeric immunoglobulin receptor (PIgR)
Programmed cell death 1 ligand 2 (PD-L2)
Proheparin-binding EGF-like growth factor (HB-EGF)
Pro-interleukin-16 (IL16)
Prolargin (PRELP)
Prostasin (PRSS8)
Protein AMBP (AMBP)
Proteinase-activated receptor 1 (PAR-1)
Protein-glutamine gamma- glutamyltransferase 2 (TGM2)
Proto-oncogene tyrosine-protein kinase Src (SRC)
P-selectin glycoprotein ligand 1 (PSGL-1)
Receptor for advanced glycosylation end products (RAGE)
Renin (REN)

Serine protease 27 (PRSS27)
Serine/threonine-protein kinase 4 (STK4)
Serpin A12 (SERPINA12)
SLAM family member 5 (CD84)
SLAM family member 7 (SLAMF7)
Sortilin (SORT1)
Spondin-2 (SPON2)
Stem cell factor (SCF)
Superoxide dismutase [Mn] mitochondrial (SOD2)
T-cell surface glycoprotein CD4 (CD4)
Thrombomodulin (TM)
Thrombopoietin (THPO)
Thrombospondin-2 (THBS2)
Tissue factor (TF)
TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2)
Tumor necrosis factor receptor superfamily member 10A (TNFRSF10A)
Tumor necrosis factor receptor superfamily member 11A (TNFRSF11A)
Tumor necrosis factor receptor superfamily member 13B (TNFRSF13B)
Tyrosine-protein kinase Mer (MERTK)
Vascular endothelial growth factor D (VEGFD)
V-set and immunoglobulin domain-containing protein 2 (VSIG2)
Olink Cadiovascular Disease III Panel
Aminopeptidase N (AP-N)
Azurocidin (AZU1)
Bleomycin hydrolase (BLM hydrolase)
Cadherin-5 (CDH5)
Carboxypeptidase A1 (CPA1)
Carboxypeptidase B (CPB1)
Caspase-3 (CASP-3)
Cathepsin D (CTSD)
Cathepsin Z (CTSZ)
C-C motif chemokine 15 (CCL15)
C-C motif chemokine 16 (CCL16)
C-C motif chemokine 24 (CCL24)
CD166 antigen (ALCAM)
Chitinase-3-like protein 1 (CHI3L1)
Chitotriosidase-1 (CHIT1)
Collagen alpha-1(I) chain (COL1A1)
Complement component C1q receptor (CD93)
Contactin-1 (CNTN1)
C-X-C motif chemokine 16 (CXCL16)
Cystatin-B (CSTB)

Elafin (PI3)
Ephrin type-B receptor 4 (EPHB4)
Epidermal growth factor receptor (EGFR)
Epithelial cell adhesion molecule (Ep-CAM)
E-selectin (SELE)
Fatty acid-binding protein, adipocyte (FABP4)
Galectin-3 (Gal-3)
Galectin-4 (Gal-4)
Granulins (GRN)
Growth/differentiation factor 15 (GDF-15)
Insulin-like growth factor-binding protein (IGFBP-1)
Insulin-like Growth Factor-Binding Protein (IGFBP-2)
Insulin-like growth factor-binding protein (IGFBP-7)
Integrin beta-2 (ITGB2)
Intercellular adhesion molecule 2 (ICAM-2)
Interleukin-1 receptor type 1 (IL-1RT1)
Interleukin-1 receptor type 2 (IL-1RT2)
Interleukin-17 receptor A (IL-17RA)
Interleukin-18-binding protein (IL-18BP)
Interleukin-2 receptor subunit alpha (IL2-RA)
Interleukin-6 receptor subunit alpha (IL-6RA)
Junctional adhesion molecule A (JAM-A)
Kallikrein-6 (KLK6)
Low-density lipoprotein receptor (LDL receptor)
Lymphotoxin-beta receptor (LTBR)
Matrix extracellular phosphoglycoprotein (MEPE)
Matrix metalloproteinase-2 (MMP-2)
Matrix metalloproteinase-3 (MMP-3)
Matrix metalloproteinase-9 (MMP-9)
Metalloproteinase inhibitor 4 (TIMP4)
Monocyte chemotactic protein 1 (MCP-1)
Myeloblastin (PRTN3)
Myeloperoxidase (MPO)
Myoglobin (MB)
Neurogenic locus notch homolog protein 3 (Notch 3)
N-terminal prohormone brain natriuretic peptide (NT-proBNP)
Osteopontin (OPN)
Osteoprotegerin (OPG)
Paraoxonase (PON 3) (PON3)
Peptidoglycan recognition protein 1 (PGLYRP1)
Perlecan (PLC)
Plasminogen activator inhibitor 1 (PAI)

Platelet endothelial cell adhesion molecule (PECAM-1)
Platelet glycoprotein VI (GP6)
Platelet-derived growth factor subunit A (PDGF subunit A)
Proprotein convertase subtilisin/kexin type 9 (PCSK9)
Protein delta homolog 1 (DLK-1)
P-selectin (SELP)
Pulmonary surfactant-associated protein D (PSP-D)
Resistin (RETN)
Retinoic acid receptor responder protein 2 (RARRES2)
Scavenger receptor cysteine-rich type 1 protein M130 (CD163)
Secretoglobin family 3A member 2 (SCGB3A2)
Spondin-1 (SPON1)
ST2 protein (ST2)
Tartrate-resistant acid phosphatase type (TR-AP)
Tissue factor pathway inhibitor (TFPI)
Tissue-type plasminogen activator (t-PA)
Transferrin receptor protein 1 (TR)
Trefoil factor 3 (TFF3)
Trem-like transcript 2 protein (TLT-2)
Tumor necrosis factor ligand superfamily Q9Y275 member 13B (TNFSF13B)
Tumor necrosis factor receptor 1 (TNF-R1)
Tumor necrosis factor receptor 2 (TNF-R2)
Tumor necrosis factor receptor superfamily member 10C (TNFRSF10C)
Tumor necrosis factor receptor superfamily member 14 (TNFRSF14)
Tumor necrosis factor receptor superfamily member 6 (FAS)
Tyrosine-protein kinase receptor UFO (AXL)
Tyrosine-protein phosphatase non- receptor type substrate 1 (SHPS-1)
Urokinase plasminogen activator surface Q03405 receptor (U-PAR)
Urokinase-type plasminogen activator (uPA)
von Willebrand factor (vWF)

Published research paper

Yew YW, Loh M, Thng STG, Chambers JC. Investigating causal relationships between Body Mass Index and risk of atopic dermatitis: a Mendelian randomization analysis. Sci Rep. 2020 Sep 17;10(1):15279. doi: 10.1038/s41598-020-72301-2.