

Genetic Studies of Prader–Willi Patients Provide Evidence for Conservation of Genomic Architecture in Proximal Chromosome 15q

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Summary

Prader–Willi syndrome (PWS) is a neurogenetic disorder associated with recurrent genomic recombination involving low copy repeats (LCRs) located in the human chromosome 15q11-q13. Previous studies of PWS patients from Asia suggested that there is a higher incidence of deletion and lower incidence of maternal uniparental disomy (mUPD) compared to that of Western populations. In this report, we present genetic etiology of 28 PWS patients from Taiwan. Consistent with the genetic etiology findings from Western populations, the type II deletion appears to be the most common deletion subtype. Furthermore, the ratio of the two most common deletion subtypes and the ratio of the maternal heterodisomy to isodisomy cases observed from this study are in agreement with previous findings from Western populations. In addition, we identified and further mapped the deletion breakpoints in two patients with atypical deletions using array CGH (comparative genomic hybridization). Despite the relatively small numbers of patients in each subgroup, our findings suggest that the genomic architecture responsible for the recurrent recombination in PWS is conserved in Taiwanese of the Han Chinese heritage and Western populations, thereby predisposing chromosome 15q11-q13 to a similar risk of rearrangements.

Keywords: Prader–Willi syndrome, chromosome15q, genomic architecture

Introduction

Prader–Willi syndrome (PWS) (MIM 176270) is a neurogenetic disorder characterized by hypotonia in early infancy, obesity in early childhood, dysmorphic features, mental retardation, and increased risk of seizures (Holm et al., 1993; Fan et al., 2009). The widely accepted genetic epidemiology from Western populations reveals that most PWS

cases (~70%) have a deletion in chromosome 15q11-q13 (Nicholls & Knepper, 2001). Some PWS cases (~25%) arise from mUPD (Robinson et al., 2000). About 2% of PWS patients carry a mutation in the PWS imprinting center, resulting in abnormal DNA methylation and gene expression (Nicholls et al., 1989; Mascari et al., 1992). Studies involving comprehensive genetic analyses of PWS are limited in Asia.

Knowledge of the molecular etiology is essential for appropriate genetic counseling of PWS patients. A significant risk of recurrence is associated with imprinting defects (Buiting et al., 1998). Moreover, several phenotype–genotype correlation studies have demonstrated that varying degrees of severity in clinical and behavioral manifestations in PWS are associated with the etiology of the patients (Cassidy et al., 1997; Webb et al., 2002; Bittel et al., 2003). Determination of the genetic subtypes is, therefore, critical for the proper care of patients with PWS.

Low copy repeats (LCRs) have been identified at the recombination breakpoints and have been implicated in mediating DNA recombination in recurrent microdeletion/microduplication syndromes (Nathans et al., 1986; Pentao et al., 1992; Kazazian, 1993; Chen et al., 1997; Reiter et al., 1998; Christian et al., 1999; Potocki et al., 2000). There are five breakpoints along 15q11-q14 that are associated with PWS (Fig. 1) (Varela et al., 2005), BP1 and BP2 are two proximal breakpoints. BP3 is located at 15q13 and is the most common distal breakpoint, accounting for the majority of PWS deletions (Butler et al., 2004). BP4 and BP5 are two breakpoint regions, which have been mapped distally to BP3, and they appear to be more complex due to breakpoint variability (Pujana et al., 2002). PWS patients with deletions are divided into four types according to deletion sizes. Type I patients have a deletion from BP1 to BP2, type II a deletion from BP2 to BP3, type III a deletion from BP1 to BP4, and type IV a deletion from BP1 to BP5 (Varela et al., 2005). Type I and type II are two major deletion types, while type III and type IV contribute to 5% of PWS cases with deletion (Varela et al., 2005). Clusters of LCRs that can act as recombination substrates are located in each of the 15q11-q14 BPs and recombination between BPs has been postulated to mediate the recurrent genomic deletion/duplication on chromosome 15, causing PWS/AS (Angelman Syndrome) or 15q13.3 microdeletion syndrome (Sharp et al., 2008; Helbig et al., 2009). The recurrent 15q13.3 microdeletion syndrome is known to be associated with mental retardation and seizures, and the proximal breakpoint maps to the LCR at BP3 or BP4 while the distal breakpoint maps to the LCR at BP5 (Sharp et al., 2008). Atypical deletion and translocation are very rare in PWS. The analysis of patients with atypical deletion through genotype–phenotype comparisons will enable us to delineate the function of individual genes. Besides genomic deletion in 15q, maternal uniparental disomy (mUPD) is the other major cause of PWS (Mascari et al., 1992; Fridman & Koiffmann, 2000).

In this study, we have identified 28 PWS patients from Taiwan. We employed methylation specific polymerase chain reaction (PCR), cytogenetic analyses, microsatellite analyses, and array CGH (comparative genomic hybridization) to classify these patients. Our study reveals a similar genetic etiology of PWS between Taiwanese and Caucasian populations. However, unlike past genetic epidemiology studies of PWS involving Asian populations, this study distinguishes deletion subtypes.

In addition, we present analyses of two PWS patients with atypical deletions. Our findings support the notion that the genomic architecture of 15q is conserved among Taiwanese and Caucasian populations and thus predisposes people to similar risk for the genetic causes of PWS.

Method and Materials

Microsatellite and Recombination Junction Analyses

DNA samples from 28 patients and their parents were obtained after the parents signed the informed consents. Twenty-two microsatellites were analyzed from the following loci: D15S541, D15S542, D15S1035, D15S18, D15S543, D15S11, D15S113, D15S986, D15S1002, D15S1019, D15S815, D15S1048, D15S898, D15S1043, D15S1031, D15S165, D15S1013, D15S1010, D15S126, D15S984, D15S115, and D15S1007. The sequences of primers were obtained from NCBI. All of the forward primers were labeled with fluorescent tags. The original PCR products were diluted 15 times and 0.3 μ l of diluted PCR products were used for capillary electrophoresis. PCR products were mixed with 0.5 μ l of GeneScanTM500LIZTM size standard (Applied Biosystems, Carlsbad, CA, USA) and 9.5 μ l of Hi-DiTM formamide (Applied Biosystems). The mixture was heated at 95°C for 3 min to denature DNA and was then fractionized by capillary electrophoresis on a 310 genetic analyzer (Applied Biosystems). The signals were collected by the 310 data collection software and analyzed by GeneMapper 4.0 (Applied Biosystems). The breakpoints of the deletion in Patient 26 were amplified by PCR using primers F26 (5'GTTGGTGGGCTAATAGAAGAGACATG3') and R26 (5'GGTCTTGCAGGTGTGACATCAAGTAC3'), deriving from unique sequences adjacent to the Agilent Technologies' probe A_18_P12147421 and probe A_14_P104444 (Genomic Workbench Standard Edition 5.0, Lexington, MA, USA), respectively.

Methylation Specific PCR

DNA samples from patients were treated with the EZ DNA Methylation-GoldTM Kit (Zymo Research, Orange, CA, USA) according to the manufacturer's protocol. The bisulfite modified DNA was amplified using either the SNRPN-M or the SNRPN-P primer sets (Kubota et al., 1997). The PCR reactions were performed using Taq polymerase (Applied Biosystems), and the PCR conditions were: 6 min at 95°C, then 35 cycles of 30 s at 95°C, 30 s at 55°C and 30 s at 72°C, followed by 7 min at 72°C. PCR products were separated in a 2% agarose gel.

Array CGH

DNA from Patients 26 and 27 was molecularly karyotyped using Agilent SurePrint G3 Human CGH 1x1M Microarray (Agilent Technologies, Lexington, MA, USA). The aCGH experiments were performed according to the manufacturer's instructions. In brief, test and reference genomic DNA samples (1.5 μ g) were labeled with ULS-Cy5 and ULS-Cy3, respectively. The labeled DNA samples were purified, combined, denatured, and pre-annealed with Cot-1 DNA and blocking reagent. They were then hybridized to the arrays for 40 h in a 65°C rotating oven at 20 rpm. Hybridization and washes were performed according to the manufacturer's instructions. The arrays were scanned at 3- μ m

resolution with an Agilent C scanner. Images were analyzed with Feature Extraction Software V10.5 (Agilent Technologies).

Results

After performing a clinical diagnosis, molecular genetic studies were conducted on a group of Taiwanese PWS patients. In order to determine the parental origin and compare the size of deletion, we performed haplotype analysis on the 28 PWS patients and their parents, using 22 microsatellite markers across 15q11.2-q25.2. Genotypes obtained from the 28 PWS patients revealed 7 patients (25%) with type I deletion, 12 patients (42.9%) with type II deletion, 2 patients (7.1%) with atypical deletion, 6 patients (21.4%) with maternal UPD and 1 patient (3.6%) with an imprinting defect.

Patient 26 showed biparental inheritance for informative markers D15S543 and D15S1019, which are located telomeric to BP2 and BP3, respectively. Additionally, Patient 26 only inherited a single maternal allele from D15S1002, located centromeric to BP3 (Fig. 2A). These results suggest that the proximal breakpoint of the deletion maps telomeric to D15S543, while the distal breakpoint maps adjacent to BP3. Using array CGH, we confirmed that the deletion breakpoint in Patient 26 positions at around base 21.47 Mb, located between the *MAGEL2* and *NDN* genes at the centromeric end, and at around 25.6 Mb of chromosome 15, located between the *GABRG3* and *OCA2* genes at the telomeric end (Fig. 2B). The deletion junction fragment in Patient 26 was subsequently amplified as an 8.5-kb PCR product using primers F26 and R26 (Fig. 3A). Sequence analysis revealed that the deletion joined nucleotides 21476484 and 25610977 from chromosome 15 (nucleotide numbering according to the hg18 assembly of chromosome 15, NCBI build 36) with four bases of microhomology (AAAG) located on each side of the breakpoints (Fig. 3B). The deletion fused the open reading frame 2 (ORF2) of a long interspersed element (LINE1) from the centromeric end with the Class 1 endogenous retroviral (ERV) sequence long terminal repeat (LTR) from the telomeric end (Fig. 3A).

The 12.4-Mb deletion in Patient 27 starts centromeric to BP1 and ends within BP5, located between D15S1031 and D15S1010 (Fig. 1). Patient 27 inherited only a single maternal allele from five informative markers (D15S542, D15S11, D15S1019, D15S1048, and D15S1013), indicating that the deletion derived from the paternally inherited chromosome (Fig. 4A). Marker D15S542 showed only maternal contribution, suggesting that the proximal breakpoint of Patient 27 most likely occurred centromeric to BP2. Furthermore, marker D15S1031 showed maternal contribution while the informative marker D15S984 showed biparental inheritance (Fig. 4A), thus suggesting that the distal breakpoint maps between marker D15S1031 and D15S984. We performed array CGH to further refine the deletion breakpoints of Patient 27. In contrast to patients with typical type IV deletion, the proximal breakpoint of Patient 27 positions centromeric to BP1, while the distal deletion breakpoint maps within BP5, at around base 30.32 Mb on chromosome 15 (Fig. 4B). Despite multiple attempts, the exact deletion junction of Patient 27 could not be determined accurately, possibly because of the existence of adjacent repetitive sequences.

In our clinical samples, we also confirmed six mUPD cases, including five heterodisomic cases and one isodisomic case. These clinical cases were initially identified as PWS based on the clinical findings; however, cytogenetic analysis revealed normal karyotypes (not shown). Methylation specific PCR analysis at the SNRPN locus revealed that all of these patients lacked the paternal specific imprints (not shown). Subsequent investigation using 16 microsatellites throughout chromosome 15q revealed the potential meiotic origin of nondisjunction (ND) in all cases of UPD and also enabled us to identify a transition in marker state between heterodisomy and isodisomy (Fig. 5; Patients 3 and 4). Out of five PWS patients (Fig. 5; Patients 1–5) with heterodisomic 15q derived from the maternal chromosome 15, three showed heterodisomy throughout the entire region with no evidence of recombination (Fig. 5; Patients 1, 2, and 5), while the other two patients showed isodisomy at some loci and heterodisomy at other loci (Fig. 5; Patients 3 and 4). In Patient 3, two microsatellite markers, D15S986 and D15S113, displayed mono-allelic signal patterns; while in Patient 4, marker D15S984 indicated inheritance of the maternally derived heterodisomic 15q with either a deletion or a segmental isodisomy. We conducted quantitative PCR analyses on marker D15S986 for Patient 3 and D15S984 for Patient 4. The results showed no significant DNA dosage variation between the patient and control, suggesting that DNA recombination or conversion leads to segmental isodisomy on the maternally derived heterodisomic 15q (data not shown). Patient 6 is the only case where isodisomic 15q likely resulted from ND in the MII stage or an error during mitosis (Fig. 5). Microsatellite analysis of Patient 6 showed isodisomic inheritance at all of the markers tested, including eight informative markers: D15S542, D15S543, D15S11, D15S986, D15S1048, D15S1043, D15S126, and D15S984 (data not shown).

Patient 28 exhibited neither detectable deletion using array-CGH analyses (data not shown) nor UPD (Figs 6A and B). Methylation specific PCR failed to detect the unmethylated paternal SNRPN allele (Fig. 6C). This suggested that an imprinting defect leading to a biallelic methylation imprint resulted in a PWS phenotype. A normal control produced a 174-bp PCR product from the methylated maternal chromosome in addition to a 100-bp PCR product from the unmethylated paternal chromosome (Fig. 6C).

Discussion

It has previously been shown that there is a higher proportion of PWS deletion cases (84–96% compared to ~70%) and a smaller proportion of mUPD cases (15% compared to 25%) reported in Asian compared to that of Western populations (Ehara et al., 1995; Kim et al., 2004; Lin et al., 2007). In this study, we investigated 28 PWS patients from Taiwan and identified 21 patients (75%) with 15q11-q13 deletion, 6 patients (21.4%) with mUPD and 1 patient with an imprinting defect (3.6%). The reason for the difference between our data and previously reported data from Asian populations is unknown. However, our result is in line with the previously reported genetic epidemiology in Western populations (Holm et al., 1993; Robinson et al., 2000; Nicholls & Knepper, 2001; Fan et al., 2009). Additionally, it should be noted that similar to what has been reported from Western populations, we also observed more type II than type I deletion patients (12:7). Past studies involving Caucasians have indicated that BP2 is responsible for most of the proximal recombination in PWS deletion cases, whereas BP3 is the distal recombination

hot spot (Butler et al., 2004; Varela et al., 2005). It is likely that the basic genomic architecture is conserved between the two ethnic groups across human chromosome 15q11-q13 and therefore contributes to the similarity in genetic etiology observed among PWS patients.

Both deletion breakpoints of Patient 26 map outside of the common breakpoint regions and there are no additional or missing nucleotides at the deletion junction (Figs 2B and 3B). Patient 26 carries paternal copies of the *MKRN3*, *MAGEL2*, and *OCA2* genes, which are absent from PWS type II deletion patients. There is no extensive homology between the DNA sequences other than the four bases of microhomology identified at the junction. Unlike recurrent rearrangement, which is attributed to nonallelic homologous recombination (NAHR) associated with LCRs (Chen et al., 1997; Lupski, 1998), nonhomologous end joining (NHEJ) is likely to be the causal mechanism of this rearrangement. Our finding is consistent with the analysis of the DNA sequence at the deletion junction caused by NHEJ in the human dystrophin gene, which revealed that five of the fourteen cases contain a short homology (2–4 bp) at the end of the junctions (Toffolatti et al., 2002).

Studying recurrent and novel deletion breakpoints can provide insight into the molecular mechanisms that lead to particular genomic disorders. Patient 27 carries a 12.4-Mb deletion of 15q11-q13 that overlaps with the type IV PWS/AS deletion patient at the distal breakpoint, BP5, but the proximal breakpoint is located between BP1 and the centromere of chromosome 15. Recombination involving BP5 had previously only been detected in a translocation patient (Varela et al., 2004), in large inverted duplicated (*inv dup(15)*) chromosomes (Wandstrat et al., 1998; Roberts et al., 2003), in some cases of interstitial duplications and triplications of chromosome 15q11-q13 (Bolton et al., 2001; Ungaro et al., 2001; Roberts et al., 2002), in one case of AS (Sahoo et al., 2007) and one case of PWS (Varela et al., 2005), and in the recently discovered recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures (Sharp et al., 2008; Helbig et al., 2009). To our knowledge, recombination involving BP5 and the genomic region centromeric to BP1 has not been identified before. It has previously been shown that the minimum proximal boundary for BP1 in PWS subjects with type I deletion is at 18.68 Mb, whereas the most distal boundary is at 20.22 Mb (Butler et al., 2008). We have now shown that the proximal breakpoint of Patient 27 positions between the centromere and BP1 at 18.36 Mb, approximately 300 kb centromeric to the known minimum proximal boundary for BP1 in PWS subjects. Furthermore, array-CGH analysis of Patient 27 shows a further reduction of \log_2 ratio of hybridization signal from -1 to -2 at nucleotides 19.10–19.43 Mb (Fig. 4B). This reduction may possibly be a consequence of homozygous or mosaic microdeletion on the maternally inherited chromosome 15. A heterozygous deletion is expected to have a \log_2 ratio smaller or equal to -1 , while a homozygous deletion is expected to have a \log_2 ratio smaller than -2 (Albertson & Pinkel, 2003). Computational analysis of the sequence from the genomic region revealed that the closest LCR and the related *HERC2* homologous segment are located telomeric to the proximal breakpoint of Patient 27, spanning the 20.2–20.3 and 18.89–18.98 Mb regions, respectively. The exact nature of the breakpoints in this patient cannot be determined, but the recombination likely entails clustering of dispersed repeats at both sides of the deletion on chromosome 15.

Patient 27 has severe loss of speech, though his comprehension seems somewhat better than what he is able to express. His phenotypes include hypertelorism, relatively prominent forehead, down-turned mouth angles and other distinctive dysmorphic features, and severe mental retardation resembling that of patients with BP3–BP5 or BP4–BP5 microdeletions of 15q13.3 (Sharp et al., 2008; Helbig et al., 2009). However, the patient has not presented with any seizures.

In this study, 6 of the 28 PWS patients (21.4%) were identified as mUPD patients. Similarly, mUPD has been shown to occur in approximately 25% of PWS patients in Western populations (Robinson et al., 1991; Mascari et al., 1992; Woodage et al., 1994). The inheritance of both copies of chromosome 15 from the mother results in the absence of expression of paternal genes at 15q11-q13 and, consequently, the PWS phenotype. Most PWS cases with UPD identified from Western populations show maternal heterodisomy for chromosome 15 and are thought to result from ND in MI, followed by postzygotic correction of a trisomic embryo (Robinson et al., 1991; Mascari et al., 1992; Mutirangura et al., 1993a; Robinson et al., 1993b, 1998; Fridman & Koiffmann, 2000; Robinson et al., 2000). Consistent with previous studies from Western populations, five of six mUPD patients with PWS from this study were found to carry heterodisomic 15q that likely resulted from ND in MI. Only one isodisomic mUPD patient with PWS was identified. The isodisomic UPD15 detected in the PWS patients was presumably due to a maternal ND in MII, followed by the loss of the paternal chromosome; alternatively, isodisomic UPD15 may be caused by postzygotic errors (Engel & DeLozier-Blanchet, 1991; Cassidy et al., 1992; Purvis-Smith et al., 1992; Mascari et al., 1992; Engel, 1993; Robinson et al., 1993a,b; Mutirangura et al., 1993b). Advanced maternal age is known to correlate with the higher frequency of ND during female gametogenesis in PWS cases (Robinson et al., 1996).

In conclusion, our molecular analysis of 28 PWS patients from Taiwan suggests that there are no etiological differences between PWS patients from Asians and PWS patients from Western populations. However, further studies involving more Asian PWS patients are required to confirm this finding given the small number of PWS patients analyzed in the current study. It has been postulated that the size and degree of homology, as well as the orientation and distance between the homologous LCR segments, are possible susceptibility factors dictating why different deletion/duplication types occur with various frequency in genomic disorders (Chen et al., 1997; Lupski, 1998). Our findings support the notion that the genomic architecture of chromosome 15q is conserved among Taiwanese and Caucasian populations and thus predisposes people to similar risks for the genetic causes of PWS. Additionally, we present analyses of two PWS patients with atypical interstitial deletions of 15q. To date, recombination between LCRs in 15q has been shown to associate with various BP combinations involved in each PWS deletion type, including BP1–BP3 (type I), BP2–BP3 (type II), BP1–BP4 (type III), and BP1–BP5 (type IV) among PWS patients (Christian et al., 1995; Amos-Landgraf et al., 1999; Christian et al., 1999; Roberts et al., 2002, 2003), and BP1–BP2, BP2–BP4, BP3–BP4, BP4–BP5, and BP3–BP5 among patients with other neurogenetic disorders (Murthy et al., 2007; Sahoo et al., 2005; Sharp et al., 2008). We believe that the delineation of the genetic basis of PWS from different ethnic backgrounds and the precise positioning of breakpoints in recurrent and novel PWS deletion patients will have great implications on

our understanding of potential ethnic differences in genomic architecture and the causal mechanisms of unique genomic rearrangement.

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List of Figures

- Figure 1 Schematic diagram showing positions of common breakpoints (BP1–BP5), location of genes and microsatellite markers as well as deletions in patients. Only Ref Seq genes (<http://www.ncbi.nlm.nih.gov/projects/RefSeq/>) are shown and their positions and sizes are not drawn to scale.
- Figure 2 Haplotype and array-CGH analyses of Patient 26. (A) Haplotype analysis of proband and his parents. The deletion region is boxed. (B) Array-CGH profile of Patient 26. The \log_2 ratio of the signals from the 15q region indicates a deletion of approximately 4.1 Mb in size (21.47–25.60 Mb). Data show \log_2 (patient/control) (x axis) against probe position (y axis). The deletion region is indicated by half-transmitted red color. The proximal breakpoint of Patient 26 is located between MAGEL2 and NDN and the distal breakpoint is located between OCA2 and GABRG3.
- Figure 3 Detection of deletion junction in Patient 26. (A) The recombination event associated with deletion in Patient 26. The sequences involved in the recombination are ORF2 of LINE1 and LTR of ERV1. The deleted chromosome segment is depicted with a dotted line. The recombinant chromosome is shown below. The deletion junction was amplified by PCR using primers F26 and R26 from the genomic DNA of the patient, but not from the genomic DNA of the patient's father or the no template control (NTC). (B) The DNA sequence of the patient (middle) and sequences of the proximal (top) and distal (bottom) regions of chromosome 15 involved in the rearrangement are shown. The 4-bp homolog is boxed. Single nucleotide variation (C/T) and copy number variation (AAGACA) are denoted by an asterisk.
- Figure 4 Haplotype and array-CGH analyses of Patient 27. (A) Haplotype analysis of proband and his parents. The deletion region is boxed. (B) Array-CGH profile of Patient 27. The \log_2 ratio of the signals from the 15q region indicates a deletion of approximately 12 Mb in size (18.36–30.32 Mb). Data show \log_2 (patient/control) (x axis) against probe position (y axis). Deviations of probe \log_2 ratios from 0 are depicted by green (decrease) and red (increase) crosses. The proximal breakpoint of Patient 27 is located 300 kb centromeric to BP1 and the distal breakpoint is located within BP5.
- Figure 5 Schematic diagram of microsatellite genotyping results for UPD patients. Microsatellite analysis shows that Patients 1–5 have maternal heterodisomy and Patient 6 has maternal isodisomy. Segmental isodisomy is observed in Patients 3 and 4.
- Figure 6 Haplotype analysis and methylation specific PCR results of Patient 28. (A) Haplotype analysis of Patient 28 and his parents. Patient 28 carries no detectable deletion or mUPD. (B) The capillary electrophoresis profiles of

seven informative markers, D15S542, D15S11, D15S986, D15S1002, D15S1019, D15S1048, and D15S1010, are shown. Orange peaks represent GeneScan™-500LIZ™ size standard. (C) Methylation specific PCR from a normal control showed the 100-bp PCR product representing the unmethylated paternal allele, in addition to the 174-bp PCR product representing the methylated maternal allele. Consistent with the clinical diagnosis of PWS, Patient 28 showed only the 174-bp PCR product derived from the methylated maternal allele.

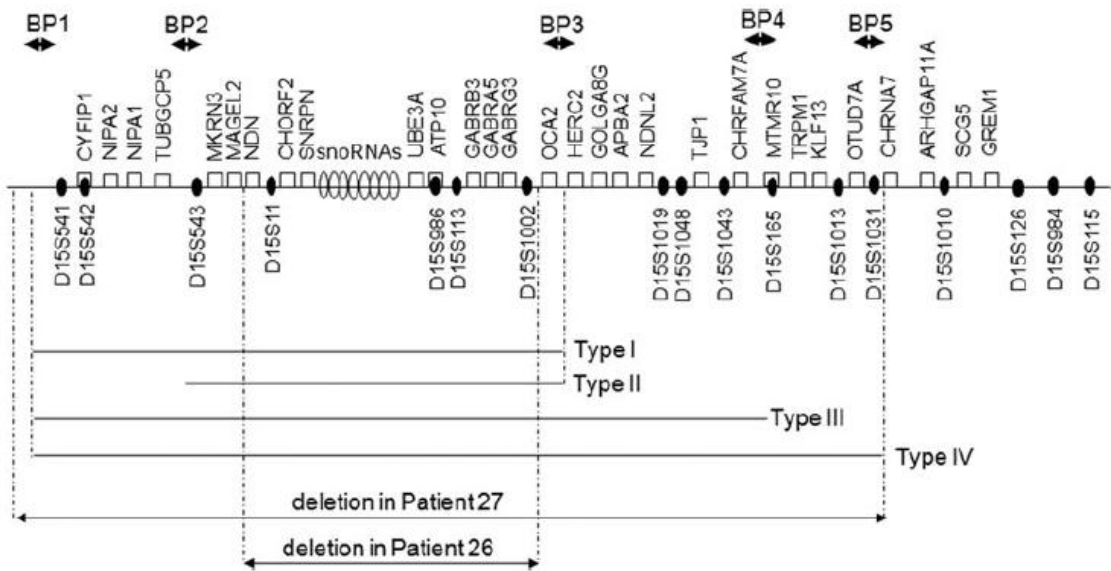


Figure 1

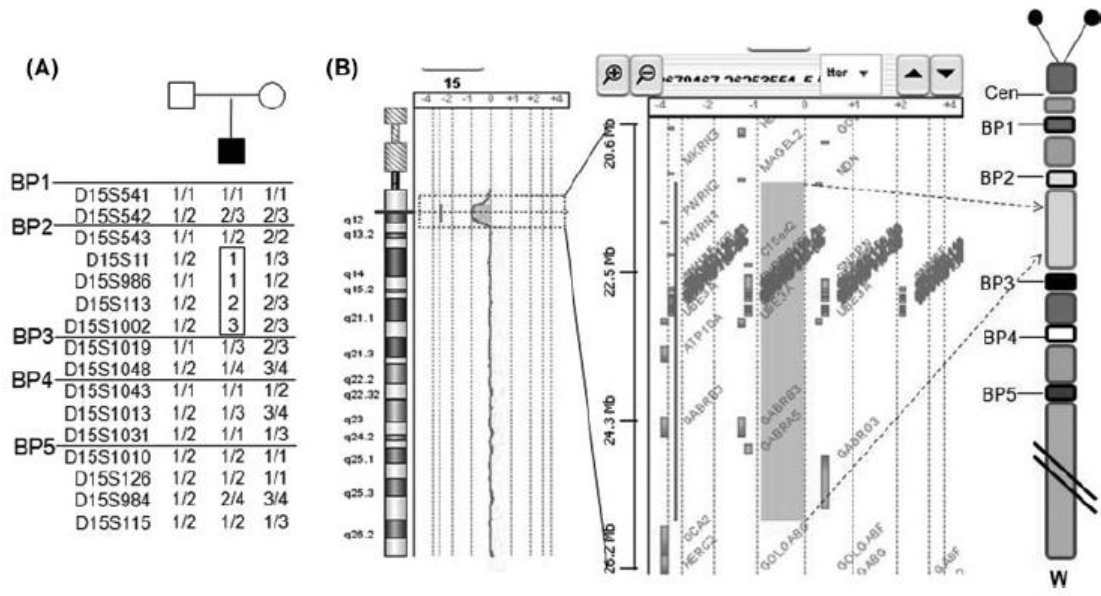


Figure 2

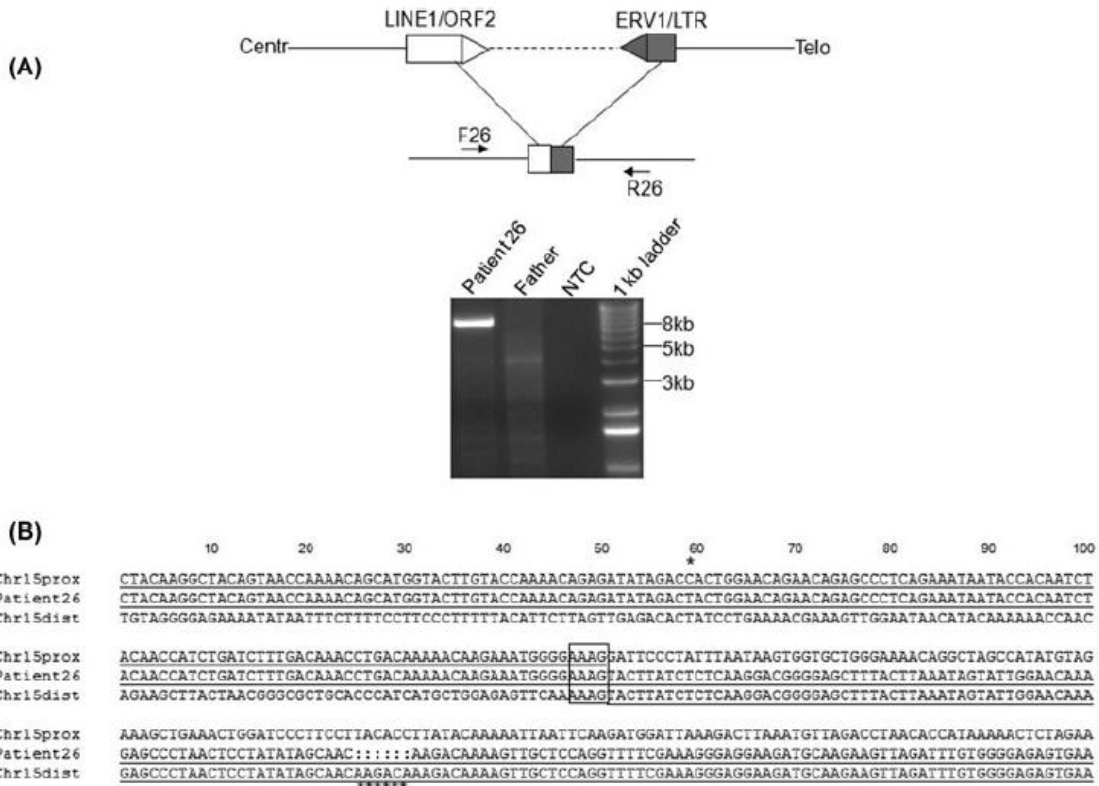


Figure 3

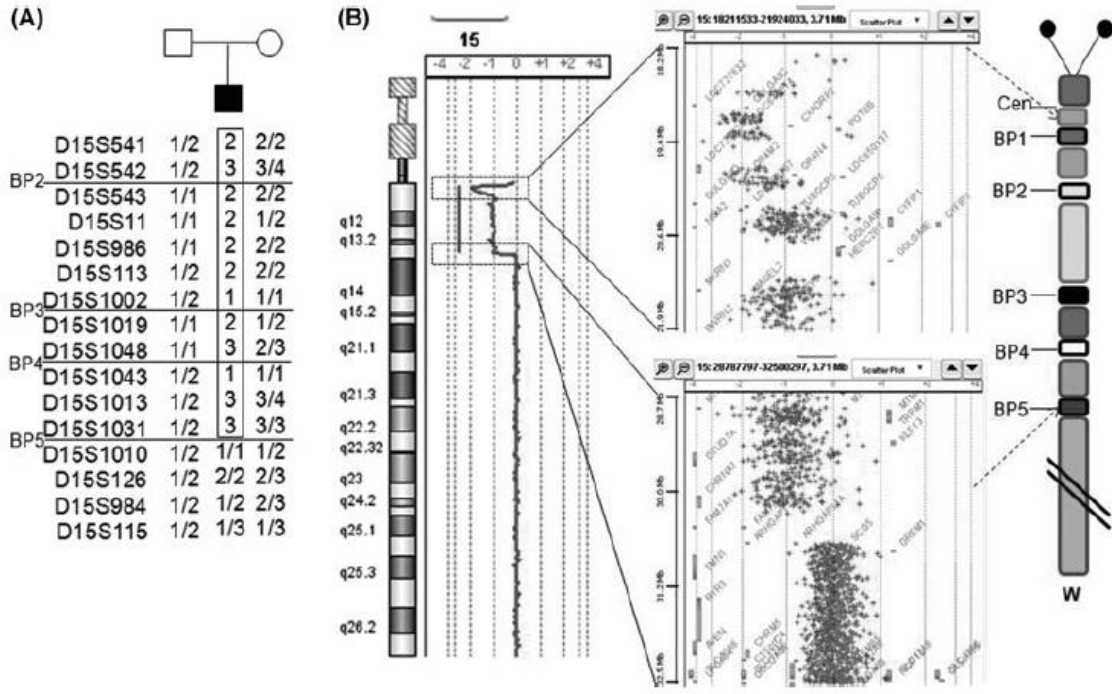


Figure 4

Marker	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
D15S541			noninformative		noninformative	isodisomy
D15S542			noninformative		noninformative	isodisomy
D15S543				noninformative	noninformative	isodisomy
D15S11			noninformative	noninformative		isodisomy
D15S986			isodisomy	noninformative		isodisomy
D15S113	noninformative		isodisomy			isodisomy
D15S1002			noninformative			isodisomy
D15S1019	noninformative					isodisomy
D15S1048	noninformative					isodisomy
D15S1043	noninformative				noninformative	isodisomy
D15S165			noninformative	noninformative	noninformative	isodisomy
D15S1031			noninformative	noninformative		isodisomy
D15S1010					noninformative	isodisomy
D15S126		noninformative				isodisomy
D15S984				isodisomy		isodisomy
D15S115		noninformative		noninformative		isodisomy




heterodisomy isodisomy noninformative

Figure 5

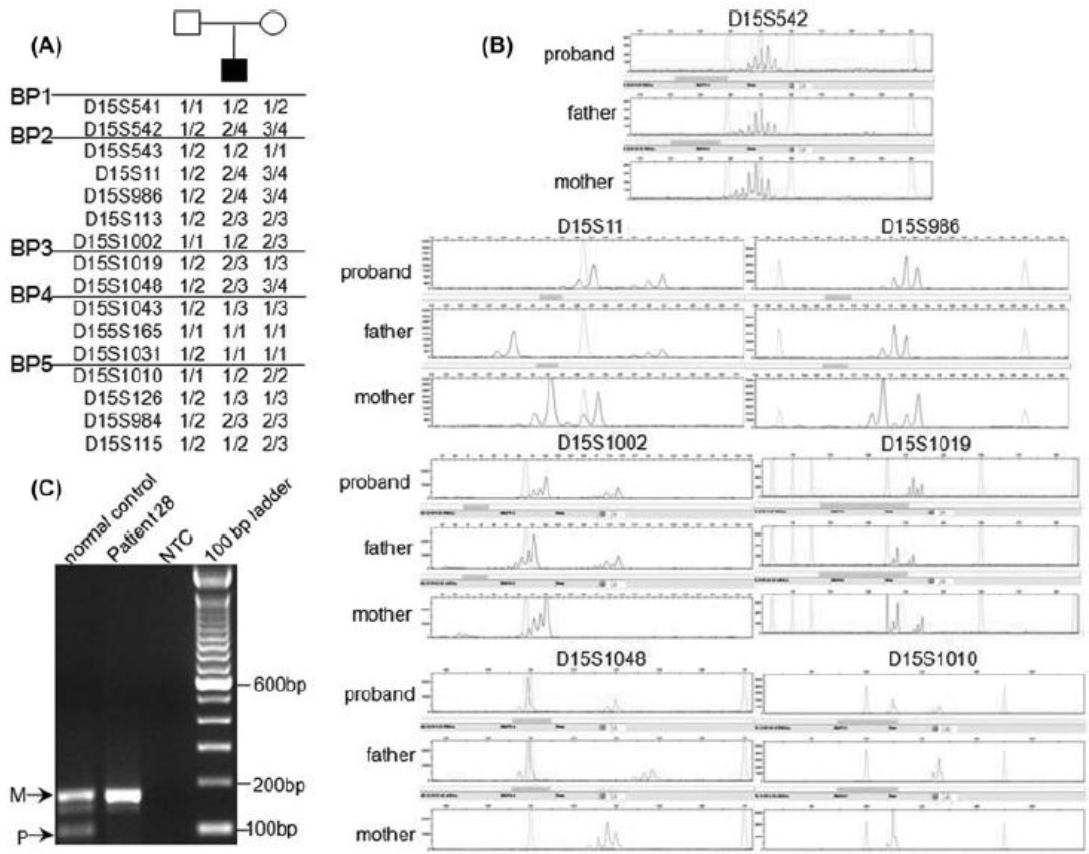


Figure 6