Human Molecular Genetics, 1998, Vol. 7, No. 5 887–894

High level of unequal meiotic crossovers at the origin of the 22q11.2 and 7q11.23 deletions

Alessandra Baumer[1,*], Fabrizio Dutly[1], Damina Balmer[1], Mariluce Riegel[1,2], Turgut Tükel[3], Malgorzata Krajewska-Walasek[4] and Albert A. Schinzel[1]

[1]Institute for Medical Genetics, University of Zurich, CH-8001 Zurich, Switzerland, [2]Service for Medical Genetics, Porto Alegre, RS, Brazil and [3]Department of Paediatrics, University of Istanbul, Turkey and [4]The Children's Memorial Health Institute, 04-36 Warsaw, Poland

Received December 23, 1997; Revised and Accepted February 6, 1998

Interstitial chromosomal deletions at 22q11.2 and 7q11.23 are detected in the vast majority of patients affected by CATCH 22 syndromes and the Williams–Beuren syndrome, respectively. In a group of 15 Williams–Beuren patients, we have shown previously that a large number of 7q11.23 deletions occur in association with an interchromosomal rearrangement, indicative of an unequal crossing-over event between the two homologous chromosomes 7. In this study, we show that a similar mechanism also underlies the formation of the 22q11.2 deletions associated with CATCH 22. In eight out of 10 families with a proband affected by CATCH 22, we were able to show that a meiotic recombination had occurred at the critical deleted region based on segregation analysis of grandparental haplotypes. The incidences of crossovers observed between the closest informative markers, proximal and distal to the deletion, were compared with the expected recombination frequencies between the markers. A significant number of recombination events occur at the breakpoint of deletions in CATCH 22 patients ($P = 2.99 \times 10^{-7}$). The segregation analysis of haplotypes in three-generation families was also performed on an extended number of Williams–Beuren cases (22 cases in all). The statistically significant occurrence of meiotic crossovers ($P = 4.45 \times 10^{-9}$) further supports the previous findings. Thus, unequal meiotic crossover events appear to play a relevant role in the formation of the two interstitial deletions. The recurrence risk for healthy parents in cases where such meiotic recombinations can be demonstrated is probably negligible. Such a finding is in agreement with the predominantly sporadic occurrence of the 22q11.2 and 7q11.23 deletions. No parent-of-origin bias was observed in the two groups of patients with regard to the origin of the deletion and to the occurrence of inter- versus intrachromosomal rearrangements.

INTRODUCTION

A number of human diseases are characterized by non-random interstitial deletions, the breakpoints of which appear to occur at 'hot-spot' regions (1). This study deals with two diseases usually associated with deletions occurring in proximity to the centromere: the CATCH 22 syndromes (2) associated with a deletion affecting chromosome band 22q11.2 and the Williams–Beuren syndrome (WBS) (3,4) associated with a deletion affecting 7q11.23.

CATCH 22 encompasses a group of related syndromes; the acronym summarizes the major clinical manifestations characteristically detected in the patients, namely cardiac defects, abnormal facies, cleft palate and hypocalcaemia in early infancy due to hypoplasia or aplasia of the parathyroid glands. The group of disorders include the DiGeorge syndrome, the velo-cardio-facial syndrome, and some forms of conotruncal cardiac defects. The vast majority of patients have a common 1.5 Mb deletion on chromosome 22q11.2 (5,6). WBS is a neurodevelopmental disorder characterized by distinctive facial features, growth and mental retardation, hypercalcaemia in infancy and a very friendly outgoing personality. The syndrome is usually associated with a deletion spanning ~2 Mb of chromosome band 7q11.23 (7,8).

A high phenotypic variability is observed in CATCH 22 and WBS. The variability in both the clinical manifestations of certain features and their severity is not easily correlated with the length of the deletions. Illustrative of this phenomenon are discordant phenotypes manifested at times in monozygotic twins (9–12) and in familial cases (6,13). The variability in expression of the two diseases, together with the prevalence of consistent deletions, are indicative of contiguous gene defects. The rare cases where much smaller deletions or translocations have been detected have provided valuable information regarding the minimal critical regions within the 'common' deletions. Critical regions have been defined in CATCH 22: a 250 kb region in the proximal region of the 'common' deletion and the recently described

*To whom correspondence should be addressed. Tel: +41 1 634 25 36; Fax: +41 1 634 49 16; Email: baumer@medgen.unizh.ch

Table 1. Probands affected by CATCH 22

patients	sex and age (years)	parental ages at birth (mother/father)	parental origin of the deletion	clinical diagnosis[2]	major clinical features[3] 1: cardiac anomalies 2: thymic hypoplasia 3: craniofacial dysmorphism 4: cleft palate 5: hypocalcemia
1 22q-14	F,F, 8[1]	47/47	maternal	VCFS (both twins)	1, 3, 4
2 22q-28	M, 3	35/35	paternal	DGS	2, 3, 5
3 22q-55	F, 2	35/30	paternal	DGS	1, 2, 5
4 22q-83	F, 2	?/33	maternal	DGS	1, 2
5 22q-100	M, 8	35/28	maternal	VCFS	1, 3
6 22q-112	M, 6	18/24	paternal	VCFS	1, 3, 4
7 22q-120	F, 6	28/32	paternal	VCFS	1, 3
8 22q-156	F, 12	27/28	paternal	VCFS	1, 2, 3
9 22q-177	M, 1	32/33	maternal	VCFS	1, 2, 3
10 22q-196	F, 2 mths	38/38	paternal	VCFS	1, 3

[1]Monozygous twins.
[2]VCFS, velo-cardio facial syndrome; DGS, DiGeorge syndrome.
[3]Major clinical features as reported by the physicians.

critical regions at distal positions (5,14–16). The minimal critical region in WBS is ~300 kb in length (17). The characterization of a number of genes mapping to the critical regions has been reported recently (17–25). Strong candidate genes in the CATCH 22 diseases are, for example, the *DGSI* gene (20), the *GSCL* gene (21), the *UFD1L* gene (25) and the *TBX1* gene (19), to name just a few of the >12 genes shown to map to the DiGeorge critical regions. Regarding WBS, many features of the disease can be explained by haploinsufficiency of the elastin gene. Other features, such as mental retardation, can be explained by further genes mapping to the common deletion, including the recently described *LIM-kinase 1* gene (18), the *WSCR1* gene, the *RFC2* gene and the *STX1A* gene (26,27).

The 22q11 and 7q11.23 deletions usually occur sporadically, with an incidence of ~1/10 000 and 1/20 000 live births, respectively (28,29). Familial cases are inherited in an autosomal dominant manner. The 22q11.2 deletion is detected in ~85–90% of clinically diagnosed CATCH 22 patients (5). In ~75% of cases, the maternal allele is affected (30). Regarding WBS, the vast majority of clinically diagnosed patients, and probably all typical WBS cases, have a deletion affecting 7q11.23 (27). No significant statistical difference is detected in the parental origin of the deletion, which has been reported to be only slightly biased in favour of maternally derived deletions (7,31). Possible parent-of-origin effects on the phenotype have been proposed in a recent study by Pérez Jurado *et al.* (7). In their group of patients, the authors observed more severe forms of growth retardation and microcephaly in association with maternal deletions (7).

The aim of this work was to gain information on the mechanisms of formation of interstitial deletions. In recent publications (31,32), it was shown that unequal meiotic crossover events occur frequently in WBS. Dutly and Schinzel (32) showed that an unequal meiotic recombination occurred at the deletion site in 10 out of 15 probands affected by WBS. The group of WBS cases was extended to 22 families in this study; significant statistical values are obtained for increased levels of recombination at the critical region, thus consolidating the results of the previous report. A high level of meiotic recombinations was also reported to occur at the critical Prader–Willi (PWS)/Angelman syndrome (AS) deletion region (33).

In this study, we are able to show that a high level of interchromosomal rearrangements also occurs in patients with the 22q11.2 deletion associated with CATCH 22. Of the 10 families included in this study, eight showed a meiotic crossover at the critical region. Furthermore, no correlation could be found in either CATCH 22 or WBS between the size of the deletions and the inter- versus intrachromosomal recombinations.

RESULTS

Parental origin of the deletions

Microsatellite markers mapping to the deleted regions were used in order to determine the parental origin of the affected chromosome. Tables 1 and 2 summarize the results obtained for the 11 probands with CATCH 22 (the probands in family 1 are monozygous twins), and the 22 probands with WBS, respectively. The parental origin of the deletions is indicated as well as the major clinical features. Of the 10 CATCH 22 families, four had a deletion affecting the maternal chromosome (40%), and six the paternal chromosome. Of the 22 WBS probands, 11 showed a maternal origin (50%) and 11 a paternal origin of the deletion.

Haplotype determination at positions flanking the deletions

The occurrence of a meiotic crossover at the critical region was investigated by analysing the segregation of grandparental haplotypes at positions flanking the deletion breakpoints. Grandparental blood samples were collected according to the parental origin of the deletions. Several markers mapping to either side of the deletions were analysed in the three-generation families. Markers located at positions possibly included in the deletions were considered only if two distinguishable alleles were detected in the proband. The markers closest to the deletion breakpoints which were informative as to the grandparental origin of the allele transmitted to the proband are summarized in Tables 3 and 4. The grandparental origin of the proximal and distal positions are indicated for each patient.

Table 2. Probands affected by WBS

patient	sex and age (years)	parental ages at birth (mother/father)	parental origin of the deletion	major clinical features 1: SVAS 2: PS 3: dysmorphic facial feature 4: hypercalcemia 5: hoarse voice 6: outgoing personality
11 WBS3 * °	M, 27	31/32	paternal	3, 5, 6
12 WBS5 * °	M, 9	35/36	maternal	1, 3, 4, 5, 6
13 WBS9 * °	F, 12	24/25	maternal	3, 4, 6
14 WBS34 * °	F, 7	21/31	paternal	3, 5, (6)
15 WBS35 * °	F, 6	27/31	maternal	2, 3, 5, 6
16 WBS38 * °	M, 6	23/26	paternal	1, 3, 5, 6
17 WBS41 * °	M, 5	29/31	paternal	2, 3, 6
18 WBS52 * °	M, 4	32/32	paternal	1, 3, 4, 5, 6
19 WBS63 * °	M, 8	18/26	paternal	1, 2, 3, 5, 6
20 WBS70 * °	F, 16	26/31	maternal	3, 5, 6
21 WBS72 °	M, 5	20/24	maternal	1, 2, 3, 6
22 WBS76 * °	M, 4	25/28	paternal	1, 2, 3, 5, 6
23 WBS81 *	M, 11	29/32	maternal	1, 3, 5, 6
24 WBS82 *	M, 5	31/31	maternal	1, 2, 3, 4, 5, 6
25 WBS84 *	M, 8	?/?	maternal	mild aortic stenosis, 3, 5, 6
26 WBS92	F, 6	?/?	maternal	3, 5, 6
27 WBS101*	M, 2	32/35	maternal	1, 2, 3, 5, 6
28 WBS102	F, 13	23/24	paternal	3, 5, 6
29 WBS112	M, 7	?/?	maternal	3, 4, 5, 6
30 WBS115	M, 6	?/23	paternal	1, 2, 3, 5, 6
31 WBS137	F, 4	25/27	paternal	1, 2, 3, 5, 6
32 WBS140	M, 5	?/?	paternal	5, (6)

[1]The major clinical findings as reported by the physicians (information regarding the occurrence of hypercalcaemia was given only in a few cases). SVAS, supravalvular aortic stenosis; PS, pulmonary stenosis

*The 15 WBS patients included in the previous study (32).

°Patients also mentioned in Robinson *et al.* (8).

Table 3. Grandparental origin of the closest informative markers flanking the 22q11.2 deletions

Cases with meiotic recombination at the deletion breakpoint

families	deletion	proximal breakpoint		distal breakpoint		genetic distances[1]
1 22q-14	maternal	F8VWFP	grandpaternal	D22S306	grandmaternal	12 cM
2 22q-28	paternal	D22S420	grandmaternal	D22S306	grandpaternal	6 cM
3 22q-55	paternal	D22S427	grandmaternal	D22S303	grandpaternal	8 cM
4 22q-83	maternal	D22S427	grandmaternal	D22S303	grandpaternal	8 cM
6 22q-112	paternal	D22S420	grandmaternal	D22S303	grandpaternal	8.5 cM
8 22q-156	paternal	D22S420	grandmaternal	D22S343	grandpaternal	16 cM
9 22q-177	maternal	D22S420	grandmaternal	D22S306	grandpaternal	6 cM
10 22q-196	paternal	D22S427	grandmaternal	D22S343	grandpaternal	13 cM

Cases with no meiotic recombination at the deletion breakpoint

families	deletion	proximal breakpoint		distal breakpoint		genetic distances[1]
5 22q-100	maternal	D22S427	grandmaternal	D22S303	grandmaternal	8 cM
7 22q-120	paternal	D22S427	grandpaternal	D22S343	grandpaternal	13 cM

[1]Approximate genetic distances according to Généthon and GDB linkage maps.

A high incidence of meiotic crossovers at 22q11.2 in patients with CATCH 22

As shown in Table 3, a significant number of 22q11.2 deletions appear to be associated with a meiotic crossover event. Figure 1 shows representative microsatellite results obtained for family 8. The proximal chromosome region is, in this case, of grandmaternal origin and the distal region of grandpaternal origin. For illustrative purposes, the marker *D22S258* is depicted in the figure because of a better electrophoretic resolution of the different sized alleles than of the marker reported in Table 3 (i.e. *D22S343*). On the basis of the average genetic distance between proximal and distal markers, the number of meiotic crossovers expected by randomly analysing 10 individuals would be 0.98. The probability of detecting, by chance, eight or more crossovers in 10 meioses is $P = 2.99 \times 10^{-7}$ (calculated considering the interference value). In this group of patients, there is no evidence for a parent-of-origin bias in the occurrence of meiotic cross-

Table 4. Grandparental origin of the closest informative markers flanking the 7q11.23 deletions

Cases with meiotic recombination at the deletion breakpoint

families	deletion	proximal breakpoint		distal breakpoint		genetic distances[1]
11 WBS3	paternal	D7S499	grandmaternal	D7S669	grandpaternal	9.7 cM
12 WBS5	maternal	D7S653	grandpaternal	D7S669	grandmaternal	9.9 cM
13 WBS9	maternal	D7S653	grandmaternal	D7S669	grandpaternal	9.9 cM
15 WBS35	maternal	D7S672	grandmaternal	D7S634	grandpaternal	11 cM
16 WBS38	paternal	D7S653	grandmaternal	D7S669	grandpaternal	5 cM
17 WBS41	paternal	D7S672	grandmaternal	D7S669	grandpaternal	6 cM
19 WBS63	paternal	D7S653	grandpaternal	D7S669	grandmaternal	5 cM
20 WBS70	maternal	D7S672	grandmaternal	D7S669	grandpaternal	10.9 cM
25 WBS84	maternal	D7S672	grandpaternal	D7S524	grandmaternal	19 cM
27 WBS101	maternal	D7S672	grandpaternal	D7S669	grandmaternal	10.9 cM
28 WBS102	paternal	D7S672	grandmaternal	D7S669	grandpaternal	6 cM
30 WBS115	paternal	D7S653	grandpaternal	D7S669	grandmaternal	5 cM
32 WBS140	paternal	D7S653	grandmaternal	D7S2518	grandpaternal	2.2 cM

Cases with no meiotic recombination at the deletion breakpoint

families	deletion	proximal breakpoint		distal breakpoint		genetic distances[1]
14 WBS34	paternal	D7S672	grandmaternal	D7S669	grandmaternal	6 cM
18 WBS52	paternal	D7S645	grandmaternal	D7S440	grandmaternal	6.8 cM
21 WBS72	maternal	D7S672	grandpaternal	D7S669	grandpaternal	10.9 cM
22 WBS76	paternal	D7S653	grandpaternal	D7S669	grandpaternal	5 cM
23 WBS81	maternal	D7S653	grandmaternal	D7S630	grandmaternal	20.8 cM
24 WBS82	maternal	D7S653	grandmaternal	D7S669	grandmaternal	9.9 cM
26 WBS92	maternal	D7S672	grandmaternal	D7S669	grandmaternal	10.9 cM
29 WBS112	maternal	D7S653	grandpaternal	D7S634	grandpaternal	10 cM
31 WBS137	paternal	D7S653	grandmaternal	D7S669	grandmaternal	5 cM

[1]Genetic distances are according to Urbàn *et al.* (31), Pérez Jurado *et al.* (7) and GDB linkage maps.

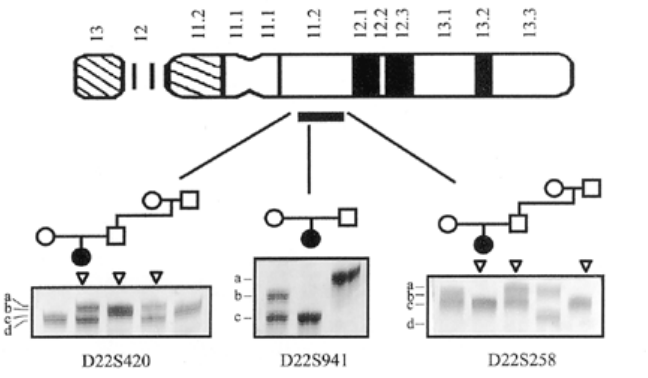

Figure 1. Representative examples of microsatellite analysis at 22q11.2 carried out for family 8. The deleted region of chromosome 22 is indicated with a black bar beside the chromosome 22 ideogram. Marker *D22S941*, located within the deleted region, illustrates the paternal origin of the deletion. Grandparental origins of the regions flanking the deletion are shown with markers *D22S420* (proximal region) and *D22S258* (distal region).

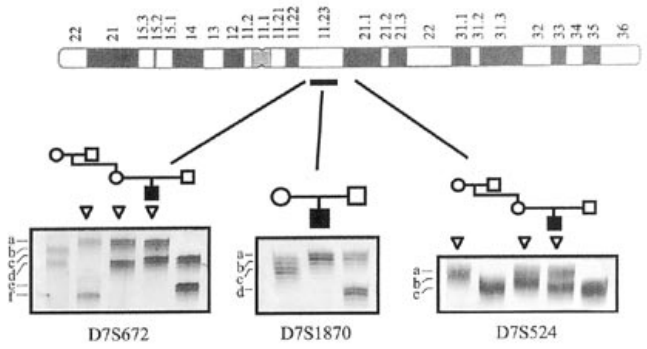

Figure 2. Representative examples of microsatellite analysis at 7q11.2 carried out for family 25. The deleted region of chromosome 7 is indicated with a black bar beside the chromosome 7 ideogram. Marker *D7S1870*, located within the deleted region, illustrates the maternal origin of the deletion. Grandparental origins of the regions flanking the deletion are shown with markers *D7S672* (proximal region) and *D7S524* (distal region).

overs: three crossovers were detected in the maternally derived deleted chromosomes, and five in those of paternal origin.

A high incidence of meiotic crossovers at 7q11.23 in patients with WBS

In this study, we have increased the number of families with a proband affected by WBS from the 15 cases reported by Dutly and Schinzel (32) to 22 families. In the earlier publication, we reported a high incidence of meiotic recombinations associated with WBS. In the group of 15 WBS patients, 10 were shown to have an interchromosomal recombination at the critical 7q11.23 region. The extra patients reported here corroborate this observation further: 13 cases are associated with a meiotic crossover (Table 4). Figure 2 shows representative results obtained for family 25. The expected number of meiotic recombinations in 22 individuals would be ~2, based on the average genetic distance

Table 5. Deletion size in CATCH 22 patients

Markers	1 **22q-14** maternal del. Mzt,M,F,GF	2 **22q-28** paternal del. P,M,F,GM,GF	3 **22q-55** paternal del. P,M,F,GM,GF	4 **22q-83** maternal del. P,M,F,GM,GF	5 22q-100 maternal del. P,M,F,GM,GF	6 **22q-112** paternal del. P,M,F,GM,GF	7 22q-120 paternal del. P,M,F,GM	8 **22q-156** paternal del. P,M,F,GM,GF	9 **22q-177** maternal del. P,M,F,GM,GF	10 **22q-196** paternal del. P,M,F,GM,GF
F8VWFP	ab,ab,bb,aa	-	-	-	-	-	-	ab,bb,aa,aa,-	-	-
D22S420	bc,cc,ab,ac	bd,cd,ab,be,ad	ni	cc,cc,ac,cc,bc	bc,bc,ac,bc,cc	cc,bc,bc,ac,bd	bc,bc,bb,ab	ad,cd,ab,ad,bc	cd,bd,ac,dd,bc	ab,bd,ad,ac,dd
D22S427	ac,ab,bc,ab	ac,ac,ac,aa,bc	ab,aa,ab,bb,aa	ac,ac,cc,ab,ac	bb,ab,bb,bc,aa	ab,ab,ab,aa,bb	cc,cc,ac,ab	ac,ac,bc,bc,-	ab,bb,ab,ab,ab	ab,ac,bc,bc,ac
D22S941	ni	ni	D	ni	-	D	D	D	D	ni
D22S944	D	ni	D	D	D	-	D	ni	D	D
D22S264	D	D	D	ni	-	ni	-	D	ni	-
D22S311	D	D	D	D	D	ni	D	ni	D	D
D22S306	bc,ab,cc,ac	ab,aa,ab,ab,bc	ab,ab,aa,aa,ab	ni	ni	ni	ab,bb,aa,ab	bc,ab,cc,ac,bc	bc,ac,bc,ac,cc	ni
D22S308	-	-	-	ab,bb,ab,ab,bb	ni	ab,aa,ab,aa,bb	-	-	bb,bb,ab,ab,bb	ab,ab,aa,ab,ab
D22S539	bb,bb,bb,ab	-	-	-	bb,bb,bb,bb,ab	-	-	-	-	-
D22S303	bb,bb,bb,ab	ad,ad,bd,ab,cd	cc,bc,bc,bb,ac	cc,bc,cc,bd,ac	aa,ab,ac,ac,bb	bd,bd,bc,ac,ab	aa,aa,aa,aa	ad,ab,dd,cd,-	bb,bc,ab,ac,ab	bb,ab,bb,ab,bc
D22S343	-	-	-	-	-	-	ab,ac,bd,cd	ab,ac,be,de,-	-	be,bc,ae,ac,de
D22S258	-	cc,bc,ac,ab,cd	-	-	bc,ac,bb,ac,ab	bd,bc,ad,ab,cd	bb,ab,bb,ab	cc,bc,ac,ad,cc	-	-
D22S315	-	ab,bc,ab,bb,ac	aa,aa,ab,-,ac	ab,bb,-,bc,bb	bc,ac,bb,bc,ab	ce,bc,ae,ad,be	-	cc,bc,ac,ad,-	-	-

The two lines indicate the probable breakpoints of the deletions. The order of the markers is from proximal (*F8VWFP*: 22q11.22–q11.23) to distal (*D22S315*: 22q11.2–q12.1).

The results obtained for the different markers are indicated as follows: D, deleted; ni, non-informative; –, not done. The alleles detected in the three-generation families are denoted arbitrarily 'a–e' according to their molecular size, whereby 'a' represents the largest allele occurring in each family. The order of the alleles is indicated for each family: Mzt, monozygotic twins (the alleles are indicated only once); P, patient; M, mother; F, father; GM, grandmother; GF, grandfather. Bold type indicates the detection of a meiotic crossover at the deletion site.

between the proximal and the distal markers. The probability of detecting 13 or more recombinations by chance in 22 meioses is $P = 4.45 \times 10^{-9}$ (calculated considering the interference value). No parent-of-origin bias in the occurrence of crossovers could be detected in the group of patients.

No apparent correlation between the sizes of the deletions and the occurrence of unequal meiotic recombinations in patients with CATCH 22 and WBS

The possibility was investigated of a correlation between the sizes of the 7q11.23 and 22q11 deletions and the occurrence of an unequal meiotic recombination. The approximate breakpoints of the deletions were analysed based on microsatellite markers mapping to the deleted regions. The common deletion usually includes markers *D22S941* and *D22S944* (34). Markers *D22S264* and *D22S311* have been reported to be deleted in almost all cases, and markers *D22S306* and D22S308 possibly deleted in a much smaller percentage of patients (34). Table 5 summarizes the results obtained for the 10 cases with CATCH 22. No detectable difference was observed regarding the length of the deletions. For all patients, markers *D22S941* and *D22S311* showed only one allele, indicative of either deletion or homozygosity, and markers *D22S427* and *D22S306* showed either one or two alleles, placing these markers outside the 'common' deletion. The distal breakpoints of the deletions in patient 7 (no meiotic crossover) and patients 1, 2, 3 and 9 (with meiotic crossovers) are all clearly located between markers *D22S311* and *D22S306*.

A largely homogeneous size of deletions associated with inter- and intrachromosomal recombinations was also seen in the patients with WBS (Table 6). Markers *D7S653* and *D7S489A* usually flanked the deletions and markers *D7S489B* and *D7S1870* showed homozygosity in almost all cases. One exception was detected; patient 13 had a larger deletion which included marker *D7S489A*. The deletion in this case was associated with a meiotic crossover event.

DISCUSSION

We show here that a significant number of 22q11.2 deletions in CATCH 22 syndromes are associated with interchromosomal rearrangements indicative of meiotic unequal crossing-over events between sister chromatids. The increase in number of patients with WBS further consolidates the previous finding that unequal meiotic recombinations underlie the formation of a high proportion of 7q11.23 deletions (32; this study).

Divergent reports have been published recently regarding the mode of formation of the deletions affecting the highly imprinted 15q11–13 region involved in PWS and AS (33,35). In one report (33), the authors demonstrated a significantly higher than random incidence of meiotic recombinations in PWS patients: five out of seven deletions were shown to be associated with unequal crossover events. The other report (35) suggests a difference in frequency of interchromosomal crossovers between AS and PWS. The incidence of meiotic crossover events detected in the PWS patients was not higher than that expected randomly, whilst all three AS patients showed an interchromosomal rearrangement at the critical region. A difference in the mode of formation of the paternal (PWS) versus maternal (AS) 15q11–13 deletion would be very interesting; however, the number of patients analysed should be increased in order to confirm this finding.

The seemingly identical size of the deletions involving either inter- or intrachromosomal rearrangements would indicate that the same 'hot-spot' regions are involved preferentially in both events. Rare variants in deletion size, such as in patient 13, would suggest that other repeats in the critical region could also be involved in misalignments, but that the intermediate structures may be less stable. Alu sequences and highly homologous sequences have been found to flank the majority of deletion and recombination sites. It can be envisaged that similar sequences are also involved in the formation of the CATCH 22 and WBS deletions. Indeed, the 3′ region of the elastin gene has been found to be very rich in Alu sequences (36), and a number of low copy repeats were shown recently to be contained in chromosome

Table 6. Deletion sizes in WBS patients

Patient	D7S653	D7S489B	D7S2476	D7S613	*ELN*	D7S2472	D7S1870	D7S489A	D7S2518	D7S2421	D7S669
11 **WBS3**	ni	D	-	-	D	-	D	ni	ni	ND	ND
12 **WBS5**	ND	D	-	-	D	-	D	ni	ND	-	ND
13 **WBS9**	ND	D	-	-	D	-	D	D	ni	ND	ND
14 WBS34	ND	ni	-	ni	D	-	D	ni	ND	-	ND
15 **WBS35**	ni	D	-	-	D	-	D	ni	ND	-	ni
16 **WBS38**	ND	ni	-	D	D	D	ni	ND	-	-	ND
17 **WBS41**	ni	D	-	-	D	-	D	ni	ND	-	ND
18 WBS52	ni	ni	D	-	D	-	D	ni	ND	-	ni
19 **WBS63**	ND	ni	ni	-	D	-	D	ni	ND	-	ND
20 **WBS70**	ND	ni	ni	-	D	-	-	ni	ND	-	ND
21 WBS72	ni	D	-	-	D	-	D	ni	ND	-	ND
22 WBS76	ND	ni	ni	-	ni	-	D	ND	-	-	ND
23 WBS81	ND	D	-	-	D	-	D	ni	ND	-	ni
24 WBS82	ND	ni	ni	-	D	-	D	ND	-	-	ND
25 **WBS84**	ni	D	-	-	D	-	D	ND	-	-	ni
26 WBS92	ND	ni	-	ni	ni	-	D	ND	-	-	ND
27 **WBS101**	ni	D	-	-	ni	-	D	ni	ND	-	ND
28 **WBS102**	ni	ni	ni	-	D	D	ni	ni	ND	-	ND
29 WBS112	ND	D	D	-	D	-	D	ni	ND	-	ni
30 **WBS115**	ND	ni	ni	D	D	-	D	ND	ND	-	ND
31 WBS137	ND	ni	ni	D	ni	ni	ni	ni	-	-	ND
32 **WBS140**	ND	D	D	D	D	-	D	ni	ND	-	ND

The two lines indicate the probable breakpoints of the deletions. The markers are listed in proximal to distal order (i.e. *D7S653*: 7q11.22 to *D7S669*: 7q21.11) according to Pérez Jurado *et al.* (7).
The results obtained for the different markers are indicated as following. D, deleted; ND, non-deleted; ni, non-informative; -, not done.
Patients indicated in bold indicated the detection of a meiotic crossover at the deletion site.

22q11 (37). Repeat sequences and highly homologous sequences are likely to stabilize chromosomal pairings and facilitate crossover events. The mechanism leading to the deletions probably involves mispairing of such elements either in sister chromatids (in the case of interchromosomal recombinations) or the creation of a loop within individual chromatids (in the case of intrachromosomal recombinations), followed by excision of the extruding loop (1).

Recurrence risk of the 22q11 and 7q11.23 deletions

The recurrence risk for healthy parents in cases where a meiotic recombination can be demonstrated is probably not higher than that for the normal population. However, it cannot be excluded that variations in the number and position of repeat sequences may result in a higher rate of unequal crossing-overs. The implications of similar mechanisms underlying both inter- and intrachromosomal deletions are that the recurrence risk for healthy parents would also be minimal in cases of intrachromosomal recombinations considering that the contribution of further endogenous or genetic factors, if at all, appears to be minimal. A slightly higher recurrence risk in these cases must, however, be taken into account due to the possibility of gonadal mosaicism in one of the parents. Indeed, recurrence of deletions has been shown not only in familial cases where one parent is a carrier for the deletion, but also in a familial case described by Eydoux *et al.* (38) with phenotypically normal parents. In this family, two siblings were shown to have a deletion on 22q11.2 by fluorescence *in situ* hybridization analysis. It would obviously be very interesting to analyse such cases at the molecular level.

Parent-of-origin bias in the occurrence of deletions and their mode of formation

A parent-of-origin bias is reported in the literature regarding the CATCH 22 deletions. The proportion of deletions affecting the maternal chromosome is estimated at ~75% (30). A slightly higher incidence of maternal deletions has also been reported in WBS (31). In the group of 32 CATCH 22 patients analysed in our Institute for parental origin of the 22q11.2 deletion, a maternal bias was not evident. The deletions affecting the maternal chromosomes (17 cases, 53%) were only marginally more frequent than those affecting the paternal chromosomes (15 cases). The percentage of maternally derived deletions (58%) in the 67 patients with WBS diagnosed at the molecular level in our institute is in agreement with the slightly higher incidence of maternal deletions reported in the literature. Taken together, the data would indicate the lack of imprinted genes in the critical CATCH 22 and WBS regions. This is also evidenced by the apparent absence of abnormal phenotypes in cases with uniparental inheritance of chromosome 22 (39). However, an interesting parent-of-origin repercussion on the WBS phenotype was reported by Pérez Jurado *et al.* (7). The authors have observed more severe forms of developmental delay in patients with maternally derived 7q11.23 deletions. This study raises the

possibility that the 7q11.23 deletions may alter an adjacent imprinting centre (7).

Regarding the mode of formation, no sex-specific occurrence of inter- versus intrachromosomal rearrangements were detected. We observed, however, a high incidence (eight out of 10) of grandmaternal alleles at the proximal positions in the CATCH 22 deletions, whereas an even distribution of grandmaternal and grandpaternal alleles was detected in WBS. A larger group of CATCH 22 patients will be needed in order to support this observation at the statistical level.

MATERIALS AND METHODS

Clinical evaluation of the patients

The patients included in this study were ascertained by clinical geneticists at our institutes or referred to us by general practitioners for molecular diagnosis.

All patients had *de novo* interstitial deletions. The major clinical findings in the 11 probands with CATCH 22 are summarized in Table 1. In the first family, the affected children are monozygous twins. The major clinical findings in the 22 patients affected by WBS are listed in Table 2.

Molecular analyses

Lymphocyte DNA was extracted by standard procedures from blood samples of the probands, their parents and, after determining the parental origin of the deletions, also from the relevant grandparents. Determination of parental origin of the deletions and haplotype analysis was carried out using microsatellite primers purchased from Research Genetics. The localization and genetic distances of the markers reported in the tables are derived from Généthon and GDB linkage maps. The microsatellite markers were used in polymerase chain reactions (PCR) consisting of 35 cycles of: denaturation for 30 s (3 min in the first cycle) at 94°C, primer annealing at 52–60°C for 45 s and primer extension at 72°C for 1 min. The reactions were carried out using a Perkin Elmer PCR cycler. The PCR products were separated on 6% denaturing polyacrylamide gels and visualized by silver staining using standard procedures.

ACKNOWLEDGEMENTS

We are grateful to the patients, their families and physicians for their valuable co-operation. In particular, we would like to thank Dr S. Basaran (Istanbul, Turkey), Dr E. Irnberger (Salzburg, Austria), Dr E. Troy (Hohenems, Austria), Dr T. Felix (Porto Alegre, Brazil), Dr A. Frank (Winterthur, Switzerland) and Professor K. Baerlocher (St Gallen, Switzerland) for referring patients to us. This study was supported by the Swiss National Foundation (grant no. 32-37798.93).

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