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The mechanisms involved in formation of deletions and duplications of 15q11-q13

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Abstract

Haplotype analysis was undertaken in 20 cases of 15q11-q13 deletion associated with Prader-Willi syndrome (PWS) or Angelman syndrome (AS) to determine if these deletions arose through unequal meiotic crossing over between homologous chromosomes. Of these, six cases of PWS and three of AS were informative for markers on both sides of the deletion. For four of six cases of paternal 15q11-q13 deletion (PWS), markers on both sides of the deletion breakpoints were inferred to be of the same grandparental origin, implying an intrachromosomal origin of the deletion. Although the remaining two PWS cases showed evidence of crossing over between markers flanking the deletion, this was not more frequent than expected by chance given the genetic distance between proximal and distal markers. It is therefore possible that all PWS deletions were intrachromosomal in origin with the deletion event occurring after normal meiosis I recombination. Alternatively, both sister chromatid and homologous chromosome unequal exchange during meiosis may contribute to these deletions. In contrast, all three cases of maternal 15q11-q13 deletion (AS) were associated with crossing over between flanking markers, which suggests significantly more recombination than expected by chance (p=0.002). Therefore, there appears to be more than one mechanism which may lead to PWS/AS deletions or the resolution of recombination intermediates may differ depending on the parental origin of the deletion. Furthermore, 13 of 15 cases of 15q11-q13 duplication, triplication, or inversion duplication had a distal duplication breakpoint which differed from the common distal deletion breakpoint. The presence of at least four distal breakpoint sites in duplications indicates that the mechanisms of rearrangement may be complex and multiple repeat sequences may be involved.

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Both Prader-Willi syndrome (PWS) and Angelman syndrome (AS) result from large interstitial deletions of chromosome 15q11-q13 in 70-75% of cases.[1-4] PWS results when the deletion occurs on the paternal chromosome and AS when the same deletion is on the maternal chromosome.[5] Both syndromes occur at a frequency of about 1/15 000-1/20 000[6-8] and therefore maternal and paternal deletions each occur in approximately 1/20 000-1/28 000 livebirths.

Although there are a few exceptions observed, the vast majority of both maternal and paternal deletions of this region are of similar size (~4 Mb) and have tightly clustered breakpoints.[5 9-11] There are two common proximal breakpoints with similar frequencies (~50% of each) in both maternally and paternally derived deletions.[12 13] It has been suggested that most distal breakpoints are included in the region covered by a single YAC.[9] The common deletion is much larger than necessary to cause the PWS or AS phenotype, as deletions of less than 7 kb including the imprinting control centre can also result in the clinically typical PWS or AS phenotype[14 15] (unpublished results). The frequent occurrence and high clustering of breakpoints in the large deletions imply an instability at these sites. It is commonly suggested that microdeletions arise through mispairing of large duplicated sequences. Repetitive DNA is abundant in the human genome, but the frequency and distribution of microdeletions does not seem to be random with regard to these sequences. Additional factors are therefore necessary to explain why certain regions of the genome are predisposed to frequent mispairing and deletion.[16] In addition, these breakpoints are not sites of high homologous recombination and cannot therefore be explained simply by a tendency to double strand breaks at these sites during meiosis I.[17 18]

Interstitial duplications[19-21] and triplications[22-24] of the PWS/AS region have also been reported. In most of these cases, maternal heterozygosity of duplicated genes is observed, indicating involvement of two different maternal chromosomes. It is therefore of interest to determine if these duplications/triplications are the result of a mechanism related to deletion events. A meiotic unequal crossover between homologous chromosomes is expected to result in the formation of reciprocal duplication and deletion products, which show recombination of flanking markers (fig 1A). Such a mechanism has been reported to be involved in duplications of 17p11.2-p12, associated with Charcot-Marie Tooth disease IA (CMT1A), and deletions of the same region which are associated with hereditary neuropathy with liability to pressure palsies (HNPP).[25 26] In contrast, an intrachromosomal

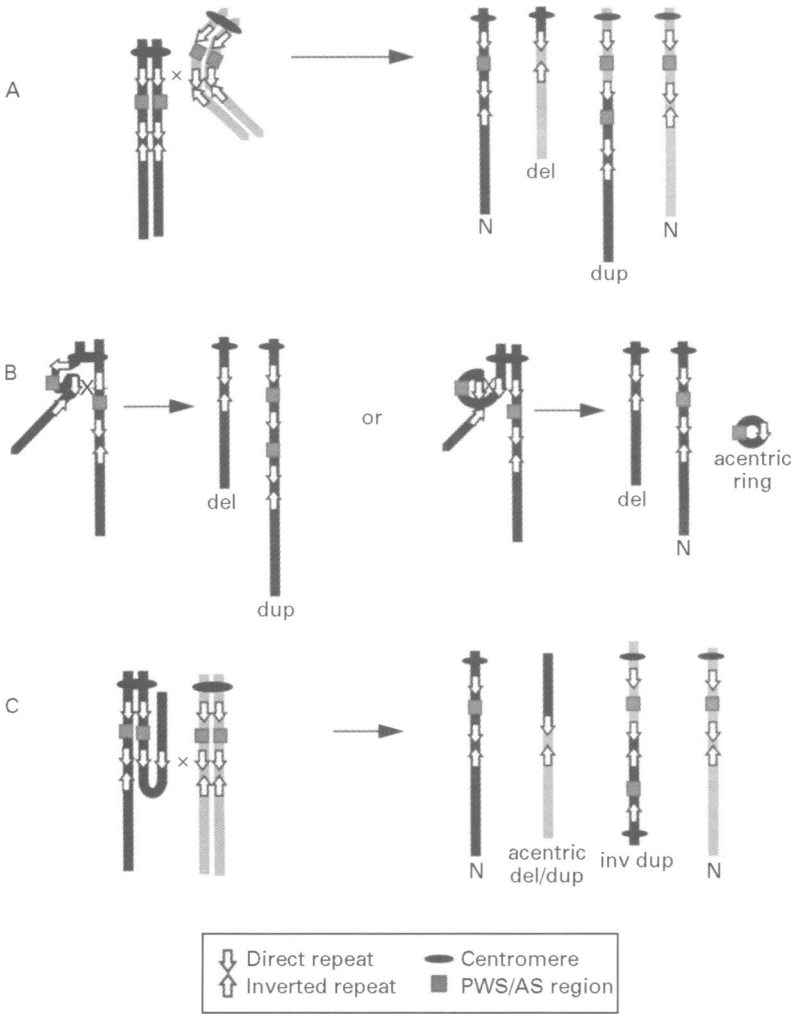

Figure 1 Models of rearrangements in chromosome 15q11-q13. (A) Classical model of unequal crossing over leading to reciprocal duplication (dup) and deletion (del) with recombination of flanking markers. (B) Unequal sister chromatid exchange (left) or intrachromatid (right) recombination may lead to deletion with no recombination of flanking markers. (C) Recombination between inverted repeats leading to inversion duplication (inv dup) derived from two different chromosomes. The presence of multiple direct and inverted repeats can lead to variable breakpoints; those shown are for illustrative purposes.

event will not result in recombination of flanking markers and should not be associated with a reciprocal duplication product, unless the crossing over is between sister chromatids (fig 1B). Interestingly, in one case of HNPP with maternal origin, the deletion was determined to be intrachromosomal by haplotype reconstruction using data from the grandparents,[27] and it was suggested that the intrachromosomal events may be specific to female meioses. In addition, the rarity of CMT1A duplications of maternal origin is not explained by a lower level of female than male meiotic recombination in this region.[28] The sex specific difference was suggested to be a result of male specific factors which help to form or stabilise the duplicated chromosome.[29] A mariner transposon-like element within the repeat has been suggested to be involved in mediating these recombination events.[30]

The 15q11-q13 region is also notable for the frequent occurrence of inversion duplications. Previous reports indicate that "small" supernumerary isodicentric inversion duplication 15q (inv dup 15) chromosomes share breakpoints in common with the two common proximal PWS/AS deletion breakpoints, while "large" inv dup 15 chromosomes tend to be of two sizes with only one breakpoint similar to the distal deletion breakpoint.[31-33] The formation of isodicentric chromosomes may also involve recombination between inverted repeats as shown in fig 1C. However, alternative mechanisms include a "U type" exchange,[34] or repair of a broken chromosome through replication and end to end fusion. Although the proposed mechanisms differ, the similarity of breakpoints between inversion duplications and deletions of this region, as well as the observation of patients carrying both a small isodicentric 15 plus a 15q11-q13 deletion[35] or duplication,[21] suggests that at least some of the causes may be related.

In order to determine if PWS/AS deletions are associated with meiotic recombination between flanking markers and hence if deletions are likely to be intra- or interchromosomal events, marker haplotypes were analysed in 20 families. Grandparental DNA was available in 18 of these, and haplotypes were inferred in the two additional families using unaffected sibs. The results exclude an unequal crossover between homologous chromosomes at meiosis as the deletion mechanism in four of six informative PWS cases. However, all three informative AS deletion cases were associated with a crossover event. A comparison of proximal and distal breakpoints between common deletions and duplications (or triplications and inversion duplications) of this same region indicates that the observed maternal duplications of this region are not the reciprocal products of deletion events, or at least that the breakpoints are much more variable. It is hypothesised that multiple mechanisms for rearrangement in this region exist and may involve unequal sister chromatid exchange or intrachromatid recombination either during or after meiosis in at least a portion of cases. The greater number of breakpoint sites associated with inversion duplications than deletions may also reflect relative location of inverted versus direct repeat sequences.

Methods

PATIENTS

Patients with 15q11-q13 deletions were ascertained through routine molecular investigations of PWS and AS patients. Deletions were diagnosed by virtue of lack of maternal or paternal inheritance of the commonly deleted RFLP probes and dosage analysis[36 37] or, usually, by microsatellite analysis.[20] Because there are no published markers more proximal than D15S1035, D15S541, D15S542, and D15S18 (order of these four polymorphisms is not known), and as these markers are deleted in half the patients,[13] only patients with the smaller deletion will be informative in this analysis (for recent mapping data in this region see Robinson *et al*[33]). In addition, many patients were uninformative even if they were intact for these markers if the parent in

Table 1 Summary of inheritance in informative PWS deletion cases

	Closest non-deleted informative marker				Intermarker distance (cM)	Crossover
	Proximal	Inheritance	Distal	Inheritance†		
PWS-47	D15S541	Grandmaternal	D15S144	Grandmaternal	23	No
PWS-RN	D15S18	Grandmaternal	D15S24	Grandmaternal	17	No
PWS-99*	D15S541	Grandparent A	D15S165	Grandparent B	17	Yes
PWS-178	D15S542	Grandpaternal	D15S118	Grandpaternal	27	No
PWS-235	D15S1035	Grandpaternal	ACTC	Grandpaternal	26	No
PWS-340	D15S1035	Grandpaternal	D15S165	Grandmaternal	17	Yes

p=0.37 to observe two or more crossovers by chance from these six meioses.
*Grandparental haplotypes inferred from sibs and cannot be designated grandmaternal or grandpaternal.
†Two additional cases with grandparents were informative distally but not for any proximal marker; one showed grandpaternal inheritance at D15S165 and one grandmaternal inheritance at D15S118.

Table 2 Summary of inheritance in informative AS deletion cases

	Closest non-deleted informative marker				Intermarker distance (cM)	Crossover
	Proximal	Inheritance	Distal	Inheritance*		
AS-V3	D15S542	Grandpaternal	D15S1048	Grandmaternal	13	Yes
AS-V14	D15S542	Grandmaternal	D15S1048	Grandpaternal	13	Yes
AS-193	D15S542	Grandmaternal	D15S165	Grandpaternal	13	Yes

p=0.002 to observe 3/3 crossovers by chance.
*Seven additional cases with grandparents were informative distally but either uninformative or deleted for proximal markers; of these, five showed grandpaternal and two grandmaternal inheritance for markers flanking the distal deletion breakpoint.

question was homozygous at these loci. In one case (PW-99), grandparents were not available but haplotypes were inferred from two sibs.

Normal maternal and paternal inheritance of microsatellite markers was observed outside the deleted region, thus excluding uniparental disomy in all cases. PWS-47 is identical to patient PWS-47 previously reported.[1] PWS-RN was previously published as HS2.[37] Cytogenetic analysis in all cases indicated that the deletions were interstitial and not associated with a translocation or other chromosomal rearrangement. In addition, the fact that proximal loci were intact excludes a deletion arising from an unbalanced cryptic translocation.

DNA ANALYSIS

Isolation of genomic DNA from peripheral blood, restriction enzyme analysis, electrophoresis, and Southern blotting were performed using standard procedures as described previously.[36 37] Probes used include pIR39 (D15S18), IR4-3R (D15S11), p3-21 (D15S10), and pCMW-1 (D15S24). PCR amplification of microsatellite loci was performed using standard conditions (usually 55°C annealing temperature). A total of 0.5-3 µl of reagent was then mixed with an equal volume of urea loading buffer (42% urea, 0.1% xylene cyanol, 0.1% bromphenol blue, and 0.1% of 0.5 mol/l EDTA) and directly loaded onto a 0.4 mm thick 6% polyacrylamide/50% urea gel. Visualisation of bands was done by silver staining of the gels. Information on microsatellite loci tested can be obtained from the Genome Data Base. All primers were obtained from Research Genetics Inc (Huntsville, AL).

The probe IR39 (D15S18) maps proximal to the common PWS/AS deletion in about 50% of patients[12] (unpublished data). Similarly, the microsatellite loci D15S541 and D15S542, which map to a YAC containing D15S18, are deleted in roughly half of PWS or AS patients.[13] The deleted region is about 4 Mb[9] and covers more than 10 cM.[18] D15S11 is located within the commonly deleted region. The probe CMW-1, detecting the D15S24 locus, maps outside (distal to) the deletion region in almost all patients[1 3] (R D Nicholls, M Mascari unpublished data). D15S165 maps distal to D15S24 (R D Nicholls, unpublished results) and less than 5 cM from the distal breakpoint. D15S1048 has also been localised near to D15S165 based on recombination in CEPH

Table 3 Extent of duplication in one tandem duplication, two interstitial triplications, and 12 inv dup15q11-q13 cases. The genetic distance of each marker in cM from D15S541 is indicated

		Patient														
		Dup4	Trip1	Trip2	ID10	ID211	ID149	ID189	ID7	ID107	ID90	ID151	ID104	ID190	ID215	ID188
Copies PWS/AS critical region	(cM)	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4
D15S12†	~17	nt	dup*	nt	nt	nt	dup*	dup*	nt	dup*	dup*	dup*	dup*	dup*	N*	**N**
D15S1019	20	ui	ui	dup*	dup*	nt	ui	nt	nt	ui	nt	nt	N*	ui	ui	**N**
D15S1048	20	ui	ui	dup*	ui	ui	ui	ui	ui	**dup**	**dup**	ui	**N**	**N**	**N**	ui
D15S165	22	dup*	ui	ui	ui	dup*	dup*	**dup**	ui	**dup**	nt	**dup**	**N**	**N**	**N**	**N**
D15S24	—	nt	dup*	nt	nt	ui	dup*	ui	nt	dup*	dup*	dup*	**N**	**N**	**N**	nt
D15S1031	22	dup*	nt	nt	**dup**	dup*	**dup**	nt	ui	dup*	dup*	dup*	ui	nt	nt	nt
D15S976	23	ui	ui	dup*	ui	nt	nt	dup*	**dup**	dup*	ui	nt	ui	**N**	ui	ui
D15S1043	23	ui	dup*	ui	**dup**	**dup**	ui	**dup**	dup*	ui	ui	ui	**N**	ui	ui	**N**
D15S1010	24	N*	ui	N	ui	**dup**	nt	ui	N*	ui	ui	n*	nt	ui	ui	ui
D15S144	27	N	ui	N	**dup**	ui	nt	nt	nt	**N**	ui	**N**	nt	nt	nt	nt

*dup or N inferred by dosage of alleles only.
†The common deletion breakpoint lies just distal to D15S12.
nt=not tested; dup (bold)=three distinct alleles were visible.
N (bold)=only one allele from heterozygous mother was transmitted.

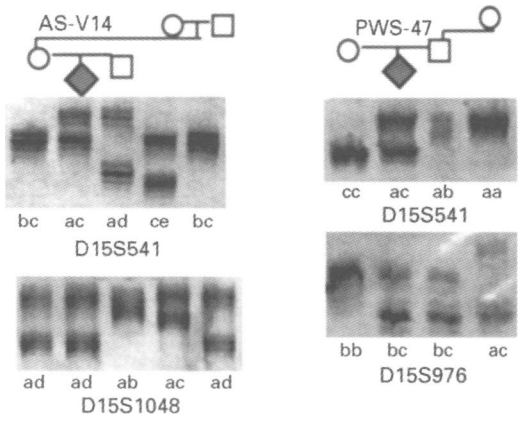

Figure 2 Sample molecular results in deletion cases. AS-V14 has inherited the grandmaternal D15S541 allele and the grandpaternal D15S1048 allele on the maternal haplotype; PWS-47 has inherited the grandmaternal allele on the father's haplotype at both D15S541 and D15S976.

and UPD15 families (W P Robinson, unpublished results). Sex specific genetic distances given in table 1 are taken from CEPH/Généthon online data and Robinson and Lalande.[18] CEPH based genetic locations are given in tables 2 and 3 for additional markers tested in this region.

Microsatellite loci were also used to delineate further the extent of deletions and duplications in this region. Lack of transmission of a paternal or maternal allele in deletion cases was considered evidence that this locus was included in the deletion, whereas heterozygosity in the patient indicated normal biparental inheritance. In cases of duplication, the presence of three distinct alleles was considered conclusive evidence that this locus was included in the duplication, whereas a dosage difference between alleles was considered suggestive of this. Dosage was measured qualitatively by comparing the intensities of bands in parents and controls. At least two independent observers judged dosage. If it was not clear to both observers whether one band was duplicated, the result was recorded as uninformative. Although this method cannot be considered 100% accurate, it is strongly supportive of the presence or absence of duplication. Lack of inheritance of both maternal alleles and an equal dosage of two alleles present in the patient was taken as definite evidence that this locus was not included in the duplication.

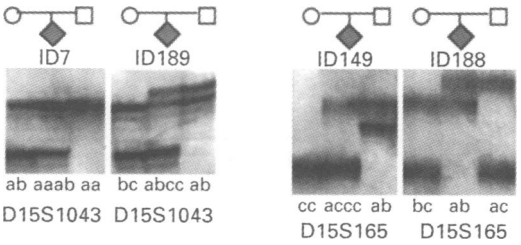

Figure 3 Sample molecular results in inv dup 15 cases. ID7 appears to have a duplication at D15S1043 by dosage only, whereas a duplication of this locus in ID189 is clearly indicated by three distinct alleles. ID149 appears to have a maternal duplication at D15S165 by dosage, whereas ID188 has inherited only one maternal allele in a single dose (as compared to the paternal allele).

Results

Sample molecular results are given in figs 2 and 3. Haplotype data for all informative families are summarised in table 1. In four of five informative PWS deletion cases with grandparents available, markers flanking the paternal deletion both proximally (D15S541, D15S542, D15S1035, or D15S18) and distally (D15S144, D15S24, or D15S118) show inheritance from the same grandparent (table 1). In two cases, the flanking markers showed origin from the grandmother and, in the other two cases, the deletion arose on the grandpaternal haplotype. Specifically, there was no evidence of crossing over between the grandmaternal and grandpaternal haplotypes within the deleted region. In contrast, case PWS-340 showed grandpaternal inheritance for D15S1035 but grandmaternal inheritance distal to the deletion breakpoint at D15S165. Thus, a crossover event between two father's chromosomes must have occurred either before or during the deletion event. In the case of PWS-99, the grandparents were not available and haplotypes were inferred from the sibs. Two possibilities exist in this family: either the patient has inherited a recombinant chromosome from the father, or both of the sibs have inherited a paternal recombinant chromosome in this region. Given a male recombination distance of 17 cM between D15S541 and D15S165, the odds are 6:1 in favour of the former (only one rather than two recombinant offspring in this region). Furthermore, as the two normal sibs share the same paternal allele at the proximal marker D15S541 and at GABRB3, the probability of the latter hypothesis is reduced as it would require a recombination specifically between GABRB3 and D15S165 in both sibs. This case has therefore been classified as a probable recombinant.

Even though two cases appear to show a recombination between the markers flanking the deletion, this does not exclude a post-meiosis I intrachromosomal origin in these cases. To calculate the probability of observing chance recombinants from cases presented in table 1, one needs to account for the genetic distance between markers in each informative case. Assuming that the probability of observing recombination between two markers corresponds to genetic distance (that is, 1 cM=1% recombination), the probability of not observing any crossovers between the flanking markers in these six cases if in fact normal levels of meiotic recombination precede the deletion event is simply:

$$p(0/6 \text{ recombinant}) = \prod_{i=1}^{N} (1-\theta_i),$$

where θ_i is the recombination fraction between informative flanking markers for case i, and N is the total number of cases. Thus, p(0/6 recombinant)= (1-0.23)*(1-0.17)*(1-0.17)*(1-0.27)*(1-0.26)*(1-0.17)=0.24. Similarly, one can consider the probability that one of the six cases would be recombinant: p(1/6 recombinant)=0.39, or that two or more crossovers would be observed: p(≥2/6 crossovers)=1-p(0/6 recombinant)-p(1/6 recombinant)=0.37. Therefore observing two recombinant cases is

well within the expected number if recombination is completely independent of the deletion event.

The result is different in the case of the three fully informative AS cases carrying maternal deletions of 15q11-q13. The female recombination distance in this region is slightly shorter than in males, and the expected probabilities of observing 0/3, 1/3, 2/3, or 3/3 crossovers by chance (as calculated above) in the three cases presented in table 2 are 0.659, 0.295, 0.044, and 0.002, respectively. However, marker results showed different grandparental origin of haplotypes on either side of the deletion breakpoints in all three cases. Thus, for the AS deletions it is unlikely that the recombination events were the result of chance and are not associated with the deletion ($p=0.002$).

It appears from tables 1 and 2 that there is no significant bias in terms of grandparental origin of the chromosomes involved in the exchange event in either the maternal or paternal deletions. Including additional cases which were informative for distal but not proximal markers, grandpaternal inheritance was seen for markers flanking the deletion distally in three of seven paternal and seven of 10 maternal deletion cases. The lack of bias in involvement of grandparental chromosomes would seem to exclude a mechanism of deletion which specifically occurred before or during resetting of the imprint on one or the other parental chromosome, unless the resetting of a paternal imprint as maternal and vice versa somehow involved exchange or association between homologues as part of the process, as has been previously suggested.[38 39]

To refine the distal breakpoints further, D15S165, D15S1043, D15S1019, D15S976, D15S1031, D15S1010, D15S144, and D15S1048 have been examined in a subset of 15 PWS or AS patients for inclusion in deletions of this region. None showed evidence for uniparental inheritance of any of these markers, with the exception of one unusual deletion patient (PW-93) previously known to be deleted distally for D15S24, but also not carrying the same proximal breakpoint as common deletions[1] (data not shown). The distal breakpoint in PW-93 occurred between D15S24, D15S1048 (both deleted) and D15S1031, D15S1043 (both heterozygous).

In contrast to the common PWS/AS deletion, duplications of the PWS/AS region frequently included markers distal to D15S12. One tandem duplication patient and two intrachromosomal triplication patients showed unequal intensities of the two amplified alleles at several loci, which is consistent with inclusion in the duplications (table 3). For many of the 12 inv dup 15q11-q13 patients examined, three distinct alleles were observed at distal loci, providing definitive proof of inclusion in the duplication. Interestingly, a minimum of four different breakpoints were observed in this region: (1) distal to D15S144, (2) between D15S1043 and D15S1010, (3) between D15S12 and D15S1019 (equivalent to the common deletion breakpoint), and (4) between GABRB3 and D15S12. Thus although inv dup 15 chromosomes can generally be distinguished as "large" (including the PWS/AS region) or "small" (excluding this region), there appears to be much more variability in breakpoint location than is seen among PWS/AS deletion patients.

Discussion

Unequal crossing over between homologous chromosomes at meiosis was excluded as the mechanism of 15q11-q13 deletion formation in four of six deletions of paternal origin. In contrast, all three informative AS cases showed evidence of recombination between markers flanking the deletion breakpoints, indicating an association with the deletion event. These results are opposite to those observed for HNPP deletions, where haplotype analysis showed the single maternal deletion case to be intrachromosomal in origin in contrast to recombination associated paternal deletions of the same region.[27] Furthermore, the CMT1A duplications (which are duplications of the same region deleted in HNPP) are almost all paternal in origin, in contrast to 15q11-q13 duplications which are all maternal in origin. As deletion breakpoints do not appear to depend on parental origin in either case, the sex and region specific differences may simply reflect differences in the resolution of certain recombination intermediates rather than representing completely distinct mechanisms. Studies on the origin of the 7q11.23 deletion associated with Williams syndrome (WBS) showed evidence for a meiotic interchromosomal exchange between markers flanking the deletion in 22 of 27 cases.[40 41] However, there was no apparent bias in parental origin of those WBS deletions which were associated with exchange.

Several hypotheses should be considered when considering the mechanism of microdeletion formation: (1) a premeiotic germline origin; (2) a meiotic event; or (3) an early somatic event.

(1) *A premeiotic germline origin.* Recurrence of an interstitial deletion from the same parent has never been reported for either PWS or AS (or any other interstitial microdeletion), making an early germline origin unlikely. A germline origin would also be likely to cause a paternal bias owing to increased cell divisions in spermatogenesis. However, the population frequency of maternal deletions ascertained through AS and paternal deletions ascertained through PWS are roughly equal (see introduction).

(2) *A meiotic origin.* Meiotic recombination in yeast is initiated by double strand breaks forming early in prophase. A chromosome instability leading to a high rate of non-homologous recombination would probably also involve double strand breaks and be expected to show a high rate of homologous recombination at the same sites. Studies of unequal recombination between repeated genes in yeast show that those sequences involved in the highest rate of non-homologous (unequal) exchange also show high rates of homologous exchange.[42] However, no excess of

meiotic recombination is apparent at the PWS/AS deletion breakpoint sites,[17 18] although the exact physical distance between D15S12 and D15S144 is not yet known. The observation that maternal and paternal deletions occur with equal frequency and involve the same breakpoint sites[12 13] is also apparently inconsistent with the striking sex specific differences in meiotic recombination in this region.[18]

The above arguments do not exclude a meiotic origin, only suggest that we may not completely understand the mechanism yet. There is evidence in yeast that exchange can occur during meiosis which is independent of exchange associated with functional chiasmata (see, for example, Hawley and Arbel[43]). Similarly, studies in *Drosophila* show evidence of recombination events owing to recombinatorial repair of transposon induced breaks.[44] Perhaps the PWS/AS deletions are instead the result of an imperfect repair mechanism invoked to repair post-recombinational breaks that tend to occur within this region. The paternal intrachromosomal versus maternal interchromosomal difference could be the result of sex specific differences in use of sister chromatids versus homologous chromosomes for repair or of how the recombination-repair intermediate is resolved. However, because of the small number of informative cases, it is not yet possible to determine if the ratio of intra- to interchromosomal events is significantly different in PWS versus AS deletion cases.

If a classical meiotic unequal crossing over fully explained the occurrence of the maternal deletions, we might expect to observe reciprocal duplications of this region. Although maternal duplications of this region are commonly observed, an examination of distal breakpoints in interstitial duplications/triplications of the commonly deleted PWS/AS region indicates a difference. The common deletion breakpoint occurs immediately distal to D15S12[9] and rarely includes D15S24[1 3] (present results). A YAC contig of the PWS region shows that D15S24 lies on a YAC which does not overlap the YAC spanning the distal deletion breakpoint.[9] D15S24 was, however, shown by FISH or molecular dosage analysis to be included in two duplication patients[20] and three tandem triplication patients.[22 23] In addition, results for one tandem duplication and two tandem triplication patients (including the case of Schinzel *et al*[23]) presented here showed unequal intensities of the amplified alleles consistent with duplication of markers distal to the common deletion (table 2). Therefore, the duplicated region in all of seven duplication or triplication cases most probably extended further than the common deletion.

Furthermore, we were able to identify a minimum of four breakpoints among inv dup 15(q11-q13) chromosomes which included the PWS/AS critical regions, one of which corresponds to the common distal deletion breakpoint. Previous studies using FISH had identified only two of these breakpoints.[33] Thus, although inv dup 15 chromosomes can generally be distinguished as "large" (including the PWS/AS region) or "small" (excluding this region), there appears to be much more variability in breakpoint location than is seen among deletion patients. Perhaps the recombination events leading to duplication and deletions are the result of the same initiating mechanism, for example, a chromosome breakage, but the resolution as a duplication versus a deletion is influenced by which distal sequences are used for repair. As a series of repeated sequences at the proximal and distal breakpoint regions have been identified in this region,[33 45 46] it may be that the location and orientation of specific repeats determines whether the recombination event will result in deletion, duplication, or inversion duplication.

(3) *A post-meiotic origin.* A post-meiotic origin also remains possible for at least some deletions and duplications. Mosaicism of 15q11-q13 deletions has been observed in association with hypomelanosis of Ito.[47 48] The presence of mosaicism for 15q11-q13 deletions within PWS patients has also been suggested,[6 49] although these results suffer from methodological errors and are not convincing. However, observable mosaicism is not necessary to postulate a post-meiotic origin. A somatic origin of non-disjunction has been inferred in multiple instances of non-mosaic trisomy and uniparental disomy.[50 51] It has also been proven that post-meiotic events are common in the formation of homologous Robertsonian translocations and isochromosomes, despite the lack of any observed mosaicism,[52] and has also been observed for de novo rearrangements[53] including PWS/AS imprinting centre deletions.[15] Furthermore, the maternal and paternal 15q11-q13 homologues appear to "pair" in late S phase of the mitotic cell cycle in lymphocytes.[39] The mechanism and function of this somatic "pairing" is unknown. However, it may provide a means to bring the two chromosomes 15 near enough to facilitate a mitotic recombination event.

Although there is an abundance of repetitive sequences in the human genome, recombination between repeats in non-homologous sites is not common. The observation of recurrent de novo deletions therefore implies several characteristics of chromatin at the breakpoint sites in addition to assuming that there exists some degree of homology between breakpoint sites. The proximal or distal deletion breakpoints or both must be sites prone to single or double strand breaks or otherwise susceptible to recombination/repair at some point in the cell or developmental cycle. The high rate of exchange between sequences 4-5 Mb apart also implies some physical proximity of these sequences in the nucleus, which can potentially be achieved simply by the nature of chromatin packaging in the region. Clearly more families need to be studied to determine if there is a parent of origin effect on the frequency of intra- versus interchromosomal involvement. Nonetheless, the initial observations indicate heterogeneity in the specific mechanism involved in the origin of 15q11-q13 rearrangements, including maternal and paternal deletions and maternal duplications, triplications,

and inv dup 15q11-q13. Although a simple unequal crossing over mechanism may be involved, it is not sufficient to explain all the data. Further investigations including identification of repetitive sequences in the proximal and distal regions are needed to determine the exact cause of instability at the breakpoint sites.

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