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Characterisation of interstitial duplications and triplications of chromosome 15q11–q13

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Abstract Chromosome 15 is frequently involved in the formation of structural rearrangements. We report the molecular characterisation of 16 independent interstitial duplications, including those of one individual who carried a duplication on both of her chromosomes 15, and three interstitial triplications of the Prader-Willi/Angelman syndrome critical region (PWACR). In all probands except one, the rearrangement was maternal in origin. In one family, the duplication was paternal in origin, yet appeared to segregate in a sibship of three with an abnormal phenotype that included developmental delay and a behavioural disorder. Ten duplications were familial, five de novo and one unknown. All 16 duplications, including two not visible by routine G-banding, were of an almost uniform size and shared the common deletion breakpoints of Prader-Willi syndrome and Angelman syndrome. Like deletions, the formation of duplications can occur in both male and female meiosis and involve both inter- and intrachromosomal events. This implies that at least some deletions and duplications are the reciprocal products of each other. We

observed no instances of meiotic instability in the transmission of a duplication, although recombination within the PWACR occurred in two members of the same family between the normal and the duplicated chromosome 15 homologues. All three triplications arose de novo and included alleles from both maternal chromosomes 15. Triplication breakpoints were more variable and extended distally beyond the PWACR. The molecular characteristics of duplications and triplications suggest that they are formed by different mechanisms.

Introduction

The proximal long arm of chromosome 15 is frequently involved in structural rearrangements, including deletions (Knoll et al. 1989), interstitial duplications (Browne et al. 1997) and triplications (Schinzel et al. 1994), and in the formation of supernumerary marker chromosomes (SMC(15); Crolla et al. 1995). Low-copy-number repeats (LCRs) have been identified within 15q in the vicinity of the commonly duplicated and deleted regions. LCRs permit unequal meiotic exchange to occur and recombination between repetitive sequences has been associated with several microduplication and microdeletion syndromes throughout the genome (Purandare and Patel 1997; Lupski 1998). The LCRs of chromosome 15 are known to be involved in the formation of deletions (Christian et al. 1999) and are also likely to mediate the other classes of structural rearrangement.

Deletions of 15q11–q13 account for approximately 70% of both Prader-Willi syndrome (PWS) and Angelman syndrome (AS) patients. The vast majority of deletions are of approximately uniform size, comprising the entire 4-Mb Prader-Willi/Angelman syndrome critical region (PWACR). There is one common distal breakpoint shared by over 95% of all deletions and two common proximal breakpoints that divide the deletions into type I (~40%) and type II (~60%; Amos-Landgraf et al. 1999; Christian et al. 1995). In contrast, the breakpoints of interstitial duplications have not yet been clearly defined.

Electronic database information:
Genome Database, <http://www.gdb.org>

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The PWACR contains a number of imprinted genes and the abnormal phenotypes associated with this region show various parent-of-origin effects. Deletions of 15q11-q13 in the paternal chromosome 15 cause PWS, whereas maternal deletions cause AS. Additional maternal copies of the PWACR produce an abnormal phenotype that is distinct from both PWS and AS and includes developmental delay, particularly with regard to speech and language problems, and behavioural problems sometimes falling within the autistic spectrum, with only mild or no dysmorphic features (Bolton et al. 2001). In contrast, paternal duplication carriers are generally unaffected (Browne et al. 1997; Cook et al. 1997; Bolton et al. 2001) and are only rarely associated with an abnormal phenotype (Mao and Jalal 2000; Mohandas et al. 1999).

In this study, we report the molecular characterisation of 16 independent interstitial duplications and three interstitial triplications with regard to their breakpoints and chromosomal origin. Individuals with additional copies of the PWACR display considerable clinical variability and

characterising the underlying rearrangements should define possible relationships between genotype and phenotype. Specifically, our aims have been: (1) to compare the proximal and distal breakpoints of the two classes of interstitial rearrangement; (2) to determine the parental and chromosomal origin; (3) to look for evidence of recombination and meiotic instability among familial duplications; (4) to relate the rearrangement breakpoints to sequence and recombination data.

Subjects and methods

Study population

Details of all the families studied are shown in Table 1. The probands were either ascertained through the Wessex Regional Genetics Laboratory or referred to us via a number of collaborators. One proband in family 5 carried a duplication on both chromosomes 15 and the proband of family 16 also carried a SMC(15) that did not contain the PWACR. All other families carried only a single rearrangement.

Table 1 Subjects

Family	Inheritance	Origin	Number	Carriers	Reference
Duplications					
1	Familial	Maternal	Two	Probands (twin daughters)	Bolton et al. (2001) Family 1 ^b , Browne et al. (1997) Family 1 ^b
		Paternal	One	Mother ^a	
2	Familial	Maternal	Five	Proband, 2 siblings, mother, maternal aunt	Bolton et al. (2001) Family 2 ^b , Browne et al. (1997) Family 2 ^b
		Paternal	One	Maternal grandmother ^a	
3	Familial	Maternal	One	Proband	Bolton et al. (2001) Family 3 ^b , Browne et al. (1997) Family 3 ^b
		Paternal	One	Mother ^a	
4	Familial	Maternal	Four	Proband, mother, 2 maternal uncles	Bolton et al. (2001) Family 4 ^b , Browne et al. (1997) Family 4 ^b
		Paternal	One	Maternal grandmother ^a	
5	Familial	Maternal	Two	1st Proband, half-sister	Bolton et al. (2001) Family 5 ^b
	Unknown	Both	One	2nd Proband	
6	De novo	Maternal	One	Proband	Bolton et al. (2001) Family 6 ^b , Thomas et al. (1999) Family 2 ^b
7	De novo	Maternal	One	Proband	Thomas et al. (1999) Family 1 ^b
8	Familial	Maternal	One	Proband	–
		Paternal	One	Mother	–
9	Familial	Maternal	Two	Proband, mother ^a	–
10	Familial	Maternal	One	Proband	–
		Paternal	One	Mother	–
11	De novo	Maternal	One	Proband	Willatt et al. (2000)
12	Familial	Maternal	Four	Proband, 2 siblings, mother ^a	–
13	Familial	Paternal	Three	Proband, sibling, father ^a	–
14	De novo	Maternal	One	Proband	–
15	De novo	Maternal	One	Proband	–
Triplications					
16	De novo	Maternal	One	Proband	Long et al. (1998)
17	De novo	Maternal	One	Proband	–
18	De novo	Maternal	One	Proband	–

^aOrigin determined by methylation-specific PCR assay

^bRefers to the family number given in the previous study

Cytogenetics

Karyotypes were determined by analysis of G-banded metaphase chromosomes harvested from peripheral blood lymphocytes. Fluorescence in situ hybridisation (FISH) studies were based on the standard method of Pinkel et al. (1988). Hybridisation signals from biotin- and digoxigenin (dig)-labelled probes were detected by using either single layers of avidin-fluorescein isothiocyanate (for biotin) or an anti-dig-tetramethylrhodamine isothiocyanate and analysed on a conventional Zeiss epi-fluorescent Axiophot microscope. Images were captured by a cooled charge-coupled device camera (Photometrics) and enhanced and analysed by using Applied Imaging MacProbe software.

Molecular studies

DNA was extracted from peripheral blood and buccal cells by a salt-precipitation technique (Miller et al. 1988). The polymerase chain reaction (PCR) was performed under standard conditions with primers spanning proximal 15q. All primers and conditions are available from the Genome Database <http://www.gdb.org>.

A quantitative fluorescent PCR assay was used to determine copy number at the locus D15S63 (Kuwano et al. 1992) and within the UBE3A gene (Malzac et al. 1998) by dividing the peak heights of both D15S63 and UBE3A products by the peak height of a single control product amplified from within the DP5.2 gene on chromosome 5 (Grodén et al. 1991). All three primer pairs were co-amplified in the same reaction and one primer from each pair was fluorescently labelled so that the products could be analysed on an ABI 377 automated DNA sequencer (Perkin Elmer).

Methylation-specific PCR assay

The parental origin of the duplications was determined by methylation-specific PCR (Zeschnick et al. 1997). The common primer was fluorescently labelled to allow quantification of the specific maternal and paternal products. When parental DNA was unavailable, this technique was used to determine parental origins of the duplication (indicated by superscript "a" in Table 1).

Nomenclature

Type I deletions/duplications are defined as having proximal breakpoints between the centromere and the two loci D15S541/D15S542, whose relative order is unknown (Christian et al. 1995). Type II abnormalities do not include D15S541/D15S542 and have breakpoints between these loci and D15S543.

Duplications and triplications are referred to as interstitial. An abnormality with an intrachromosomal origin has arisen from a single chromosome and, in the absence of parents, this is assumed to be the causative mechanism for cases with identical alleles at all loci tested within the duplication. An interchromosomal error involves both homologues of the transmitting parent. This is assumed to be the causative mechanism when three different alleles are observed at one locus, even if parental DNA is unavailable for confirmation.

Results

Cytogenetics

Routine cytogenetic analysis, at a band level of over 550, was able to identify all duplications and triplications except those in families 7 and 13. PWACR duplications in these individuals were subsequently detected by PCR and/or FISH during additional studies undertaken because of

their abnormal phenotypes. FISH and/or PCR analysis also showed that, in all cases, the duplications and triplications involved the PWACR and were not pseudogene expansions. Retrospective cytogenetic analysis in families 7 and 13 did not identify these duplications clearly. The size of the pericentromeric area of 15q varies greatly within the normal population and this can make duplications of the PWACR difficult to observe cytogenetically. A slight difference in the pericentromeric region was observed in both families but this was considered to be within the limits of variation seen in normal individuals. FISH analysis in family 16 showed that, in this triplication, the middle repeat was inverted (Long et al. 1998).

Characterisation of breakpoints

Breakpoint analysis was performed by using PCR at microsatellite loci throughout 15q11-q14 and the results for all 18 families are shown in Fig. 1. The interpretation of results for families 5 and 8 was complex (see later) but, including these families, all duplications appeared to have a single distal breakpoint between D15S217 and D15S1019. Proximally, there were exactly equal numbers of type I and type II breakpoints. In contrast, the breakpoints of the triplications were more variable, although, by molecular analysis, the three copies of the PWACR present in each triplication appeared to be the same size as one another.

Parental and chromosomal origins

Parental origins of all duplications and triplications were determined from microsatellite data and/or by using methylation specific PCR. Both methods were in agreement whenever tested in the same individual. In 17 of 18 families, the proband(s) carried additional maternal copies of the PWACR (in family 5, one of the two probands carried both a maternal and a paternal duplication). In the remaining family (family 13), the proband's duplication was of paternal origin. In 10 of the duplication families, one or more relatives carried the same duplication, which was either maternally or paternally inherited. There was only one family in which we could directly demonstrate the founder mutation (family 8). Therefore, we ascertained six demonstrable de novo duplications, five of maternal origin (all in probands) and one of paternal origin (proband's mother, family 8; see Table 2). There was also a paternal duplication of unknown origin in a proband in family 5 and, of the nine familial cases in which the founder mutation could not be demonstrated, the earliest generation tested carried a paternally inherited duplication in six cases and a maternally inherited duplication in three. Therefore, while duplications arise in both male and female meioses, the relative frequency of de novo duplications in each is unclear.

Eleven of the 16 duplications appeared to have arisen from an interchromosomal event, whereas the remaining five cases had an intrachromosomal origin. Eight of the

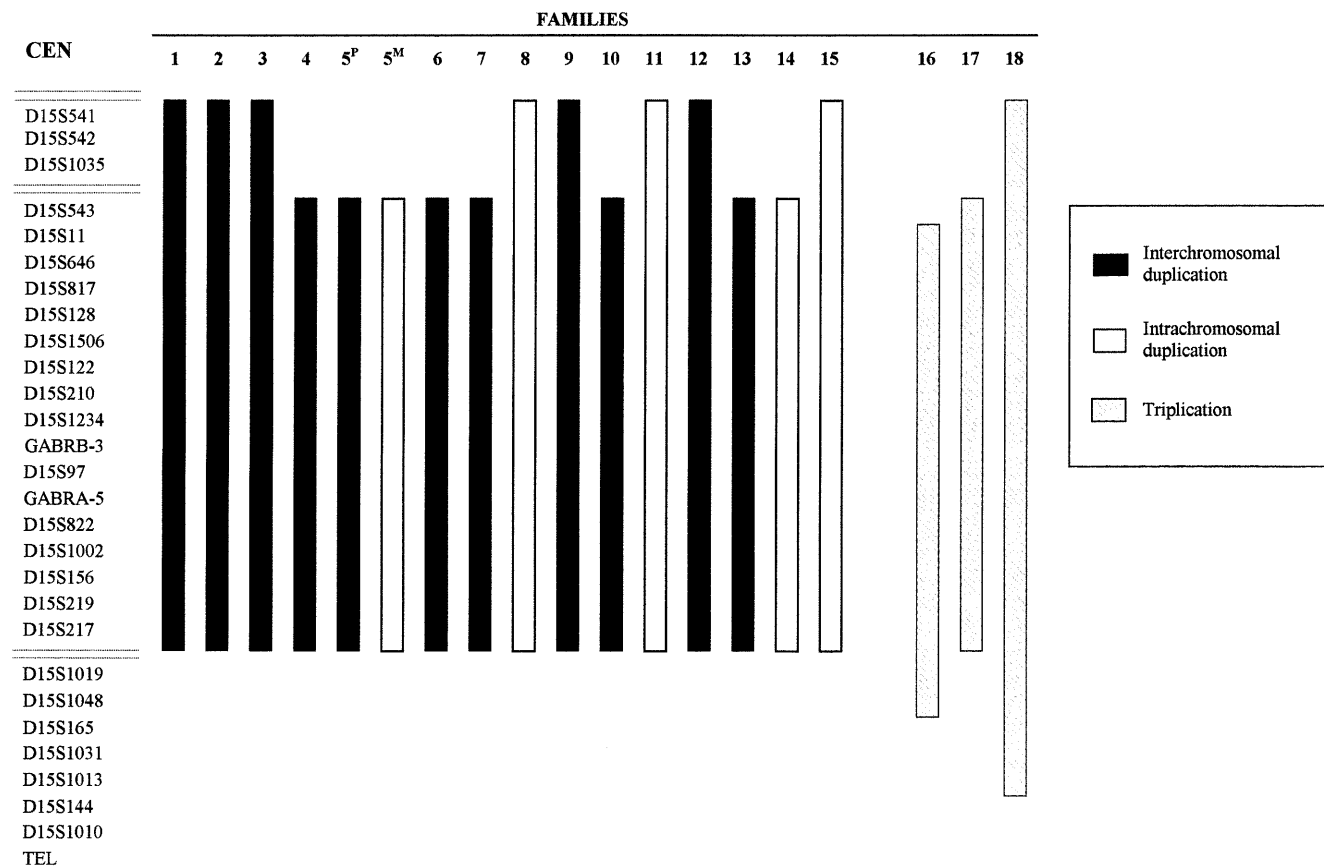


Fig. 1 Origin and breakpoints of all duplications and triplications studied. Microsatellite markers are shown in order from centromere to telomere. *Dotted lines* show the positions of the common breakpoints, 5^p , 5^m duplications of paternal and maternal origin respectively in this family

interchromosomal duplications were familial, two de novo and one unknown, whereas among the intrachromosomal duplications, two were familial and three de novo (see Table 3).

All three triplications had arisen de novo and microsatellite data showed that all the triplications included alleles derived from both maternal chromosome 15 homologues.

Meiotic instability

There has been one report of an interstitial duplication that expanded during meiosis when transmitted from a carrier mother to her son (Guererri et al. 1999). Among our ten familial cases, there was no evidence for meiotic instability: duplication size appeared to be unchanged in a total of 30 meioses. In familial cases, it is also possible to follow the transmission of the duplicated alleles through a family. In family 4, we observed two instances of recombination between duplicated and normal chromosome 15 homologues in the five meioses studied (see Fig. 2).

Table 2 Chromosomal origins of duplications: de novo cases

Family number	Parental origin	Breakpoints	Chromosomal origin
6	Maternal	Type II	Inter
7	Maternal	Type II	Inter
8 ^a	Paternal	Type I	Intra
11	Maternal	Type I	Intra
14	Maternal	Type II	Intra
15	Maternal	Type I	Intra

^aThe mother of the proband in family 8 whose duplication is known to have originated on her paternal chromosome 15

Table 3 Chromosomal origins of duplications: all cases

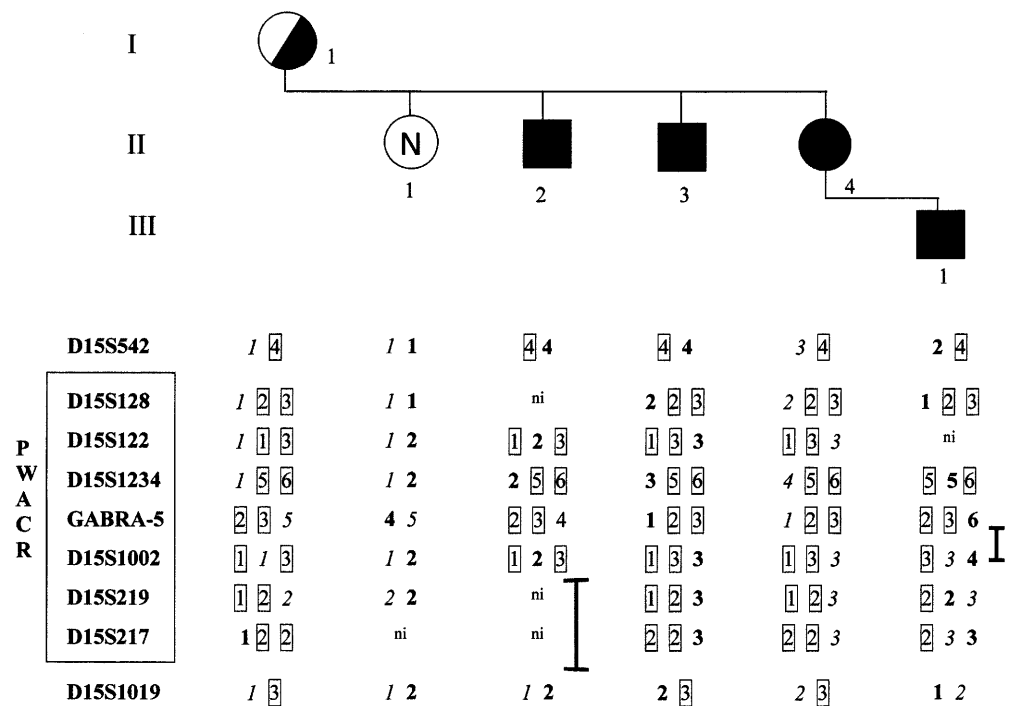
Duplications	Interchromosomal	Intrachromosomal
Familial	8	2 ^a
Unknown	1	0
De novo	2	4 ^a
Total	11	5

^aThis duplication is included as de novo and familial, hence the discrepancy in the total given under *Intrachromosomal*

Family 5

By FISH, two of the three half-sisters had a single interstitial duplication of the PWACR, whereas the third sister had two interstitial duplications, one on each chromosome

Fig. 2 Pedigree and allele data for family 4 showing recombination between duplicated and normal chromosome 15 homologues in individuals II-2, between D15S1002 and D15S1019, and III-1, between GABRA5 and D15S1002 (bars). *Italic, bold and boxed numbers* represent different haplotypes, *italic* normal, *boxed* duplication, *bold* paternal alleles from the unaffected father. *Black filled symbols* indicate maternally inherited duplication carriers, *half-filled symbol* paternally inherited duplication carrier, *N* normal individual, *ni* non-informative alleles



15 homologue. Unfortunately, no parental DNA was available. Quantitative PCR confirmed the presence of three and four copies of the PWACR, respectively. The parental origin of the single duplications was shown to be maternal by methylation-specific PCR. In the sister with two abnormal chromosomes 15, the ratio of the amplified maternal and paternal bands was normal. This excludes maternal uniparental disomy (UPD) and so we have interpreted this as inheritance of an interstitial duplication, i.e. two copies of the PWACR from each parent. Both maternal and paternal duplications were the same size with type II proximal breakpoints. At each locus within the duplicated region, the maternal alleles common to the three sisters could be subtracted, leaving the remaining paternal allele(s). All informative alleles within the common maternal duplication were homozygous, implying an intrachromosomal origin. For the paternal duplication, the alleles were heterozygous at D15S543 and D15S11, implying an interchromosomal origin and confirming that the two duplications arose independently. Multiple rearrangements involving proximal 15q have been reported previously, most frequently involving SMC(15) in association with another abnormality (Long et al. 1998; Abeliovich et al. 1995). However, in these previously reported cases, the rearrangements were likely to have arisen through a common mechanism.

Family 8

The proband and his mother were known to have an interstitial duplication from FISH and quantitative PCR. The mother carried an intrachromosomal type I duplication transmitted de novo from her father. Interpretation of the

microsatellite data in the proband was complicated by a segment of homozygosity, approximately 20 cM in length and including most of the 4-Mb PWACR cassette. Between D15S11 and D15S217, the proband inherited the same haplotype from his father and maternal grandfather. Consanguinity between the proband's parents was denied. However, this most probably represents autozygosity from the previous mating of two distantly related individuals who shared a chromosome segment identical by descent. Both parents transmitted this segment so that the proband received three copies of the same ancestral haplotype between D15S11 and D15S217. Such long homozygous segments, up to 77 cM in length, may be relatively common in the human genome (Broman and Weber 1999) but have not been previously reported for chromosome 15.

Family 13

The proband inherited her duplication from her father. Her phenotype included developmental delay and a behavioural disorder. She was originally referred in 1997, aged 19 months, with global developmental delay and was found to have a normal karyotype. Fragile X was also excluded. The duplication was subsequently identified during a research study of patients attending an autism assessment clinic where she was found to have an autistic spectrum disorder with difficulty in the areas of social interaction and repetitive stereotyped patterns of behaviour. The duplication was also present in one elder sister, aged 8, who had speech and language delay and who is awaiting assessment for persistent behavioural difficulties. It was not present in the third sister, aged 10, who had delayed

early milestones, academic difficulties, which now appear to have resolved, and no behaviour disorder.

Discussion

Rearrangements involving proximal 15q are relatively common and are thought to involve unequal meiotic recombination between repeat sequences flanking the PWACR. However, we show that interstitial duplications and triplications involving the PWACR are different in terms of their size and chromosomal origin. Therefore, the formation of chromosome 15 rearrangements is likely to be complex and to involve several different mechanisms. Interestingly, in one proband, the duplication was paternal in origin. Paternal duplications are only rarely associated with an abnormal phenotype (Mao and Jalal 2000; Mohandas et al. 1999). However, the identification of an additional case suggests that this association may not be coincidental. In the remaining 17 families, the probands carried additional maternal copies of the PWACR.

Breakpoints

Interstitial duplications share the same common breakpoints with deletions, whereas triplications include sequences distal to the PWACR and share breakpoints with large SMC(15). In contrast to our finding of a uniform duplication size, some breakpoint heterogeneity has been reported previously (Bundey et al. 1994; Robinson et al. 1998; Guerreri et al. 1999). This may be attributable to incomplete breakpoint determination or the misinterpretation of triplications as duplications, because these abnormalities are virtually indistinguishable by standard G-banding. All four triplications characterised by Ungaro et al. (2001) were initially interpreted and/or reported as duplications, as was one of ours. However, in exceptional cases, other duplication breakpoints may exist and we cannot exclude breakpoint heterogeneity below the level of mapping with microsatellites.

Formation of rearrangements

Approximately 2/3 of the interstitial duplications were interchromosomal in origin, the remaining 1/3 being intrachromosomal. The formation of duplications in both male and female meiosis can involve either two chromosome 15 homologues or two sister chromatids from a single chromosome 15. Since we have observed recombination between duplicated and normal chromosome 15 homologues, it is possible that some of the inherited duplications that appear to be interchromosomal may have arisen from an intrachromosomal event that recombined with a normal chromosome 15 during a subsequent meiosis. Since deletions share the same breakpoints and are formed via the same mechanisms as duplications (Carrozzo et al. 1997; Robinson et al. 1998), some, if not all duplications and

deletions, are the reciprocal products of each other. Therefore, unlike rearrangements in Charcot-Marie-Tooth type I or hereditary neuropathy with liability to pressure palsies, duplications and deletions of the PWACR can occur in both male and female meiosis and involve both inter- and intrachromosomal events.

In addition to chromosome 15, interstitial triplications have been reported for chromosomes 2, 5, 7 and 9. In all patients tested, the triplication was maternal in origin. Proposed mechanisms include U-type exchanges among three chromatids (Wang et al. 1999) or through several different meiotic events occurring sequentially (Rivera et al. 1998). Alternatively, triplications may be formed via illegitimate recombination between a normal chromosome 15 and an unstable large SMC(15) intermediary (Schinzel et al. 1994). This is supported by the location of triplication breakpoints and the presence of a PWACR negative SMC(15) in the proband of family 16 (Long et al. 1998). Presumably, the formation of a triplication via an SMC(15) would result in a triplicated segment carrying alleles from both chromosome homologues of the transmitting parent, even if the SMC(15) intermediary was itself intrachromosomal in origin (Wandstrat and Schwartz 2000).

Unequal recombination is involved in the formation of all unbalanced inter- and intrachromosomal rearrangements. However, although the level of recombination in proximal 15q is well above the genome average, especially given the proximity to the centromere, the common breakpoints are not sites of recombination hotspots (Robinson et al. 1993; Robinson and Lalande 1995). Therefore, it appears that the PWACR rearrangements are not caused by an overall excess of recombination, but rather that, when recombination does occur at these sites, it is highly prone to errors. Similarly, although male and female recombination in proximal 15q is very different, interstitial duplications and deletions can occur in both male and female meioses.

Clinical phenotype associated with additional copies of the PWACR

The abnormal phenotype associated with interstitial duplications is highly variable, even within the same family (Bolton et al. 2001). However, this cannot be explained on the basis of gene content, because of the practically uniform size of duplications. For triplications and large SMC(15), there is also no clear relationship between rearrangement size and clinical severity (Mignon et al. 1996; Webb et al. 1998). The normal phenotype associated with most paternal duplications (Cook et al. 1997; Schroer et al. 1998; Guerreri et al. 1999; Bolton et al. 2001) suggests that the causative genes are imprinted and expressed only from the maternal allele. This is slightly paradoxical because, whereas there are seven well-characterised genes and at least nine other transcripts that are paternally expressed in this region (Lee and Wevrick 2000), there are only two maternally expressed genes, viz. UBE3A (Rougelle et al. 1997; Vu and Hoffman 1997) and ATP10C (Meguro et al.

2001), and one putative maternally imprinted transcript (Lee and Wevrick 2000).

Abnormal phenotypes have previously been reported for two paternal duplications (Mao and Jalal 2000; Mohandas et al. 1999) and two paternal triplications (Cassidy et al. 1996; Ungaro et al. 2001). There is further evidence from our families that additional paternal copies of the PWACR may cause an abnormal phenotype. In family 13, a paternal duplication appears to segregate in a sibship of three with an abnormal behavioural phenotype. Additionally, in family 5, the individual carrying a paternal and a maternal duplication has a more severe phenotype than her two half sisters who carry only a maternal duplication (Bolton et al. 2001). However, affected individuals are generally ascertained with non-specific developmental delay and, since the abnormal phenotype associated with additional maternal copies of the PWACR is so variable, it is not possible to determine unequivocally whether additional paternal copies of the PWACR are coincidental to the phenotype in these few individuals or actually causative. It is possible that certain paternal rearrangements cause an abnormal phenotype because of an associated imprinting defect, whereby a relaxation of imprinting control also allows some expression of maternally imprinted genes. Duplications and triplications, with insertion of 4 Mb and 8 Mb genomic DNA, respectively, could alter chromatin structure so that a particular region partially escapes imprinting control (Muralidhar et al. 1999). Expression studies will be required to test the hypothesis that imprinting defects underlie the abnormal phenotypes in paternal duplication carriers.

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