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Molecular mechanism for duplication 17p11.2—the homologous recombination reciprocal of the Smith-Magenis microdeletion

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Recombination between repeated sequences at various loci of the human genome are known to give rise to DNA rearrangements associated with many genetic disorders[1]. Perhaps the most extensively characterized genomic region prone to rearrangement is 17p12, which is associated with the peripheral neuropathies, hereditary neuropathy with liability to pressure palsies (HNPP) and Charcot-Marie-Tooth disease type 1A (CMT1A; ref. 2). Homologous recombination between 24-kb flanking repeats, termed CMT1A–REPs, results in a 1.5-Mb deletion that is associated with HNPP, and the reciprocal duplication product is associated with CMT1A (ref. 2). Smith-Magenis syndrome (SMS) is a multiple congenital anomalies, mental retardation syndrome associated with a chromosome 17 microdeletion, del(17)(p11.2p11.2) (refs 3,4). Most patients (>90%) carry deletions of the same genetic markers and define a common deletion[5–7]. We report seven unrelated patients with *de novo* duplications of the same region deleted in SMS. A unique junction fragment, of the same apparent size, was identified in each patient by pulsed field gel electrophoresis (PFGE). Further molecular analyses suggest that the *de novo* 17p11.2 duplication is preferentially paternal in origin, arises from unequal crossing over due to homologous recombination between flanking repeat gene clusters and probably represents the reciprocal recombination product of the SMS deletion. The clinical phenotype resulting from duplication [dup(17)(p11.2p11.2)] is milder than that associated with deficiency of this genomic region. This mechanism of reciprocal deletion and duplication via homologous recombination may not only pertain to the 17p11.2 region, but may also be common to other regions of the genome where interstitial microdeletion syndromes have been defined.

We have shown previously that recombination between flanking repeat gene clusters (SMS–REPs) leads to the SMS deletion by identifying a novel junction fragment of the same apparent size in multiple patients[8]. Several independent studies have also identified repeat gene clusters flanking common microdeletion breakpoints in Williams[9–11], Prader-Willi/Angelman[12,13] (PWS/AS) and DiGeorge/velocardiofacial[14,15] (DGS/VCFS) syndromes. It is probable that the flanking repeat gene clusters observed in these microdeletion syndromes predispose to homologous recombination events, making those regions susceptible to chromosome deletion, as is the case for SMS. We investigated the hypothesis that homologous recombination and unequal crossing over between SMS–REPs causes duplication of 17p11.2 as the reciprocal event of the SMS deletion.

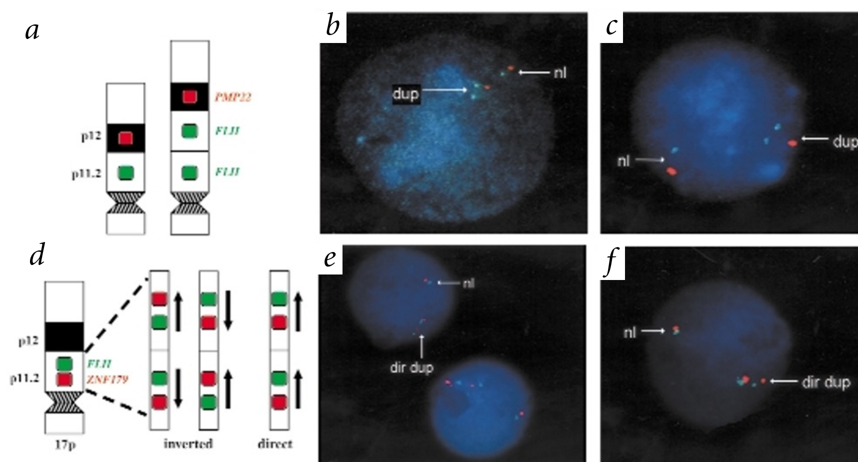

Fig. 1 Two-colour FISH analysis using *PMP22*, *FLII* and *ZNF179* probes. The *PMP22*-containing probe was detected with anti-digoxigenin conjugated to rhodamine (red), the *FLII* cosmids were labelled with biotin and detected with avidin conjugated to fluorescein isothiocyanate (green). The interphase nuclei were counterstained with DAPI (blue). ***a***, Idiogram of chromosome 17p with location of FISH probes. Left, normal chromosome 17. Right, duplication of 17p11.2, the region containing *FLII*. FISH results from lymphoblast cell lines of patients 990 (***b***) and 1192 (***c***) are shown. The normal chromosome 17 (nl) displayed one red and one green signal, and the abnormal chromosome (dup) displayed one red signal and two green signals, indicating a duplication of the *FLII* locus. ***d***, Ideogram of chromosome 17p. Left, 17p with the *FLII* and *ZNF179* locations indicated within 17p11.2. Right, three diagrams of hypothetical orientations for duplication including *FLII* and *ZNF179* loci. A direct duplication of 17p11.2 revealed a distinct pattern by FISH. FISH results from patients 1251 (***e***) and 1192 (***f***) are also shown. The *ZNF179* locus was detected with anti-digoxigenin conjugated to rhodamine (red), whereas *FLII* was detected as above. The normal chromosome 17 (nl) displayed one red and one green signal, whereas the abnormal direct dup(17)(p11.2p11.2) chromosome (dir dup) showed a red-green-red-green signal pattern. Patient 1006 (not shown) was previously reported to have a tandem duplication of 17p11.2 on one chromosome 17, and a deletion of *PMP22* on the homologous chromosome[27].

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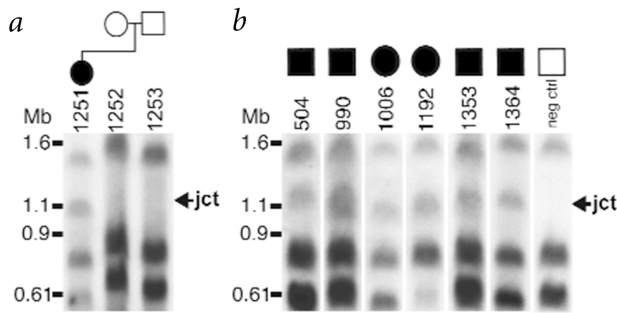

Fig. 2 PFGE detection of novel 17p11.2 duplication junction fragments. PFGE analysis detected the 17p11.2 duplication junction fragment in seven unrelated 17p11.2 duplication patients. The Southern blot was hybridized with a *CLPSMCR* cDNA probe, which identified an ~1.1-Mb junction fragment (arrow, jct) present in duplication patients. ***a***, The *de novo* junction fragment in one family. ***b***, A similar size junction fragment in all duplication patients examined. *Saccharomyces cerevisiae* chromosomes were used for size markers. Pulsed field data on 990 and 1006 were previously reported[8,27].

Chromosome analysis revealed an apparently increased band size for the 17p11.2 region in seven patients ascertained for developmental delay. We performed FISH analysis with proximal 17p probes. The myelin gene *PMP22* maps within the 1.5-Mb region in 17p12 that is typically duplicated in CMT1A (ref. 16). *FLII*, the human orthologue of *Drosophila melanogaster flightless-I* (*fliI*), maps to the SMS region in 17p11.2 and is deleted in all patients with SMS (refs 17,18). FISH studies indicated duplication of the SMS region, but not the CMT1A region, in all seven patients (Fig. 1*a–c*, and data not shown). We used two probes specific for the SMS common deletion region, *FLII* and *ZNF179* (ref. 19), to distinguish direct versus inverted duplications (Fig. 1*d*). Unequal crossing-over of nonallelic, directly repeated sequences between sister chromatids or between two homologous chromosomes is predicted to generate tandem duplication. FISH results showed direct (tandem) duplications in all seven patients tested (Fig. 1*e*,*f*, and data not shown). These results are consistent with the predicted outcome of unequal crossing over between direct repeats, causing a tandem duplication of 17p11.2, and inconsistent with an inverted duplication.

We used PFGE analyses with an SMS–REP probe to identify a rearrangement-specific junction fragment. SMS–REP is a region-specific, low-copy repeat gene cluster that contains at least four genes, *CLPSMCR* (for coactosin-like protein from the Smith-Magenis critical region), *TRESMCR*, *KERSMCR* and *SRPSMCR*, and appears to span more than 200 kb (ref. 8). Southern analyses of *Not*I-digested genomic DNA from the seven 17p11.2 duplication patients and their parents, using the same *CLPSMCR* probe that revealed an SMS deletion junction fragment[8], identified an approximately 1.1-Mb *de novo* junction fragment in all patients (Fig. 2*a*, and data not shown). PFGE analysis using a somatic cell hybrid cell line retaining the duplication chromosome detected the *de novo* junction fragment (data not shown). The junction fragment appears to be identical in size in all patients, within the limits of resolution of PFGE analysis (Fig. 2*b*). Two patients with cytogenetic duplications involving 17p12, who were also shown to be duplicated for the *PMP22* probe using FISH (ref. 20), did not show evidence for a duplication-specific junction fragment using the *CLPSMCR* probe (data not shown), indicating that DNA recombined in these larger duplications is distinct from that for dup(17)(p11.2p11.2).

We determined the parental origins and the mechanism for duplication of 17p11.2 by microsatellite marker analysis (Fig. 3). Among the seven duplication patients, six families were informa-

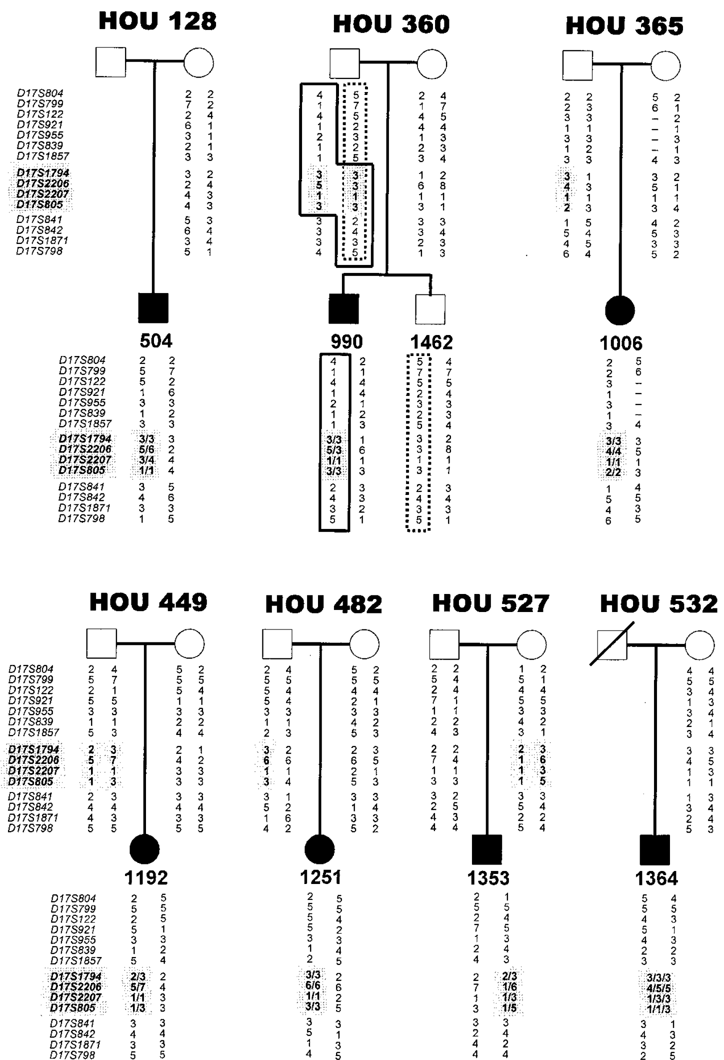

Fig. 3 Haplotypes of seven duplication patients and their parents. The markers within the SMS common deletion region and their genotypes are shaded. Seven unrelated families (HOU 128, HOU 360, HOU 365, HOU 449, HOU 482, HOU 527 and HOU 532) are shown. The duplication in patient 504 is likely to be derived from paternal interchromosomal recombination because, for the informative locus (marker *D17S2206*), the mother contributes only one allele to her child. Patients 990 and 1192 inherited two distinct paternal alleles and one maternal allele, thus the duplications in these patients are generated by interchromosomal rearrangement between the two paternal homologues. The haplotype generated by unequal crossing over of paternal chromosomes is outlined in bold in patient 990. Haplotype analysis of the unaffected brother (1462) of patient 990 in family HOU 360 enables one to determine the phase of the alleles on the paternal chromosome. The paternal haplotype not subjected to unequal crossing over was inherited by the brother of 990, 1462, and is demarcated with dashed lines. Patients 1006 and 1251 reveal a double dosage for the markers inherited from one of the paternally derived chromosomes, suggesting that the duplication in these patients are generated by paternal intrachromosomal rearrangements. A minus sign in patient 1006 and her mother indicates a deletion determined by dosage of the alleles (as previously demonstrated by FISH analysis[27]). The duplication in patient 1353 is generated by maternal interchromosomal rearrangement. The origin of duplication in patient 1364 can not be determined.

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Table 1 • Clinical findings of duplication 17p11.2

Patient	Age	Gender	Short stature	Mental retardation[a]	Behavioural abnormalities	Dental abnormalities	Seizures	Other findings
504[b]	6 y 6 m	M	yes	borderline (74)	hyperactive	yes	no	hypotonia, poor balance dysmorphic features[c]
990	9 y 10 m	M	no	mild (65)	autistic[d]	yes	by EEG	submucous cleft palate bifid uvula
	11 y 3 m			variable[e]	non-autistic[d]			
1006[b]	14 y	F	yes	mild (62)	attention deficit	yes	no	growth hormone deficiency
1192	10 y 6 m	F	yes	mild (65)	attention deficit	yes	febrile x1	oral apraxia microcephaly
1251	3 y 2 m	F	no	low average (83)	no	no	yes	dysmorphic features[f]
1353	17 y	M	yes	mild	hyperactive obsessive/compulsive	yes	no	hypotonia (early)
1364	41 y	M	yes post puberty	mild	hyperactive	yes	no	obesity as adult notably good balance

[a]Full-scale IQ results are given in parentheses. [b]Patients 504 and 1006 are reported in refs 20, 21 and 27, respectively. [c]Triangular face, downslanting palpebral fissures, mandibular and maxilary hypoplasia, smooth philtrum, high arched palate with thick palatine ridges, posteriorly angulated and prominent ears. [d]Childhood autism rating scale: age 9 y 10 m, 'autistic'; age 11 y 3 m, 'non-autistic'. [e]Results varied from the moderate range (55) on the Wechsler Math Composite, to the low average range (84) on the Wechsler reading composite, to the normal range (102) on the test of non-verbal intelligence (TONI-2). [f]Triangular face, mild frontal bossing, beaked nose, smooth philtrum and high arched narrow palate.

tive. Of these, five showed that the rearranged chromosome was of paternal origin. For two cases the duplication arose through an intrachromosomal event. In four of six cases, the recombination resulting in the duplication occurred between homologous chromosomes (interchromosomal). In one duplication patient (1353), the duplication was derived from a maternal interchromosomal recombination, as markers from each maternal homologue were represented within the duplicated region in this patient. The haplotype data demonstrated unequal crossing over between homologous chromosomes associated with the duplication in family HOU 360. This fully informative family had an unaffected sibling, which allowed for phasing of the alleles. The same markers duplicated in these patients were contained within the common SMS deletion, suggesting involvement of the same genomic region in both rearrangements (data not shown).

The clinical findings in the seven patients studied with dup(17)(p11.2p11.2) are shown (Table 1). The phenotype is relatively mild with generally normal appearing facies (except in patients 504 (ref. 21) and 1251), mild to borderline mental retardation and behavioural difficulties. A proportion of patients have short stature (5/7; height below the fifth percentile for age) and dental abnormalities (6/7) such as malocclusion and crowded teeth. No major organ developmental abnormalities were seen in these patients, in contrast with patients deleted for this region[2].

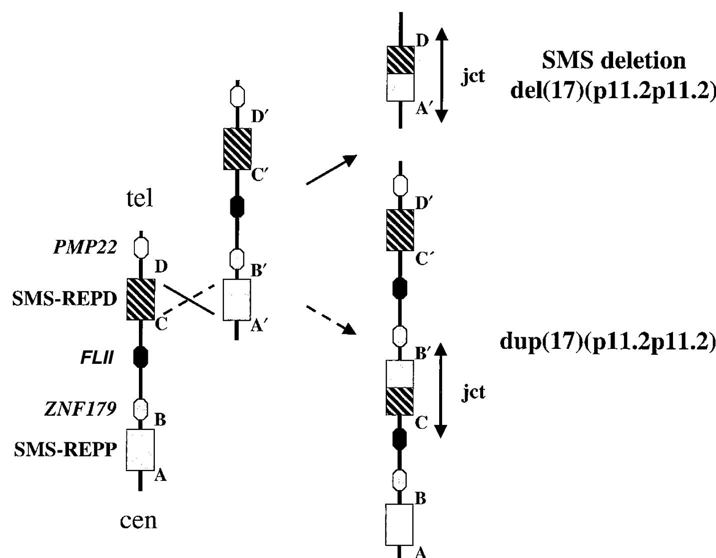

Fig. 4 Genomic rearrangements of 17p11.2 resulting from recombination between proximal (SMS–REPP, rightward grey cross hatch) and distal (SMS-REPD, leftward black cross-hatch) repeat gene clusters. The SMS–REPs, *PMP22* (open oval), *FLII* (black oval) and *ZNF179* (grey oval) are represented on each homologous chromosome. SMS–REPM (ref. 8) is omitted from the figure for simplicity. Capital letters (A–D and A′–D′) refer to the flanking unique sequences on the homologues; A on the centromeric side and D on the telomeric side. The proximal and distal SMS–REPs can misalign during meiosis as illustrated. An unequal crossing-over event occurs between misaligned SMS–REPs via homologous recombination. There are two predicted reciprocal recombination products: (i) the SMS deletion (solid arrow); and (ii) the duplication of 17p11.2 (dashed arrow). Each has a unique junction fragment (jct) by PFGE.

Our results on seven patients with *de novo* dup(17)(p11.2p11.2) show a novel junction fragment that is specific to these patients and to their rearranged chromosomes, and of similar size within the limits of resolution of PFGE. The same probe that revealed an SMS deletion-specific junction fragment detected the junction fragment in the duplication patients. Unequal crossing over of flanking markers was demonstrated by haplotype reconstruction. The genetic markers duplicated in these patients are the same as those deleted in SMS patients with the common deletion. Our data are consistent with the hypothesis that homologous recombination and unequal crossing over between SMS–REPs cause the 17p11.2 duplication and the SMS deletion as reciprocal recombination products (Fig. 4). Supportive of the predicted outcome of the unequal crossing-over event, the hybridization pattern of two-colour FISH using two gene probes derived from within the SMS common deletion region exhibited a pattern implying a direct duplication. Our data also suggest that *de novo* dup(17)(p11.2p11.2) may preferentially occur during paternal gametogenesis, although additional duplication patients must be analysed before a firm conclusion can be derived. In contrast with the findings in CMT1A,

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in which the paternal rearrangements were exclusively interchromosomal and the maternal rearrangements were exclusively intrachromosomal[22], we report two paternal intrachromosomal duplications and one maternal interchromosomal rearrangement.

We have defined a clinical syndrome based on a shared molecular structure among patients. This represents a paradigm shift in medical genetics in that common clinical findings of patients are usually the impetus for delineation of a syndrome and subsequent discovery of its molecular basis. Duplication of segments of the human genome may eventually be shown to be responsible for many human traits. The human genome project will delineate genome architectural features, such as low-copy, region-specific repeats, which may enable prediction of regions susceptible to rearrangements associated with genomic disorders. Recent reports have postulated that specific genetic loci with flanking repeated gene clusters are associated with other recurrent microdeletions[9–15,23,24]. It remains a distinct possibility that most interstitial chromosomal microdeletion syndromes have corresponding microduplication syndromes that represent the reciprocal recombination product.

Methods

FISH analysis. We performed two-colour FISH as described[25] on interphase nuclei of lymphoblast cell lines derived from the patients. Probes for the *PMP22* locus in the CMT1A region[20], for *FLII* in the SMS region[17] and the *ZNF179* locus in 17p11.2 (ref. 19) were used as reported.

PFGE analysis. High molecular weight DNA was isolated in agarose plugs from peripheral blood samples, somatic cell hybrid cell lines and Epstein-Barr virus-transformed lymphoblastoid cell lines established from controls and patients[26]. For Southern analysis, we used the 1.1-kb *Hin*dIII fragment from the cDNA clone 41G7A, which contains the 3´ end of the coding region and part of the 3´ UTR, as the *CLPSMCR* probe.

Marker genotypes. We determined parental origin and chromosomal mechanism of the duplication by microsatellite analysis. Oligonucleotide primer sequences were obtained from the Genome Database (http://www.gdb.org), and the 5´ end of forward primers were specifically end-labelled with fluorescent dyes of 6-FAM, TET or HEX (Applied Biosystems). We carried out PCR in a final volume of 50 µl containing genomic DNA (200 ng), Tris (10 mM, pH 8.3), KCl (50 mM), $MgCl_2$ (1.5 mM), 0.001% gelatin, dNTPs (250 µM each; Boehringer), primers (0.4 µM each), AmpliTaq (2 U; Perkin Elmer) and 10% DMSO (*D17S2206* and *D17S2207* only). Initial denaturation was at 95 ºC for 5 min followed by 35 cycles of denaturation at 95 ºC for 30 s, annealing at 55 ºC for 30 s, extension at 72 ºC for 30 s and a final extension at 72 ºC for 7 min. Genotypes were analysed according to the manual of Linkage Mapping Set (Applied Biosystems). We visualized PCR products by 2% agarose electrophoresis and diluted 5–20 times according to the band intensity. We mixed PCR products (1.5 µl) with formamide loading dye and TAMRA 500 markers (Applied Biosystems), and electrophoresed on 5% denaturing polyacrylamide gel in the model 377-96 DNA sequencer (Applied Biosystems). Their sizes and relative intensities were calculated with Genescan (ver. 2.1) and Genotyper (ver. 2.0) software (Applied Biosystems). For the quantitative analysis of four polymorphic markers within the SMS common deletion region, we performed 20–25 cycle PCR and compared the height ratios of upper and lower alleles of each genotype with those of the normal control with the same genotype.

Somatic cell hybrids. We carried out polyethylene glycol fusion between the lymphoblastoid cell line from duplication 17p11.2 patient 990 and a thymidine kinase-deficient (TK^-) hamster cell line, A23 (ref. 6). For this fusion, 24 independent clones were isolated with cloning rings and transferred to a 24-well microtitre plate. We obtained cells representing each clone by trypsinization of a confluent well of a 24-well plate, transferred them to a 6-well plate and then to T25 flasks. Two-colour FISH was used to analyse the hybrids and identify those retaining the 17p11.2 duplication chromosome.

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