Letters to the Editor

SHOX point mutations in dyschondrosteosis

Céline Huber, Veronica Cusin, Martine Le Merrer, Michèle Mathieu, Véronique Sulmont, Nathalie Dagoneau, Arnold Munnich, Valérie Cormier-Daire

Dyschondrosteosis (DCS) has been recently ascribed to mutations of the SHOX gene on the pseudoautosomal region of the X and Y chromosomes. Most cases are accounted for by large scale deletions and only two point mutations have been hitherto identified in exon 4 (R195X and Y199X). Here, we show that point mutations in various regions of the SHOX gene also play an important role in the pathogenesis of the disease.

A total of 22 affected subjects belonging to eight families were included in the study. Inclusion criteria for affected status were short stature (2 SD below normal) with short forelimbs and distal radioulnar deformity on forearm x rays.

The 22 patients and their relatives were genotyped using microsatellite DNA markers of the pseudoautosomal region (CA–SHOX, DXYS233, DXYS234, DXYS228). Linkage studies supported the mapping of the disease gene to Xp22.3 in all families and hemizygosity at the CA–SHOX locus was observed in three families (families 1–3, data not shown).

In families 4–8, PCR amplification and sequence analysis of the five translated exons of the SHOX gene (table 1) led to the detection of five different mutations which consistently cosegregated with the disease (table 2). In family 4, a two base pair deletion in exon 2 led to a frameshift and a translation termination at codon 77. In families 5 and 6, base changes at nucleotides 334 and 445 respectively created a stop codon in exon 3 (Q112X and E149X). In family 7, a GT insertion at codon 161 led to a premature stop codon. All four mutations resulted in a premature translation termination. Finally, in family 8, a C→T transition in exon 4 changed an arginine into a cysteine in the protein (R173C). This mutation was found in the affected mother and her three affected children but neither in the healthy father nor in 90 control chromosomes.

We have reported on SHOX deletions and a nonsense mutation in DCS (seven deletions, one nonsense mutation). Studying eight additional DCS families, we describe here three deletions and five point mutations of SHOX. Taken together, these data suggest that SHOX deletion is the most frequent common disease causing mechanism in DCS (10/16, 62.5%) and that point mutations also account for a significant fraction of our patients (6/16, 37.5%).

This study also supports the view that haploinsufficiency is the most frequent mechanism in DCS (10 deletions and five nonsense and frameshift mutations in 15/16 families). Among the five novel mutations reported here, only one was a missense mutation resulting in an amino acid change in the homeodomain of SHOX. Interestingly, this family was not clinically different from the others. Indeed, all patients had short stature and x ray deformity of the forearms although intrafamilial variability was consistently observed with males being usually less severely affected.

In conclusion, this study shows that point mutations of the SHOX gene account for a significant number of DCS patients and shows that haploinsufficiency is the most frequent disease causing mechanism in DCS.

Table 1 Detailed PCR conditions for SHOX exon amplification. The exon numbers have been assigned according to Blaschke and Rappold1

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<tr>
<th>Exon</th>
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<td>Exon 2 5’</td>
<td>GAGAGGCGGGGACCTCACCACCA</td>
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<td>Exon 6a 5’</td>
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<tr>
<td>Exon 6a 3’</td>
<td>ATCCACTTGGTACAGGAGTTT</td>
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<td>376</td>
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Table 2 SHOX point mutations identified in DCS patients

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<th>Sequence change</th>
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<td>Family 4</td>
<td>106–107 del CG</td>
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<td>Family 5</td>
<td>334 C→T</td>
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<td>Family 8</td>
<td>517 C→T</td>
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Phenotypic heterogeneity of CYP1B1: mutations in a patient with Peters’ anomaly

Andrea Vincent, Gail Billingsley, Megan Priston, Donna Williams-Lyn, Joanne Sutherland, Tom Glaser, Edward Oliver, Michael A Walter, Godfrey Heathcote, Alex Levin, Elise Héon

Congenital glaucoma refers to a genetically heterogeneous group of distinctive clinical diseases characterised by increased intraocular pressure most often associated with increased corneal diameter, corneal oedema, and consequent visual impairment. Primary congenital glaucoma (PCG) is associated with a primary angle defect, whereas secondary congenital glaucoma is associated with a more generalised developmental anomaly of the anterior segment such as seen in Peters’ anomaly. The inheritance of PCG is usually autosomal recessive.

Peters’ anomaly consists of corneal opacity, defects in the posterior structures of the cornea, and iridocorneal and/or keratolenticular adhesions, and it most frequently occurs sporadically. Over 50% of subjects develop glaucoma in childhood. Numerous aetiologies have been proposed including chromosomal abnormalities, teratogens, and mutations in the eye developmental genes PAX6 and PITX2. However, large subsets of Peters’ anomaly cases are without molecular characterisation.

Primary congenital glaucoma has been linked to chromosomes 1p36 (GLC3B) and 2p21 (GLC3A), but only the GLC3A gene has been identified. This gene, CYP1B1, encodes a broadly expressed cytochrome P450 enzyme (P4501B1) whose natural substrate is unknown. It is proposed to be the major gene for disease-causing mutations in primary congenital glaucoma. A variety of chain terminating and missense CYP1B1 mutations have been described. We report two novel mutations in CYP1B1 in a patient who had Peters’ anomaly with secondary congenital glaucoma.

Case report

The subject, a male of Native Indian (Mohawk)/French Canadian background, presented with a history of bilateral cloudy corneas and tearing since birth. Examination at 3 weeks of age showed bilateral corneal oedema with central corneal opacities, superficial pan-angle defect, and iridocorneal adhesions with a well-formed anterior chamber (fig 1, above). Iris was present for 360°. The posterior stroma was absent centrally, and there was a basophilic granular deposit containing a small amount of extracellular melanin. Descemét’s membrane was not identified. The position of the fetal sutures and suggested the existence of previous keratolenticular adhesions during development. Peters’ anomaly was identified in this half of the button. However, transmission electron microscopy showed a recognisable Bowman’s membrane, though abnormal and containing scattered kerocytes. Also a small segment of Descemét’s was observed, although thin (approximately 1.5 µm) with a rather poorly defined banding pattern. No endothelial cells were seen.

DNA extraction followed standard protocols. Amplification of the coding sequence of CYP1B1 used previously published primers. Additional primers for exon 3 were designed from the genomic DNA sequence (Acc No U56438). Forward primer: 5’-ctataaagcttcctcaag-3’, reverse primer: 5’ttgtcgaagatctggtg-3’. Mutational analysis of the amplicons by single strand conformational polymorphism (SSCP) and direct cycle sequencing used protocols previously described. We identified two novel CYP1B1 mutations in exon 2 making our subject a compound heterozygote for the missense mutations 3807T→C, which is predicted to result in the amino acid change Met1Thr (ATG→ACG), and the nonsense mutation 3976G→A, which

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is predicted to truncate the P4501B1 polypeptide (Trp57Stop, TGG→TAG) and possibly cause nonsense mediated decay of the CYP1B1 mRNA (fig 2). This latter mutation was seen in the mother’s DNA who was clinically normal.

The father was not available for testing. These mutations were not seen in 100 normal controls of mixed ethnicity. Furthermore, mutational analysis of PITX2 and PAX6 using previously described protocols failed to document any pathogenic sequence change in this patient. Ten additional unrelated subjects with Peters’ anomaly were screened for mutations in CYP1B1, PITX2, and PAX6. Only one patient showed a sequence change in PAX6 and none in CYP1B1.

Discussion
Peters’ anomaly is a congenital abnormality that shows a wide range of histopathological changes. It has been proposed that a normal Descemet’s/endothelial unit is necessary for maintenance of the integrity of the corneal stroma. The posterior stromal abnormalities seen in our patient may be a consequence of the abnormal segment of Descemet’s membrane. The nature of the basophilic deposit in the posterior stromal defect remains uncertain but may represent denatured lens protein associated with the presumed developmental keratolenticular adhesion noted at the time of the corneal transplant. The absence of Bowman’s membrane on light microscopy, albeit not complete, is also a characteristic feature of Peters’ anomaly.

It has been suggested that both Peters’ anomaly and primary congenital glaucoma arise from defective neural crest cell migration in the 4th to 7th week of fetal development, during which period the anterior segment of the eye forms. The relationship between these two diseases is otherwise not well understood. This is the first documentation of CYP1B1 mutations causing a phenotype other than congenital glaucoma. We report two new CYP1B1 mutations, the combination of which is associated with a different phenotype, Peters’ anomaly. These findings strengthen the potential role of this gene in anterior segment eye development. One of the CYP1B1 mutations identified (3976G→A, Met1Thr) would probably disrupt translation initiation, as the methionine codon surrounded by a Kozak sequence is crucial for ribosomal recognition. The next conserved Kozak sequence where translation could be initiated occurs in frame, but would result in truncation of 131 amino acids from the amino terminus of the protein. Translation can be initiated at non-AUG codons, including an ACG, but this is extremely rare and requires favourable mRNA secondary structure. The second mutation in this patient (3976G→A) results in a premature stop codon (Trp57Stop). Expression of this allele would therefore result in a protein truncated by 486 amino acids and is thus also likely to represent a functional null allele.

Our observations suggest that a normal CYP1B1 product is required for differentiation of the anterior segment, and that some cases of Peters’ anomaly and PCG may share a common aetiology. Homozygous CYP1B1 null mutations have been described in cases of congenital glaucoma; however, insufficient clinical information is available to establish...
phenotype-genotype correlations. We hypothesise that the severity of the Peters’ phenotype described correlates with the extent of predicted protein truncation.

The spectrum of the phenotypes associated with CYP1B1 mutations is broader than anticipated as is the genetic heterogeneity of Peters’ anomaly. The findings reported here suggest that the role of CYP1B1 is not solely confined to the pathogenesis of primary congenital glaucoma but may play a more general role in eye development. This work emphasises the genetic heterogeneity and the complexity behind anterior segment development disorders and early glaucoma.

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Role of TP53 P72R polymorphism in human papillomavirus associated premalignant laryngeal neoplasm

Leena-Maija Aaltonen, Ren Wei Chen, Stina Roth, Antti A Mäkitie, Heikki Rihkanen, Antti Vaheri, Lauri A Aaltonen

Laryngeal papillomatosis, a disease in which malignant transformation takes place in 3-7% of patients, is caused by low risk human papillomavirus (HPV) types 6 and 11. The mechanism by which HPV affects TP53 involves the HPV E6 oncoprotein, which has been shown to bind TP53 protein and cause its degradation through the ubiquitin pathway. It has been proposed that subjects with homozygous arginine (Arg) at position 72 in the TP53 amino acid sequence are more susceptible to HPV associated uterine cervical and cutaneous tumorigenesis than are heterozygotes, and that the TP53 Arg allele is susceptible to TP53 E6 mediated degradation regardless of whether the infection is by a low risk or high risk HPV type. However, although these findings could not be confirmed in studies of large populations of women with premalignant and malignant cervical neoplasia, recently Zehbe et al reported an association between TP53 codon 72 Arg homozygotes and development of cervical cancer.

Our study is based on Finnish adult onset (age at diagnosis ≥17 years) laryngeal papilloma patients treated at Helsinki University Central Hospital, Department of Otorhinolaryngology, during the years 1975-1994. Diagnosis of laryngeal papillomatosis was confirmed by detection of HPV DNA by polymerase chain reaction (PCR) from patients' laryngeal biopsies. Because only paraffin embedded biopsies (n=59) were available, and only 29 of them were HPV positive, results on fresh frozen HPV positive biopsies of an additional 13 randomly selected adult onset patients treated during the years 1997-1999 were included.

The TP53 proline 72 arginine (P72R) polymorphism was analysed from lymphocyte DNA by sequencing the genomic PCR product of exon 4 of these 42 adult onset patients. The results were confirmed by digestion of the PCR products. All data derived from the patient samples by sequencing and by digestion analysis were identical. Our reference population was 186 cancer free anonymous blood donors and their samples were analysed by digestion only. No differences existed in Arg and Pro/Arg allele frequencies between patients and controls (table 1).

Of the HPV 6 positive patients, 15/27 (56%) were Arg homozygotes, and of the HPV 11 positive, 7/10 (70%). In addition, three patients' biopsies were both HPV 6 and 11 positive, and two patients had some other HPV type in their laryngeal biotype. Based on experimental studies with HPV 11 and analysis of skin cancers representing mixed HPV types, Storey et al proposed that in addition to high risk HPV types, Arg homozygosity predisposed to tumours promoted to low risk HPV types as well. This conclusion is not in accordance with our data. Although HPV 11 E6 may induce degradation of TP53 Arg in vivo, this mechanism is not necessarily involved when laryngeal tumours are caused by HPV 6 or 11 virus types. These findings argue against a role for the P72R polymorphism in this HPV associated premalignant neoplasm and against the hypothesis that TP53 Arg affects the susceptibility of TP53 to E6 mediated degradation also in infections caused by low risk HPV types.

Table 1 TP53 polymorphism in adult onset laryngeal papilloma patients and controls

<table>
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<th>Amino acid at position 72</th>
<th>Patients (n=42)</th>
<th>%</th>
<th>Controls (n=186)</th>
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<td>Arg</td>
<td>24</td>
<td>57</td>
<td>48</td>
<td>47</td>
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<td>Pro/arg</td>
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<td>75</td>
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<tr>
<td>Pro</td>
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<td>12</td>
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No significant differences were detected between Arg and Pro/Arg amino acid sequences at position 72 in patients and controls. The Pro allele was more common in controls than in patients (Fisher's exact test, two tailed).

Predisposing chromosome for spinocerebellar ataxia type 6 (SCA6) in Japanese


The autosomal dominant cerebellar ataxias (ADCA) are a group of neurodegenerative disorders which can be classified into three major categories on the basis of their clinical features and mode of inheritance. SCA type III is a pure cerebellar syndrome that is genetically heterogeneous and includes spinocerebellar ataxia type 5 (SCA5), SCA6, SCA10, and SCA11. The gene responsible for SCA6 has been identified as coding for the subunit of the P/Q type voltage dependent calcium channel (CACNA1A). Moderate CAG expansion in the coding region causes the disorder, with the number of CAG repeats being originally reported as 21-27 in mutant alleles (n=8) and 4-16 in control alleles (n=950). Subsequent studies have indicated that the range of pathologial expansion in SCA6 alleles varies from 20 to 33. The CACNA1A gene was first identified during the search for specific mutations causing familial hemiplegic migraine (FHM) and episodic ataxia type 2 (EA2). The gene product has four transmembrane domains and glutamine repeats are located at the C-terminal side of the intracellular segment. Missense mutations of these transmembrane domains and deletions or splice mutations leading to a truncated protein are responsible for FHM and EA2, respectively. The CACNA1A gene is predominantly expressed in Purkinje cells and granule cells of the cerebellum and is essential for the survival and maintenance of normal function by these neurons. Biochemical mechanisms leading to the development of SCA6 are not fully understood. However, the fact that slowly progressive ataxia is often observed in EA2 indicates that a small glutamine expansion in the SCA6 gene also disturbs the function of P/Q-type calcium channels, leading to selective neuronal degeneration in the cerebellum.

The pathogenic expansion in SCA6 is relatively small compared with those in other SCAs caused by triplet repeat expansion, but there is still a significant inverse correlation between the age at onset and the number of repeats in SCA6. Some homozygotes for the SCA6 mutation show a more severe phenotype, but others do not. Unlike other SCAs with long CAG repeats, the expanded SCA6 allele is known to be relatively stable during meiosis and mitosis, with some exceptions. The cardiac feature of SCA6 is slowly progressive ataxia, but exceptions have been reported.

The frequency of SCA6 varies between white ethnic subgroups, with a range of 0% to 15.2%. In Japan, the frequency varies between regions, ranging from 5.9% to more than 30%. In Hokkaido, the north-ernmost island of Japan, SCA6 accounts for 30% of 161 families with ADCA, the highest frequency reported to date. These findings prompted us to search for a possible founder chromosome in Japanese SCA6, and to determine whether there are any alleles predisposing to the generation of SCA6 mutation.

Material and methods

Twenty one unrelated Japanese SCA6 families were investigated. Twelve non-consanguinous families and one consanguinous family have already been reported elsewhere, while eight families were newly added in this study. Thirteen of 21 families reside in Hokkaido, while the other eight families come from various other areas of Japan. The ancestors of the Hokkaido families moved to this island approximately a century ago from various, random other areas (data not shown). Altogether, 58 subjects were clinically affected, 35 were asymptomatic, and 10 had married into these families. In addition, 25 patients without family members available for testing were recruited from Hokkaido; a family history of ataxia was positive in 18 and negative in seven patients. Among the total of 83 patients, the mean age at onset was 49.6 (SD 11.6) years, ranging from 19 to 75 years.

After informed consent was obtained, high molecular weight DNA was extracted from peripheral white blood cells according to the method of Zhuchenko et al; polymerase chain reaction (PCR) amplification of CAG containing segments in the CACNA1A gene was performed using primers S-5-F1 and S-5-R1. S-5-F1 was end labelled with 6-FAM (PE Biosystems). After PCR amplification of genomic DNA using a PE9600 thermal cycler (PE Biosystems), the CAG repeat polymorphism was analysed using an ABI PRISM 377 gene sequencer equipped with GeneScan® software version 2.0 (PE Biosystems). The number of CAG repeats was determined with reference to the product size of the sequenced alleles.

To construct haplotypes carrying the CACNA1A gene, D19S840, D19S1150, D19S226, and D19S885 were analysed. These four microsatellites cover a 4 cM interval containing the entire CACNA1A genome from the telomeric to the centromeric end. D19S1150 is located in intron 7 of the gene (fig 1). Polymorphism of these microsatellites was analysed using an automated gene sequencer® and the alleles of each microsatellite were numbered according to the product size. The CA repeat sequence of D19S1150 was determined using Genome Database information (accession No 1320259). After purification on a Microcon-100 spin column (Amicon), PCR
products of homozygotes for D19S1150 were directly sequenced using a BigDye Terminator Cycle Sequencing Kit (PE Biosystems), with p858 FOR as the forward primer.

In addition to these microsatellites, we examined two single nucleotide polymorphisms (SNPs) in the coding region of the gene: one (A/B system) was a G to A substitution at position 2369 in exon 16, and the other (C/D system) was a G to A substitution at position 1457 in exon 8 (fig 1). A pair of primers, Yb-1 (5’-TCCACAGCTGCATCTCC AAG-3’) and Yb-2 (5’-ACCCCTCCCTTGAG CCCCT-3’), generated a 270 bp fragment covering the site of position 2369 in exon 16 (A/B system). This site was recognised by the $H_{ga}$ restriction enzyme. The SNP at nt 1457 in exon 8 (C/D system) was detected by mismatch PCR. Another primer pair, Ym-1 (5’-ATACTCTGGCTTTTCTATGC-3’) and Ym-2 (5’-TTTCATCCTCGGCGAGGATCGA CCTCTTCTGCTTTTGAGATCGA-3’), generated a 170 bp fragment that included a $C_{la}$ restriction site. PCR was done for 30 cycles in a total volume of 20 µl with 1.7 mol/l N,N,N’,N-trimethyl glycine (Wako) under the same conditions as for amplification of microsatellites, except that denaturation, annealing, and extension were each done for 60 seconds. To facilitate introduction of a restriction site, $r_{Th}$ DNA polymerase (PE Biosystems) was used with the Ym-1/-2 primer pair. The annealing temperature was set at 58°C for the Yb-1/-2 pair and 52°C for the Ym-1/-2 pair. After digesting the PCR products for one hour at 37°C with 1 U of either $H_{ga}$ or $C_{la}$, the alleles at each polymorphic site were determined by agarose gel electrophoresis.

We constructed haplotypes for the mutant SCA6 alleles (SCA6 chromosome) in 21 families based on their family structures and map order of 19p13 markers. Differences in allele frequency between the affected and control haplotypes were analysed using the $\chi^2$ test and $p<0.05$ was considered statistically significant. Unrelated normal Japanese subjects (mostly residents of Hokkaido) served as controls. In addition, three normal subjects who had married into the affected families from outside Hokkaido were included as controls. Both the SCA6 patients and controls were from the same ethnic background.

For phase unknown samples, such as the controls (unrelated normal subjects, n=172) and the SCA6 patients (n=25) for whom family samples were not available, estimation of haplotype frequency was performed by the maximum likelihood method using a simplified version of the GENEF computer program (J-M Lalouel, unpublished data). Procedures for generating the haplotype have been described in full by Jeunemaitre et al. Briefly, two polymorphisms were chosen to generate the haplotype, followed by sequential inclusion of one polymorphism at a time. Haplotypes showing a frequency below 1/4N (where N is the sample size) were eliminated during the process, and then the haplotype frequency was re-examined. Simple $\chi^2$ tests of homogeneity were applied for statistical comparison between cases and controls.

**Results**

Among the 21 families, 58 affected subjects and nine asymptomatic subjects of risk age carried the expanded CAG repeat. The geno-
typing data for the 12 previously reported families are included in the present analysis. Among these 21 families, one asymptomatic subject of risk age was homozygous for 21 repeat alleles and the others were all heterozygotes with both expanded and normal alleles. No cases of the unstable transmission of expanded alleles were observed. The 25 other patients without family samples were all heterozygotes for SCA6 mutations. The mean CAG repeat size of the mutant alleles was 23.1 (SD 2.1) (n=93 SCA6 alleles), with the range being 21-33. There was a significant inverse correlation between age at onset and the number of CAG repeats (n=83 patients with known age at onset; \(\gamma = -0.706, R^2 = 0.499, p<0.0001\), Pearson’s product moment method). When polynomial analysis was used, a significant correlation was also obtained (R^2=0.539, p<0.0001). The number of CAG repeats in unrelated normal alleles ranged from 4 ∼ 18 (n=388), with a peak of 13 (24.5% of the total); 64.2% of the control alleles had 11-13 repeats and 7.0% had 15 repeats or more.

After construction of the D19S840-D19S1150-C/D-A/B-D19S226-D19S885 haplotype in the affected families, we found that the same haplotype (major haplotype) cosegregated with affected status in each family (table 1). In the D19S1150-C/D-A/B haplotype, either “5-C-B” (17 families, 81%) or “1-C-B” (four families, 19%) was selectively associated with SCA6 chromosomes. Sequencing showed that allele 1 of D19S1150 had (CA)6AA(CA)17, and allele 5 had (CA)6AA(CA)21.

We first compared the allele frequency of each polymorphism for unrelated control chromosomes with SCA6 chromosomes deduced from the affected families. Three intragenic markers, an intronic microsatellite (D19S1150) and two SNPs in exons 8 and 16, showed significant differences in allelic frequency between the affected chromosomes and controls (p<0.0001, table 2). Even two extragenic microsatellites, D19S226 and D19S885, showed a significant difference (p<0.0001 and p<0.005, respectively). These results indicate that there was significant linkage disequilibrium between SCA6 mutations and these markers. In order to determine the profile of the CACNA1A gene haplotype, we then analysed the D19S1150-C/D-A/B polymorphism in controls (172 unrelated normal subjects) and 25 SCA6 patients for whom family data were unavailable. After genotyping, we performed

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<td>P90</td>
<td>2</td>
<td>5</td>
<td>C</td>
<td>B</td>
</tr>
</tbody>
</table>

ND: phase not determined.

### Table 2: Linkage disequilibrium between SCA6 mutation and 19p13 polymorphic markers from the SCA6 chromosomes of 21 unrelated families

<table>
<thead>
<tr>
<th>Locus</th>
<th>Polymorphism</th>
<th>No of alleles</th>
<th>Associated allele</th>
<th>Frequency (No)</th>
<th>Control chromosome</th>
<th>Frequency (No)</th>
<th>Difference</th>
<th>(\chi^2)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D19S840</td>
<td>9</td>
<td>2(203 bp)</td>
<td>55% (11)</td>
<td>44.6% (318)</td>
<td>NS</td>
<td>0.47</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D19S1150</td>
<td>9</td>
<td>5(158 bp)</td>
<td>81% (17)</td>
<td>16.8% (388)</td>
<td>51.23</td>
<td>p&lt;0.0001</td>
<td>22.63</td>
<td>0.0005†</td>
<td></td>
</tr>
<tr>
<td>nt1457 in exon 8</td>
<td>2</td>
<td>C</td>
<td>100% (2)</td>
<td>64.5% (344)</td>
<td>11.33</td>
<td>p&lt;0.001</td>
<td>0.77</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>nt2569 in exon 16</td>
<td>2</td>
<td>B</td>
<td>100% (21)</td>
<td>71.8% (344)</td>
<td>8.18</td>
<td>p&lt;0.005</td>
<td>10.47</td>
<td>p&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>D19S226</td>
<td>13</td>
<td>10(245 bp)</td>
<td>57% (12)</td>
<td>14.0% (318)</td>
<td>22.79</td>
<td>p&lt;0.0001</td>
<td>0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D19S885</td>
<td>7</td>
<td>2(175 bp)</td>
<td>70% (14)</td>
<td>32.3% (318)</td>
<td>10.47</td>
<td>p&lt;0.005</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data from SCA6 chromosomes from 20 unrelated families.†Fisher’s exact probability test.‡Definitions of the biallelic system are shown in fig 1[f1].NS: not significant.
haplotype estimation by the maximum likelihood method on samples for which the phase was not determined. Estimated haplotype frequencies were compared between SCA6 patients and controls (table 3). The results of this analysis were as follows: (1) the frequencies of 5-C-B and 1-C-B haplotypes were significantly higher in patients than in controls (49% vs 11%, \( \chi^2=46.69, df=1, p<0.001 \) and 5% vs 0%), indicating that the SCA6 mutant allele in these 25 patients was most likely to carry either haplotype 5-C-B or 1-C-B; (2) the frequency of the C-B haplotype was significantly higher in patients than in controls (70% vs 45%, \( \chi^2=11.14, df=1, p<0.001 \)); (3) C-B was the most frequent haplotype in controls (45% of 344 alleles); and (4) the D-B haplotype frequency was 27% in controls, but 0% in patients.

**Discussion**

The present study disclosed several findings about the genetic background of SCA6 in the Japanese. First, study of SCA6 families showed that only two haplotypes, "5-C-B" (81%) and "1-C-B" (19%), were significantly associated with the affected chromosomes (SCA6 chromosomes), and that the allele frequencies of each locus on these chromosomes was significantly different from those of controls. Second, 5-C-B was also the most frequent haplotype in probands (49%, n=50 chromosomes), indicating that one of the two haplotypes in each patient can be expected to be this common haplotype. Third, all of the affected haplotypes carried the C-B haplotype, which was the most frequent haplotype in control chromosomes (45%). The significantly high frequency of the 5-C-B haplotype among the probands implies that their SCA6 mutation also resides on this haplotype, as was found in the affected families. However, since we could not determine directly which chromosomes (haplotypes) were the site of the SCA6 mutation, the possibility that haplotypes other than C-B carry the mutation cannot be completely excluded.

In Hokkaido, the majority of residents including the present subjects are descendants of immigrants from various areas of Japan and share a single ethnic background. Taking these historical data and the results of our genetic analyses into account, there is a possible founder effect in the subjects from Hokkaido and also in those from other areas of Japan. Judging from our data, these results favour the hypothesis that the expanded SCA6 alleles in the Japanese population originated from a chromosome with a C-B haplotype, which is the most frequent haplotype in controls (45% of alleles). The most plausible scenario is as follows. First, the SCA6 mutation occurred on a chromosome with the 5-C-B haplotype. At some point thereafter, the removal of four CA repeats occurred, an event which changed the haplotype from 5-C-B to 1-C-B. This is supported by the finding that the CAG repeat size of mutant SCA6 alleles is more variable on 5-C-B chromosomes than on 1-C-B chromosomes (22-33 vs 21-24).

In the SCA6 allele, 7 and 11-13 CAG repeats are the predominant alleles in normal populations, regardless of ethnic background. Alleles with 15 CAG repeats or more are quite rare in European/American populations. However, alleles with 15-19 repeats are not infrequently observed in the Japanese population, having a range of 5.9% to 7.0% (present study, n=388). A recent study indicated that, in dominant SCAs caused by triplet repeat expansion including SCA6, the frequency of large alleles in a normal population is correlated with the relative prevalence in different ethnic groups. These data suggest the possibility that such large alleles are a potential reservoir for full mutant alleles, which may explain the high prevalence of SCA6 in the Japanese. It would be worthwhile to determine whether such intermediate SCA6 alleles in the normal Japanese population have a C-B haplotype.

Recent recurrent mutations of at risk chromosomes are considered to be potential founders in several CAG triplet disorders. In Huntington's disease (HD), haplotype studies on a cohort of families have shown that only 41% were derived from either one of two common ancestral haplotypes while the rest were from independent mutations. De novo expansions from intermediate alleles have also been reported in HD. In Machado-Joseph disease (MJD/SCA3), haplotype analyses using intragenic SNPs have shown several ancestral mutations, and normal chromosomes with intermediate expansions in a prevalent population carry the same haplotype that is shared with the affected chromosomes in that population. On the other hand, in DRPLA, a single predisposing haplotype was selectively associated with the affected chromosome and with normal chromosomes carrying a larger expansion. The frequency of the allele with the pre-
disposing haplotype is considered to be correlated with the prevalence of DRPLA in different ethnic groups. Several different founder haplotypes for SCA6 have been identified in white populations. In addition, de novo expansion from the intermediate alleles has been reported. Observation of these triplet repeat diseases suggests that the number of founder haplotypes is associated with the degree of instability of the predisposing chromosomes, which leads to pathogenic repeat expansion.

Despite extensive ongoing investigation, the molecular mechanism responsible for the instability of expanded repeats remains unknown. Our study showed that the majority of Japanese SCA6 mutations are derived from a C-B haplotype pool. This implies the possibility that some cis acting factor plays a role in promoting instability of CAG repeats in the SCA6 gene. A similar mechanism has been postulated through the study of DRPLA.

Brock et al. reported that the expandability of elongated CAG triplet repeats was strongly correlated with their location within CpG islands and with the GC content in the flanking sequence of CAG repeats. Their study provides insight into the molecular basis of cis acting factors, which modify the instability of expanded triplet repeats. However, in SCA6 as well as DRPLA, the molecular mechanism leading to full expansion from a particular predisposing chromosome is not fully understood.

To understand the molecular mechanism of SCA6 mutation better, our conclusions need to be confirmed through the study of different ethnic groups.

We thank members of the families participating in this study, and Drs K Shima (Sapporo Minami National Hospital), T Hamada, T Fukuzaka (Hokukyai Neurological Hospital), and others for referring the families. This work was supported by a Grant in Aid for Scientific Research on Priority Areas and a Grant for Referring the Families. This work was supported by a Grant in Aid for Scientific Research on Priority Areas and a Grant in Aid for Scientific Research (A) and (B2) from the Ministry of Education, Science, Sports and Culture, Japan, and a Grant for Research on Ataxic Diseases from the Ministry of Health and Welfare, Japan. This work was presented at the 124th Annual Meeting of the American Neurological Association on 10-13 October 1999, Seattle, Washington, USA.
Prenatal testing for Huntington's disease: experience within the UK 1994-1998

Sheila A Simpson, Peter S Harper on behalf of the United Kingdom Huntington's Disease Prediction Consortium

Huntington's disease (HD) is an adult onset, autosomal dominant disorder with onset of symptoms usually in the fourth or fifth decade. The classical triad of clinical features, movement disorder, cognitive impairment, and personality and psychiatric disorder, cause serious management problems. There is significant morbidity within the affected families, especially for those who themselves are at risk of developing the disease. HD affects about 5000 people in the UK and about five times that number are considered to be at 50% risk of developing the disease.

Since the mapping of the locus for Huntington's disease on chromosome 4 in 1983, followed by the identification of the gene and its expanded polyglutamine repeat in HD in 1993, it has been possible to offer accurate tests for HD. Prenatal tests and presymptomatic testing for adults at risk of HD are available at genetic centres throughout the world.

There are two common approaches to prenatal testing in HD. Direct testing involves investigating for the presence of the mutation in a pregnancy. This gives an accurate result. If the status of the at risk parent has not been ascertained, then this may produce predictive information about that person.

In exclusion testing, the at risk grandparental chromosome 4 locus is excluded using linkage analysis. This test preserves the 50% risk of the parent, and allows a pregnancy at low risk to continue. In this situation, pregnancies that share the risk of the parent would be terminated. However, should the at risk parent not develop HD, a normal pregnancy would have been lost.

Given the technical feasibility of prenatal mutation testing and the severity of the disorder, it might be expected that prenatal diagnosis would be frequently requested.

Tyler et al reviewed a group of referrals for exclusion testing in pregnancy, and surveyed a group of subjects at 50% risk of developing HD about their attitudes to prenatal testing. They concluded that the demand for such testing was likely to be small. We considered it important to assess this demand in relation to that for presymptomatic testing, and since the numbers recorded by individual centres were small, to collect the data on a UK basis.

In Britain, the UK Huntington's Disease Prediction Consortium was created to monitor the use of molecular testing in HD, to evaluate the developing service, and to ensure the highest standards were applied to the procedure. Several studies before the introduction of predictive testing reported the views of those at risk of HD. These showed that between 56% and 80% of at risk subjects would undergo predictive testing once it was available. Uptake of such testing has been considerably less than this, 9-15% with some exceptions.

Table 1 Prenatal tests and their outcome (UK) 1994–1998

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Exclusion tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outcome: low risk</td>
<td>13</td>
<td>4</td>
<td>3</td>
<td>8</td>
<td>6</td>
<td>34</td>
</tr>
<tr>
<td>Outcome: high risk</td>
<td>7</td>
<td>5</td>
<td>8</td>
<td>9</td>
<td>6</td>
<td>35</td>
</tr>
<tr>
<td>Outcome: uninformative</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>9</td>
<td>11</td>
<td>17</td>
<td>12</td>
<td>72</td>
</tr>
<tr>
<td>Direct tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outcome: low risk</td>
<td>8</td>
<td>4</td>
<td>12</td>
<td>10</td>
<td>12</td>
<td>46</td>
</tr>
<tr>
<td>Outcome: high risk</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>8</td>
<td>11</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>6</td>
<td>13</td>
<td>18</td>
<td>23</td>
<td>74</td>
</tr>
<tr>
<td>Total (all tests)</td>
<td>37</td>
<td>15</td>
<td>24</td>
<td>35</td>
<td>35</td>
<td>146</td>
</tr>
</tbody>
</table>

1994-1998

www.jmedgenet.com
The consortium has recorded all presymptomatic predictive tests since testing began in 1988, a total of 2937 up to the end of December 1997. Less than 50% of these have been unfavourable results. Data on all prenatal tests recorded in the period 1994 to 1998 form the basis for the study reported here. Anonymised data on each test performed in pregnancy were collected on an annual basis from each of the member centres of the United Kingdom Huntington’s Disease Prediction Consortium. Information on referrals during pregnancy that did not lead to testing did not form part of this study.

One hundred and forty six prenatal tests were recorded in the years 1994 to 1998 (table 1). This includes one twin pregnancy.

Forty five percent of tests (65) occurred where the parent was aware that they had the mutation for HD. Nine of these parents were described as having clinical features of the disease at the time of the pregnancy.

Fifty four percent of tests (78) were carried out in pregnancies where the parent was at 50% risk. Eight of these were accomplished using mutation detection, that is, the at risk parent was prepared to find out that they too had the mutation as a result of investigation of the pregnancy. In three cases, presymptomatic predictive testing was performed during the pregnancy and before the pregnancy was investigated.

In two cases, the parent was at 25% risk, that is, the grandparent was asymptomatic but at 50% prior risk.

Sixty six (43%) unfavourable results have been produced, but only 61 terminations performed. In one of the four cases where the pregnancy continues, the pregnancy shares the parent’s 50% risk as the result was produced using exclusion testing. In the remaining three cases, the mutation was detected in the fetus.

There was one report of miscarriage as a result of the prenatal investigation, in a twin pregnancy.

The decision to investigate a pregnancy for an adult onset disease (and possibly terminate that pregnancy) is never an easy one, and lately the families have expressed much hope that a cure will be found for the disease and therefore they would be able to avoid termination of a pregnancy. HD can be variable in severity, and undoubtedly experience of later onset disease, which may be less severe, can encourage couples not to investigate their pregnancies.

Many people who present for predictive testing already have at least one child. The trend is for these subjects not to test pregnancies that occur after they have received an unfavourable result, since they would then have children at 50% risk and children whose status was certain.

The trauma associated with termination of any pregnancy is considerable. Tolmie et al. described a group of UK families (an earlier group who are not included in this study) who had difficulty with their decision to terminate an at risk pregnancy following exclusion testing. Three of nine high risk pregnancies were continued. Clarke et al showed the problems of producing predictive information for an adult onset incurable disease. In cases where an unfavourable result is produced, but the pregnancy continues, these children will grow up with certainty of information about their status, having had no choice in the decision. In addition, their parents are aware of their status, and their prejudices and those of society will undoubtedly be disadvantageous for them. There are only four such cases in this series.

There are couples who have undergone tests on as many as five pregnancies in their efforts to ensure that no child of theirs would have to suffer the problems of being at risk as they had themselves (S Simpson, personal communication).

Many couples express their anxiety about any child of theirs growing up with an affected parent, even if it were known that the child would not be at risk because of prenatal testing. These people may have grown up in a family where alcoholism, suicide, and divorce are common. Those who have had an unfavourable result by predictive testing and then have chosen to have a pregnancy are in the minority in this group.

Other options are available to those who know they have the mutation and who wish to have children. In at least one case, after the termination of a high risk pregnancy, the couple chose artificial insemination by donor. However, couples who have attempted to adopt or foster have had considerable difficulties because of the at risk status of one of the partners.

It is now becoming possible to offer preimplantation diagnosis to couples who do not wish investigation of an established pregnancy. This technique remains at a research stage; only preliminary data are available for its use and the rate of successful implantation of the fetus remains low. Nevertheless some patients in the clinic setting have expressed interest, but accurate figures are not available.

For those at risk who decide not to undergo presymptomatic predictive testing, the opportunity to ensure that the gene is not transmitted to the next generation remains with the use of exclusion testing. For some subjects their commitment is absolute (see above) and they have repeated attempts to have a pregnancy with a
low risk. Forty nine percent of the total tests in this series were performed using exclusion testing, although there has been an increasing number of tests using direct mutation testing in the last three years (fig 1). These data provide evidence that many at risk subjects would prefer not to know whether or not they are going to develop HD, but such is their experience of the disease and their at risk status that they wish to prevent the birth of an at risk child.

Maat-Kieviet et al.8 described the experience in The Netherlands of 72 prenatal tests in Huntington's disease. As in our study, a trend towards an increasing number of direct tests has been observed, although exclusion testing is still seen as a useful tool for those who do not wish predictive testing.

In a survey of subjects from Germany11 who were at risk of HD, over 67% indicated that they would wish to undergo presymptomatic predictive testing themselves, but only 45% would wish to use prenatal diagnosis. Twenty seven percent of those questioned stated they could not use prenatal diagnosis because they felt they could not terminate a pregnancy.

In South Africa, 59 subjects who had undergone predictive, diagnostic, or prenatal testing for HD were reported.20 The two affected pregnancies were aborted, from a group of 10 who had undergone prenatal tests.

In a review of international data, Evers-Kiebooms et al.21 showed that more prenatal tests took place where the male was at risk. This may indicate that the female role of child care was recognised by the families, in that if the male were subsequently affected, child care would continue.

Only a minority of those at risk of Huntington's disease in the UK have chosen to prevent the transmission of the disease by the use of prenatal diagnosis. There may be lack of knowledge among the at risk population about the tests available22 and older family members and spouses occasionally deliberately withhold information about the presence of the disease within the families. This may also help to explain the low uptake of presymptomatic predictive testing in the UK, but it does not explain why the number of those requesting prenatal diagnosis is so small in comparison with the number of requests for predictive testing.

The families in general express great hope for the future23 for treatment or prevention of HD and the low uptake of prenatal testing may reflect this. The continued use of exclusion testing in pregnancies, despite the ready availability of accurate direct testing, also shows the usefulness of exclusion testing for those who do not wish to obtain accurate information about their own status, but who are unwilling to risk transmission of the disease.

The support of the Huntington's Disease Association and all consortium members, who produced these results, is gratefully acknowledged.

Pregnancy outcome and long term prognosis in 868 children born after second trimester amniocentesis for maternal serum positive triple test screening and normal prenatal karyotype

I Witters, E Legius, K Devriendt, P Moerman, D Van Schoubroeck, A Van Assche, J-P Fryns

Measurement of maternal serum alphafetoprotein (ms AFP), human chorionic gonadotrophin (ms hCG), and unconjugated oestriol (uE₃) at the beginning of the second trimester of pregnancy is a well established screening test for Down syndrome (trisomy 21). Previous studies have described the association of abnormal levels of ms AFP and ms hCG with a variety of problems and complications of pregnancy, such as preterm delivery, fetal growth retardation, and fetal death, and severe hypertensive disorders in pregnancy. Over the past years, we have noted in the genetic clinic that several children with syndromic and non-syndromic forms of MCA-MR were born after a pregnancy with a positive maternal serum triple screening test and a normal prenatal karyotype. Therefore, we decided to perform the present study and collected data on the pregnancy outcome and the physical and psychomotor development of 868 children born after second trimester amniocentesis for positive maternal serum triple screening test with a normal prenatal karyotype. We found a significantly increased incidence of complex multiple congenital anomalies syndromes (17, 1.95%) in the children.

Material and methods

During the period from 1 January 1993 to 31 December 1995, 995 women had amniocentesis performed at the Leuven Centre for Human Genetics and showed normal chromosome results and normal amniotic fluid AFP. Maternal serum triple tests and amniocenteses were performed in different centres. In October 1998, a questionnaire (available on request) was mailed to the 995 women with a list of questions about the outcome of pregnancy, the perinatal history, and the physical and psychomotor development of their children. A total of 870 patients (87.5%) answered the questionnaire. They gave birth to 868 children (864 singletons, six twin pregnancies, four spontaneous abortions, and four intrauterine deaths). Further medical information was also obtained from the relevant obstetricians and paediatricians. All children with a major congenital malformation, as an isolated finding or as part of a multiple congenital malformation (MCA) syndrome or sequence, were examined by the same clinical geneticist (JPF) and/or the same fetal pathologist (PM).

Results

Isolated (minor and major) congenital anomalies were present in 28 children (3.23%) (table 1). Twelve children (1.38%) had an isolated major congenital malformation and half of these (0.69%) were isolated congenital cardiac defects. The most interesting result of the study is the high incidence of so called multiple congenital anomalies (MCA) syndromes. In 17 children (1.95%), a complex MCA syndrome was diagnosed in the neonatal period: six MCA syndromes with monogenic inheritance, two chromosomal syndromes not diagnosed in the prenatal period (one 22q11 deletion, one 16q deletion mosaic), and nine children with MCA-(MR) syndromes/sequences of hitherto unknown aetiology (table 2). Two of these 17 children died in the perinatal period (a patient with Coffin-Siris syndrome and one with Fryns syndrome); two others (a child with 16q deletion mosaicism and one with MCA/MR syndrome and complex cardiopathy) died at the age of 18 months and 1 year, respectively. Of the 13 surviving MCA children, seven are moderately to severely mentally retarded (table 2, children 1 (3), 3 (4), 3 (6), 3 (7), and 3 (8)) and two mildly to moderately mentally retarded (table 2, children 2 (2) and 3 (2)). Only the two children with Pierre-Robin sequence, one child with Bartter syndrome, and one child with Wiedemann-Beckwith syndrome are mentally normal.

Table 1 Types of isolated minor and major malformations

<table>
<thead>
<tr>
<th>Minor malformations</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral club foot</td>
<td>2</td>
</tr>
<tr>
<td>Unilateral hip dysplasia</td>
<td>2</td>
</tr>
<tr>
<td>Multiple cutaneous haemangiomata</td>
<td>1</td>
</tr>
<tr>
<td>Preauricular appendix</td>
<td>1</td>
</tr>
<tr>
<td>Unilateral proso</td>
<td>1</td>
</tr>
<tr>
<td>Unilateral cataract</td>
<td>1</td>
</tr>
<tr>
<td>Skull asymmetry</td>
<td>1</td>
</tr>
<tr>
<td>Thyroglossal cyst</td>
<td>1</td>
</tr>
<tr>
<td>Hypospadias grade II</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>16 (1.84%)</td>
</tr>
<tr>
<td>Major malformations</td>
<td></td>
</tr>
<tr>
<td>Cardiopathies</td>
<td></td>
</tr>
<tr>
<td>Tetralogy of Fallot</td>
<td>1</td>
</tr>
<tr>
<td>ASD</td>
<td>1</td>
</tr>
<tr>
<td>VSD</td>
<td>4</td>
</tr>
<tr>
<td>Urological malformation with ureter duplex</td>
<td>2</td>
</tr>
<tr>
<td>Communicating hydrocephalus</td>
<td>1</td>
</tr>
<tr>
<td>Liver haemangioendothelioma</td>
<td>1</td>
</tr>
<tr>
<td>Spondylocostal dystostosis</td>
<td>1</td>
</tr>
<tr>
<td>Unilateral hand malformation with complete syndactyly of two fingers (superdigit)</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>12 (1.38%)</td>
</tr>
</tbody>
</table>
We conclude that a positive triple screening test result selects a group of pregnancies at risk for serious multiple congenital anomaly syndromes with the same efficiency as for numerical chromosomal abnormalities. These data need confirmation by further studies.

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Table 2. Diagnosis in the 17 children with complex malformations.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Letters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pierre-Robin sequence</td>
<td>(1)</td>
</tr>
<tr>
<td>Spastic diplegia-adducted thumbs</td>
<td>(2)</td>
</tr>
<tr>
<td>MCA syndrome with pre- and postnatal growth retardation, body asymmetry, renal malformation, conductive deafness</td>
<td>(3)</td>
</tr>
<tr>
<td>MCA syndrome with macrocephaly, ventriculomegaly, VSD</td>
<td>(4)</td>
</tr>
<tr>
<td>MCA syndrome with microbraincophaly, facial dysmorphism, ASD, VSD</td>
<td>(5)</td>
</tr>
<tr>
<td>MCA syndrome with complex cardiopathy, facial dysmorphism, pre- and postnatal growth retardation (died at age 1 year)</td>
<td>(6)</td>
</tr>
<tr>
<td>MCA syndrome with facial dysmorphism/AVSD</td>
<td>(7)</td>
</tr>
</tbody>
</table>

Table 3. Results of the study group compared with chromosomal anomalies in women with positive triple test (unpublished data).

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Letters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal karyotype</td>
<td>Abnormal karyotype</td>
</tr>
<tr>
<td><em>No</em></td>
<td>%</td>
</tr>
<tr>
<td>MCA/MR syndromes</td>
<td>17</td>
</tr>
</tbody>
</table>

| Chromosomal anomalies | 29 | 2.2 | 1.41–3.00 |

*Same malformation complex in deceased sister.*

Discussion

The general incidence in liveborns of most of the complex malformation syndromes diagnosed in this study, for example, Fryns syndrome, Coffin-Siris syndrome, and Wiedemann-Beckwith syndrome, is very low (less than 1 in 10,000 to 1 in 20,000 live births). So far, only a few case studies have been reported on positive maternal serum positive triple test screening and the occurrence of a MCA/MR syndrome, for example, Rubinstein-Taybi syndrome, in the liveborn child.

The findings in the present study show that the incidence of rare so-called MCA/MR syndromes, diagnosed at birth, is significantly increased in children born after maternal serum positive triple test screening (with a normal karyotype after amniocentesis).

The number of MCA/MR syndromes (17 out of 868 children = 1.95%) found in our study group compares well with the number of autosomal unbalanced structural or numerical anomalies found in a control group of 1316 newborns referred to our centre for maternal serum positive triple test screening (29 of 1316 = 2.20%) (unpublished data) (table 3). This indicates that triple test screening for Down syndrome with the evaluation of maternal serum hCG, uE3, and AFP in the second trimester of pregnancy selects a broader group of pregnancies at risk for serious MCA (MR) syndromes.
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J M Meck
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A syndrome of overgrowth and acromegaloidism with normal growth hormone secretion is associated with chromosome 11 pericentric inversion

Constantine A Stratakis, Maria L Turner, Antony Lafferty, Jorge R Toro, Suvimol Hill, Jeanne M Meck, Jan Blancto

An acromegalic phenotype in late childhood or early adulthood is shared by a variety of clinical conditions, including growth hormone (GH) excess.1 Exclusion of an abnormality of the somatotrophic axis in a young patient with acromegaloid features should lead the differential diagnosis towards diagnoses such as pachydermoperiostosis (MIM 167100)1–3 or insulin mediated pseudohormegaly, a disorder associated with severe insulin resistance.4 In the absence of insulin resistance and findings characteristic of pachydermoperiostosis, such as thickening of the periostium (visible mostly in skull x rays) or the skin, acrolysis, or alopecia4,5,7 another genetic syndrome associated with acromegaloid features may be considered.8,15 These are rare conditions, having each been described in individual kindreds, and their causes remain unknown. Inheritance, when present, appears to be as an autosomal dominant trait. They are almost always associated with abnormalities of the skin, the mucosa, and its appendages, such as keratitis,6 thickened mucosa,12 hypertrichosis,12 and cutis verticis gyrata.13

In this report, we identify a chromosomal anomaly that was confirmed by fluorescence in situ hybridisation (FISH) in a patient with acromegaloid features and his family. The patient, his mother, and sibs participated in protocol 97-CH-0076 (National Institute of Child Health and Human Development, National Institutes of Health (NIH)) and consented to the proband’s photographs for the purposes of medical education and publication.

Case report

The proband was a 14 year 3 month old male (fig 1) who was referred to our clinic with the diagnosis of possible acromegaly. He was born at term after an uncomplicated pregnancy. His birth weight was 5018 g (over the 95th centile), his length was 60 cm (over the 95th centile for a newborn and on the 50th centile at 1 month old boy), and his weight over the 95th centile; his facies and body habitus were acromegaloid but the second trimester; relation to fetal weight and preterm delivery. Prenat Diagn 1995;15:1041–6.

References


The patient had normal tooth spacing and an absence of acral enlargement (fig 1). The function and size of the lacrimal glands were normal. Oral examination showed a normally sized tongue. The size of the submandibular salivary glands was normal on examination of the neck. Thyroid, heart, and abdominal examinations were normal with no evidence of nodules, cardiac dysfunction, or organomegaly, respectively. The skin texture was normal, albeit somewhat oily; the patient had acne, as shown in fig 1B. Genital examination and pubic hair were normal (Tanner V); testicular volume was 25-30 ml, bilaterally.

The evaluation of the patient’s somatotrophic axis is presented in table 1. All assays were performed at Endocrine Sciences (California Hills, CA) or Covance Laboratories (Vienna, VA). It included responses to both stimulation (arginine, clonidine, and L-Dopa) and suppression (oral glucose tolerance test (oGTT)) tests of GH, thyrotrophin releasing hormone (TRH) stimulation testing, and insulin-like growth factor type 1 (IGF-1) levels. The patient’s 24 hour, every 20 minute GH secretion pattern was also obtained (data not shown). Radiological and skin biopsy examinations were also performed to exclude pachydermoperiostosis. Pituitary imaging and sonograms of the abdomen, liver, kidneys, and testes (data not shown) were also obtained. During the patient’s evaluation at the NIH, his bone age was estimated; it was then compared with previous recordings of the patient’s bone age at different chronological ages, obtained elsewhere (table 2).

The patient lacked the characteristic for acromegaly mandibular and acral enlargements (fig 1), but he had prominent supraorbital ridges and generally coarse facies. His laboratory evaluations, including oGTT, TRH stimulation, 24 hour GH secretion, and IGF-1 levels, were normal (table 1). Pituitary magnetic resonance and other imaging studies, which included an echocardiogram and renal ultrasonography, were also normal (data not shown). The patient did, however, have an advanced bone age (table 2). The clinical information that was available for the other members of the family is summarised in fig 2.

To search for features of pachydermoperiostosis, radiological imaging of the skull and the extremities and a skin biopsy were obtained. The former showed normal bones, without any signs of periosteal proliferation or new bone growth; the fingers were normal, without any

Figure 1  Clinical findings in the patient: (A) Symmetrical overgrowth. (B) Acromegaloid facies. (C) Lack of prognathism despite coarse facies and prominent supraorbital ridges. (D) Normally spaced teeth. (E, F) Absence of acral enlargement.
widening indicative of clubbing (data not shown). A biopsy of skin obtained from the left forearm showed normal histology (data not shown). Adnexal structures were of the expected number and size (hair follicles and eccrine glands). Furthermore, the mucin content in this biopsy did not differ from normal controls.4

**Table 1** Clinical profile of the proband

<table>
<thead>
<tr>
<th>oGTT</th>
<th>Proband (III.2)</th>
<th>Mother (I.1)</th>
<th>Sister (III.1)</th>
<th>Half brother (III.3)</th>
<th>Maternal uncle (II.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (minutes)</td>
<td>Glucose (mg/dl)</td>
<td>Insulin (µIU/ml)</td>
<td>GH (ng/ml)</td>
<td>TSH (µIU/ml)</td>
</tr>
<tr>
<td>0</td>
<td>87</td>
<td>16.1</td>
<td>2.8</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>15</td>
<td>97</td>
<td>26.6</td>
<td>0.8</td>
<td>18.4</td>
<td>18.4</td>
</tr>
<tr>
<td>30</td>
<td>90</td>
<td>34.9</td>
<td>0.3</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>45</td>
<td>60</td>
<td>34.6</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>101</td>
<td>21.1</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>94</td>
<td>21.9</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>180</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak GH to clonidine</td>
<td>10.1 ng/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak GH to arginine</td>
<td>15.6 ng/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-1</td>
<td>429 ng/ml (age related normal range 286–660, mean 403)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-2</td>
<td>348 ng/ml (age related normal range 245–737, mean 491)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GHBP</td>
<td>199 pmol/l (normal values are less than 50 pmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GHBP</td>
<td>139 pmol/l (age related normal range 52–783 pmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C peptide</td>
<td>1.2 ng/ml (normal fasting values 0.4–2.1 ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** Bone versus chronological age in the proband

<table>
<thead>
<tr>
<th>Chronological age</th>
<th>Bone age</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 6/12</td>
<td>14</td>
</tr>
<tr>
<td>13 4/12</td>
<td>16</td>
</tr>
<tr>
<td>14 9/12</td>
<td>19</td>
</tr>
</tbody>
</table>
The patient’s mother and sisters were also briefly examined, although they declined further investigation. The mother and one of the sisters had a height in excess of the 95% centile. Both shared clef palate defects with the proband but had less obvious acromegaloïd features. The mother’s GH, GHRH, and IGF-I levels and oGTT responses were also normal (data not shown).

High resolution karyotype analysis was obtained by standard methods from the patient, his mother, father, and three sisters. Probes for fluorescence in situ hybridisation (FISH) were nick translated and hybridised on metaphase chromosomes that were prepared from peripheral lymphocytes, as previously described. Ten metaphases were scored for every probe examined. The commercially available probe for the MLL gene at 11q23 was labelled with digoxigenin (Oncor Inc, Gaithesburg, MD) and detected with fluorescein (Oncor, Gaithesburg, MD). Chromosome identification was accomplished through cohybridisation with a chromosome 11 telomere specific probe (11pter) that was labelled with Spectrum Green (Vysis, Naperville, IL). Images were obtained with a Zeiss Axiophot microscope, equipped with a “Cytovision” imaging system (Applied Imaging, Pittsburgh, PA), as previously described.

The peripheral blood karyotype of the proband showed that, on one of the chromosome 11 pair, the chromatin near the tip of the p arm had a pattern similar to that of the distal 11q region (fig 2), suggesting a pericentric inversion of chromosome 11 or a 46,XY,inv(11)(p15.3;q23.3) karyotype. FISH with two probes that hybridise to the 11p telomere (11pter) and to the MLL gene on 11q23, respectively, showed that, on one chromosome 11, the two probes were positioned on the p arm, with the MLL probe centromeric to the 11pter probe; the two probes hybridised to their expected positions (11pter on the distal p arm and MLL on the distal q arm) on the other chromosome 11 (fig 4). Classical and molecular cytogenetic analysis showed the same abnormality in the patient’s mother and one of his sisters (data not shown); these studies were normal in the patient’s father and his other two sisters, none of whom had this phenotype.

Discussion

No chromosomal abnormality or genetic locus has previously been described in the various syndromes associated with acromegaloïdism. The present report identified a pericentric inversion of chromosome 11 that segregated with acromegaloïd features and other abnormalities in one family. This finding may assist in the identification of gene(s) responsible for these conditions, only after proper clinical classification of acromegaloïdism.

Acromegaloïdism describes a highly heterogeneous group of disorders. In general, acromegaly and gigantism, the two syndromes of GH excess, share several features with pseudoacromegaly caused by severe insulin resistance. The distinction between these two groups of disorders is made by the laboratory finding of insulin resistance in pseudoacromegaly. Pachydermoperiostosis and the other
syndromes associated with acromegaloïdism have neither insulin resistance nor GH excess, the exception being an apparently coincidental GH-producing pituitary adenoma found in a patient with pachydermoperiostosis.\textsuperscript{16} The differentiation of pachydermoperiostosis from the other conditions is made radiologically or histologically or both. Patients with this condition have evidence of periosteal thickening of the skull or the long bones, digital clubbing, and/or acrolysis, similar to that seen in hypertrophic pulmonary osteoarthropathy or inherited acrophy.\textsuperscript{11,17,18} In addition, several cutaneous findings, including papular mucinosis, hyperkeratosis, and generalised hypertrophy of epidermal appendages,\textsuperscript{11,17,18} are distinctive features suggestive of pachydermoperiostosis. Cutis verticis gyrata (multiple furrows on the scalp and/or forehead) is also often seen in association with pachydermoperiostosis.\textsuperscript{11,18} Periostitis without acromegaloïd features or any skin involvement has been reported and is referred to as Curranaro disease;\textsuperscript{19} however, this condition should not be considered in the differential diagnosis of acromegaloïd syndromes.

Other reports of families with an acromegaloïd phenotype include a Kirghizian family with arthritis, osteolysis, keratitis, and other ectodermal findings with autosomal recessive inheritance (MIM 221810;\textsuperscript{20} a Canadian family with thickened oral mucosa and acral hyperextensibility (MIM 102150);\textsuperscript{21} and an Irish kindred with coarse facies and generalised hypertrichosis since childhood.\textsuperscript{12} The family reported by Dallapiccola et al\textsuperscript{22} was similar to the one reported by Hughes et al.\textsuperscript{10} As in most of the other patients with non-pachydermoperiostosis acromegaloïd syndromes,\textsuperscript{14,15} the features of acromegaly in our family were confined to the face. On the other hand, the proband had a history of a cleft palate and a diaphragnatic hernia. Neither of these congenital anomalies has been reported in other patients with acromegaloïd syndromes. Although the diaphragmatic defect was not present in any of the patient’s affected relatives, variable cleft palate was present in all of them.

The aetiology of acromegaloïdism in our family remains unclear. The overgrowth and advanced bone age of the proband support the notion of aberrant expression of an unknown growth factor. The presence of an unidentified growth factor in the serum of patients with acromegaloïdism has been suggested before.\textsuperscript{23} A substance of approximate molecular weight of 70 000 daltons was found in the sera of five subjects with acromegaloid features. Its growth promoting activity was shown by determining in vitro. It was shown to be independent of epidermal, nerve, or fibroblast growth factors and growth hormone.\textsuperscript{24} Although the present study could be used to suggest that perhaps the gene coding for this factor is on chromosome 11, the patients studied by Ashcraft et al.\textsuperscript{25} had pheno- types different from those of our patients; at least one of them had acral thickening similar to that seen in acromegaly or pseuodacromegaly and another had, perhaps, true acrome- galy.\textsuperscript{25}

It is also possible that the genetic defect in our kindred is not a growth factor per se, but rather a signalling molecule that participates in a related pathway. It was recently found, for example, that patients with insulin resistance and pseudoacromegaly have a defect in phosphoinositide 3-kinase signalling,\textsuperscript{26,27} leading to insulin resistance and activation of mitogenic pathways stimulated by the insulin receptor.\textsuperscript{28}

The genetic defect responsible for the phenotype in our family could also involve inappropriate repression of a gene because of fusion of genetic material from another sequence on chromosome 11, the interruption of an inhibitory state (imprinting or a related state), or the deletion of suppressive elements. Similar abnormalities have been described in patients with chromosome 11 cytogenetic defects that involved growth factors and other genes mapped to this chromosome\textsuperscript{29} and would also explain the variability of the phenotype seen in our family. Examples include Beckwith-Wiedemann syndrome that maps to 11p15.5 and is believed to result from relaxation of imprinting of this area leading to IGF type 2 (IGF-2) overexpression.\textsuperscript{30} The 11p15.3 breakpoint of the pericentric chromosomal inversion in our kindred, however, is quite distal to the IGF-II and related loci.\textsuperscript{31,32} A search of the available databases failed to find any other imprinted sequences or known growth factor genes that map to the breakpoints of the inversion.

In conclusion, we present a family with variable clinical expression of acromegaloïdism associated with other developmental defects that segregate with a pericentric inversion of chromosome 11. This report may lead to the identification of new gene(s) on chromosome 11 that are important for midline fusion and growth of facial structures.

This work was (in part) presented in an abstract form at the 80th Annual Meeting of the Endocrine Society, New Orleans, LA, June 1999. We thank Dr G Evans (McDermott Center for Human Growth & Development, UT Southwestern Medical Center at Dallas, Dallas, TX) who provided information related to his work on the karyotype of the proband. We are indebted to his family and, in particular we thank the proband’s mother whose inquisitive nature helped to identify this chromosomal anomaly.

Maternally inherited duplication of the possible imprinted 14q3 region

Cécile Mignon-Ravix, Francine Mugneret, Christiana Stavropoulou, Danielle Depetris, Philippe Khau Van Kien, Marie-Geneviève Mattei

The existence of parent of origin differences in the expression of some genes, a process known as genomic imprinting, has been recognised and documented over the past several years. This epigenetic marking process results in the differential expression of normal genes, depending on whether they are of maternal or paternal origin. A number of human disorders have been identified as resulting from alterations in genomic imprinting. One type of genetic abnormality which can unmask genetic variant in this region may help the identification of imprinted genes.

Nevertheless, no imprinted genes have yet been identified on chromosome 14, and the location of the imprinted region on human chromosome 14 remains unclear.

Analysis of parental origin effects in human trisomy for chromosome 14q or monosomy for the same chromosome makes the region 14q23-q32 a candidate region for containing imprinted genes. Moreover, the observation of a partial duplication of 14q in a developmentally delayed girl with minor abnormalities and her phenotypically normal father led Robin et al. to propose that the 14q23.3-q31 region may be imprinted. As genotype-phenotype correlations in patients with aneuploidy for this region may help the identification of imprinted genes on human chromosome 14, we report here a direct 14q31 duplication observed in a child with mild developmental delay and his phenotypically normal mother. This duplication was also detected in five relatives with a normal phenotype and maternal inheritance through three generations.
Case report

Our patient, a 2 month old boy, was referred following a cyanotic episode. He was the first child of healthy, unrelated parents. The family history was uneventful and he was born by caesarean section. Birth weight, length, and head circumference were 3515 g, 54 cm, and 33 cm, respectively. At the age of 2 months, clinical examination showed dysmorphic features (macrosomia, metopic suture, neck hyperextension without opisthotonos, large nose, prominent philtrum, gingival hyperplasia, and bilateral palmar simian crease) and axial hypotonia with moderate distal hypertonia. EEG and MRI scan of the brain, medullary ultrasound, abdominal ultrasound, pulmonary and skeletal x-ray, ECG and retinal examination were all normal. The patient walked at 20 months. By the age of 27 months, clinical evaluation of the patient showed very slight dysmorphism, slight overgrowth (+2 SD), moderate psychomotor delay with specific speech delay, hypotonia, and behavioural and sleep disturbance.

Routine R and G banding techniques, performed on metaphases from PHA stimulated lymphocytes of the proband, showed an elongated chromosome 14 with an extra G positive band on the long arm (fig 1). High resolution banding suggested that the patient had a duplication of band 14q31. The same 14q duplication was found in the proband’s mother and four other members of his family, all of whom had a normal phenotype (fig 2A).

Fluorescence in situ hybridisation (FISH) was performed with various probes, in order to characterise the rearrangement. A chromosome 14 specific paint, used as recommended by the manufacturer (ONCOR), confirmed that the extra material originated from chromosome 14 (data not shown). FISH experiments using YAC probes estimated to cover the 14q31 band were performed, in order precisely to define the duplicated chromosomal region in the proband. The YAC clones 813e6, 930c12, 749g4, 815d5, 872e3, 756a4, 945d2, 856g8, and 965b9, obtained from the CEPH (Centre d’Etude du Polymorphisme Humain), were labelled with biotin-14dCTP or digoxigenin-11dUTP by random priming (Bioprime DNA labelling system, Life Technologies and Dig high prime, Roche Diagnostics), then hybridised on metaphase chromosomes or interphase nuclei. The hybridised biotin signals were made visible with fluorescein labelled avidin, and the digoxigenin signals were visualised with rhodamine labelled anti-digoxigenin antibody following standard protocols.12 The results are summarised in fig 2B. The more proximal YACs (813e6 and 930c12), as well as the more distal ones (945d2, 856g8, and 965b9), gave signals of equal intensity on the normal and the duplicated chromosomes 14, indicating the presence of only two copies in the patient’s genome. In contrast, YACs 749g4, 815d5, 872e3, and 756a4 showed two signals...
of different sizes, the larger one being located on the duplicated chromosome 14. In order to confirm these results, two YACs with different FISH patterns were cohybridised on the same metaphase preparations: YAC 815d5 (green signal) presumed to be included in the duplication and YAC 965b9 (red signal) presumed to map outside the duplication. As expected, the green signal (815d5) on the duplicated chromosome 14 was larger than the red signal (965b9) on the same chromosome (fig 3A, B). The same experiment was performed on interphase nuclei, where the undercondensation of the chromatin allows increased resolution. This allowed the detection of two red signals (YAC 965b9) and three green signals (YAC 815d5), clearly showing a duplication of the region covered by YAC 815d5 (fig 3C). Different YAC probes were hybridised with metaphase spreads from subjects III.2 and III.3, confirming the presence of the same 14q duplication in the proband and his relatives.

In order to determine whether the duplication is direct or inverted, the proximal and distal duplicated YACs (749g4 and 756a4 respectively) were labelled differently, hybridised to interphase nuclei, and visualised with specific fluorochromes. Alternate green and red signals observed in nuclei show that the two copies of the duplication are organised in direct orientation on chromosome 14 (fig 4).

In order to gain insight into the mechanism that gave rise to this duplication, we used mic-
Letters

proband is probably unrelated to the duplicated segment. Moreover, duplication of the 14q31 band, when maternally inherited, does not seem to be associated with an abnormal phenotype.

It has been suggested by Robin et al.9 that a similarly duplicated chromosome 14 (duplication 14q24.3-q31), if paternally transmitted, does induce an abnormal phenotype. This parent-of-origin dependent phenotypic disparity associated with similar duplications suggests a possible imprinting effect. The fact, however, that the duplications are not identical allows three different hypotheses to be formulated with respect to imprinting effects.

Firstly, the imprinted genes could be located outside the 14q24.3-q31 interval. This region may contain genes that do not have a deleterious effect if overexpressed, and duplication will therefore produce no phenotypic effect. In that case, the association of the 14q duplication with an abnormal phenotype, both in the proband described here and in that described by Robin et al., would be merely coincidental.

Secondly, the imprinted genes could be located in band 14q24.3 and not in band 14q31. In that case they would be duplicated in the patient described by Robin et al.9 but not in the family described here. In such a situation, no parent of origin phenotypic effect would be associated with a 14q31 duplication. Then, a 14q31 duplication of paternal origin would be associated with a normal phenotype, as we show to be the case here for a 14q31 duplication of maternal origin. This hypothesis is supported by the fact that G positive bands, such as the 14q31 band, contain very few genes.11

Thirdly, imprinted genes could be located in band 14q31 and duplicated both in the family reported here and in that of Robin et al.9 If this were the case, both observations are in agreement and suggest that imprinted genes from this region are paternally expressed. However, the presence of paternally expressed genes only is not consistent with the observation that the maternal UPD(14) phenotype is moderate compared to the paternal UPD(14) phenotype. This would suggest that maternally expressed genes are located elsewhere in the 14q24.3-q31 region. Unfortunately, it is not possible to choose between these three hypotheses because no case of paternally inherited 14q31 duplication has yet been described.

So far, only a few genes have been mapped to the human 14q31 band (NCBI http://www.ncbi.nlm.nih.gov/), and none of them has been shown to be imprinted. Those that have been mapped include the genes encoding galactosylceramidase (GALC), the thyroid stimulating hormone receptor (TSHR), the suppressor of lin-12 (C elegans)-like (SELIL), neurexin III (NRXN3), and a member of the G protein coupled receptor superfamily (GPR68/OGRI).

Further studies of chromosome 14 aneu- somy are needed in order to define the imprinted region better and identify the corresponding genes.
We would like to thank the family for its participation, Dr A Nivelon-Chevaller and Dr C Robinet for their kind collaboration in the clinical study of the patient, and Dr M Mitchell for reviewing the English language. This work was supported by INSERM and the Association pour la Recherche contre le Cancer (ARC).


A novel missense mutation in the GTPase activating protein homology region of TSC2 in two large families with tuberous sclerosis complex

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Tuberous sclerosis complex (TSC) is an autosomal dominant disorder (OMIM 191092) characterised by autism, seizures, mental retardation, benign tumours of the brain, heart, kidney, lung, and skin, and malignant tumours of the kidney.1 TSC has a wide range of phenotypic variability, with some subjects severely affected and others only mildly affected. There are two TSC genes, TSC1 on chromosome 9q342 and TSC2 on chromosome 16p13.3.13 Approximately two thirds of cases of TSC appear to result from de novo germline mutations. In 1994, an extended four generation family with 19 affected members was reported in which 34 members (17 affected with TSC and 17 unaffected) underwent both physical and psychiatric assessments.1 The majority of the affected subjects had mild physical expression of TSC, but there was significant clustering of neuropsychiatric disorders among affected subjects compared with their unaffected relatives. The disorders that were over-represented included mood disorder, anxiety disorder, and autism. The largest difference was observed in anxiety disorder, which was seen in 10 of the affected subjects and in two of the unaffected subjects (p=0.016). One affected child had pervasive developmental disorder and one had autism. Analysis of this family suggested that TSC could present phenotypically with mild physical signs and symptoms, but with significant neuropsychiatric disease. Linkage to the TSC2 gene locus on chromosome 16p13.3 was shown with a lod score of over 3.4

We report here the identification of a missense mutation in exon 34 of the TSC2 gene in affected members of this family. We also examined a second four generation family5 from the same geographical area as the first family but not known to be related to them. The same exon 34 mutation was found in affected members of the second family.

Methods
To search for mutations in the coding regions of the TSC2 gene we used single strand conformation analysis (SSCP). The primers amplifying each of the 41 exons of TSC2 and the PCR conditions have been described previously. The PCR products were run on MDE gels (AT Biochem). To maximise the detection of variant bands, each PCR product was run on two gels, one without glycerol and one with 5% glycerol. Samples in which variant bands were detected were reamplified and sequenced. This

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members of the first family. Subjects 201, 212, and 302 are unaffected members of the second family. Subjects 715 and 717 are affected family members from the first family, TS-15. This family, from the same geographical area as the first family, includes 24 affected subjects.

The same exon 34 change found in the first family was found in both of the affected members that we tested and none of six unaffected members. We also analysed TSC2 exon 34 from 57 unrelated subjects without a personal or family history of TSC. None of these controls had the A4508C change, indicating that this is not a common genetic polymorphism.

Discussion

The A4508C mutation is predicted to change amino acid 1503 from glutamine to proline. This amino acid is identical among the human, mouse, rat, fugu, and Drosophila homologues of tuberin, the product of the TSC2 gene (fig 2). Exons 34 to 38 of TSC2 encode a region of tuberin with homology to rap1 GTPase activating protein (GAP). Tuberin has been shown to have GAP activity for both rap1 and rapb. The A4508C is the first missense mutation identified in exon 34 of TSC2. Ten TSC2 missense mutations in the GAP domain have been previously reported (one in exon 35, one in exon 36, five in exon 37, and three in exon 38). These are described in detail in the online TSC Variation Database (http://expmed.bwh.harvard.edu/ts/). Only one of the previously reported GAP domain missense mutations (in exon 38) was found in a familial, rather than a sporadic, case of TSC.

In the first family with the exon 34 mutation, mild physical signs of TSC were associated with significant neuropsychiatric symptoms including pervasive developmental disorder and autism. The second family has not yet had formal neuropsychiatric evaluations. However, like the first family, many affected subjects appear to have a mild form of TSC. For example, hypomelanotic skin macules (white spots) or white skin freckles were the only known manifestation of TSC in 10 of the 19 affected subjects in the first family (52%) and 12 of the 23 affected subjects in the second family (52%).

Several of the mildly affected subjects in these families might not have been recognised as having TSC had they not been related to the index patients. This raises the possibility that people in the general population with neuropsychiatric disease who lack the classical signs of TSC could also have germline missense changes within the GAP domain of TSC2. TSC is associated with autism, hyperactive behaviour, sleep disorders, and aggressive behaviour. Both mutations and polymorphisms in the TSC genes could therefore be considered candidate susceptibility or genetic modifier alleles for any of these disorders. TSC2 may be of particular interest as a possible susceptibility locus for autism because autism appears to have a strong genetic component.

Multiple genome wide screens have been performed, two of which have identified
potential autism susceptibility loci on chromosome 16p, 12q, 17q, the location of TSC2.

In summary, we have identified a novel mis-
sense change at a highly conserved residue
within the region of GTPase activating domain
homology of the TSC2 gene in two four
generation TSC pedigrees with a total of more
than 40 affected members. This is, to our
knowledge, by far the largest known group of
TSC patients carrying the same mutation.

Therefore, we anticipate that these families will
be important in the future identification of
modifier gene effects in TSC. In one family, an
association of TSC with significant neuropsy-
chiatric disease has already been documented.
Further studies will be required to understand
biochemically the functional consequences of
this exon 34 missense mutation and to charac-
terise more completely the clinical and neu-
ropsychiatric manifestations of TSC in these
families. Understanding the relationship be-
 tween naturally occurring germline TSC2
mutations and neuropsychiatric disease could
eclipsed the underlying biology of TSC and
potentially facilitate studies aimed at preven-
tion and/or early diagnosis.

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Interstitial deletion of 3p22.2-p24.2: the first
reported case

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Autosomal deletions or chromosomal haploin-
sufficiency syndromes are observed in 1 in
7000 live born infants and may cause multiple
malformations, growth failure, and mental
retardation. Deletions on the short arm of
chromosome 3 have been reported in 35 cases
and have been divided into two groups: delec-
tion 3p syndrome with breakpoints be-
tween 3p12-24 and 3p25 and proximal del-
tion 3p syndrome with different breakpoints
between 3p11 and 3p21.2. The first reported case of an
interstitial deletion of chromosome 3p22.2-24.2 in
a 6 year old male with developmental
delay is presented here.

Case report
The proband was the fourth child born, in
England, to healthy, unrelated, white parents.
There was no family history of note. He was
born vaginally following spontaneous onset of
labour at 41 weeks of gestation after an
uneventful pregnancy and weighed 3140 g
(10th centile). A murmur was noted shortly
after delivery and echocardiography confirmed
the presence of a small, perimembranous ven-
tricular septal defect. His early milestones were
uneventful pregnancy and weighed 3140 g
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after delivery and echocardiography confirmed
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tricular septal defect. His early milestones were
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uneventful pregnancy and weighed 3140 g
(10th centile). A murmur was noted shortly
after delivery and echocardiography confirmed
the presence of a small, perimembranous ven-
tricular septal defect. His early milestones were
unu
night and help with toileting in the daytime. He had no other health problems.

On examination at the age of 5.5 years, his height was 100.5 cm (4 cm <3rd centile), weight 16.2 kg (3rd-10th centile), and head circumference 52.7 cm (75th centile). He had mild bilateral fifth finger clinodactyly. He had midface hypoplasia and a prominent forehead, shown in figs 1 and 2. He had posterior angulation of his ears and a thin upper lip. His nipples were widely spaced. His big toes were short and relatively broad and his fourth toe curled under the third toe (fig 3). The fifth toenail was hypoplastic and there was a wide gap and deep groove between the first and second toes. There were no other dysmorphic features and the remainder of the examination was unremarkable.

Audiological assessment showed a right uni-lateral sensorineural hearing loss (40-45 dB loss). X-ray of his foot was carried out because of the unusual appearance of the toes. No significant bony abnormality was seen.

CYTOGENETIC AND FISH STUDIES

Cytogenetic G banded studies on the peripheral blood lymphocytes showed a de novo interstitial deletion of bands p24.2 to p22.2 in the proximal short arm of chromosome 3 (banding level of 550). The resulting karyotype was a de novo 46,XY,del(3)(p22.2p24.2) (fig 4). This finding was confirmed on examination of 60 cells.

Fluorescence in situ hybridisation (FISH) studies were used to confirm the cytogenetic deletion. A whole chromosome 3 painting probe (Oncor, Inc) showed continuous hybridisation along both the short and long arms of both homologues of chromosome 3. Locus specific YAC clones (906g05 locus D3S3714/D3S3680, 938g11 locus D3S1266, 937h04 locus D3S3564, 792d07 locus D3S3678, 802g01 locus D3S658, Max Planck Institute for Molecular Genetics) overlapping the cytogenetic breakpoints showed loss of signal for the locus D3S1266 and D3S3564. The final karyotype has been interpreted as: de novo 46,XY,del(3)(p22.2p24.2)[60].

ish(wcp3, D3S3714/D3S3680x2, D3S1266−, D3S3564−, D3S3678x2, D3S658x2).

Discussion

Terminal 3p deletions have been described in 24 cases and interstitial proximal 3p deletions in 11 cases and are felt to show distinct phenotypes. In the terminal 3p deletion syndrome, the patients have pre- and postnatal growth retardation, mental retardation, and developmental delay. A number of craniofacial anomalies are also described, including flat occiput, triangular face, hypertelorism, epicanthic folds, synophrys, ptosis, broad and flat nose, and downturned corners of the mouth. The patients also have small hands and supernumerary digits. In the proximal 3p deletion syndrome, the patients have a characteristic facial appearance with narrow forehead, epicanthic folds, short palpebral fissures, broad nasal bridge, and low set, poorly formed ears. Developmental delay is also described. Joint abnormalities may be present.
including decreased joint mobility, ulnar deviation of the hands, camptodactyly, and calcaneovalgus deformity of the feet.

There have been no deletions published spanning the region 3p22.2-p24.2. In the case reported here, the proband had a number of mild dysmorphic features, global developmental delay, and short stature. These features are frequently described in patients with chromosome deletions and they are likely to be related to the monosomy of the region p22.2-p24.2. This is apparently the first case report with this deletion, which is surprising as, although the features are relatively mild, the combination of growth failure and developmental delay usually leads to a paediatrician checking the karyotype, as these are features associated with chromosomal abnormalities. In this case, it may be that the early response of the patient to effective intervention by the child development team delayed any further testing. The deletion may usually be associated with a milder phenotype and so chromosome analysis is not undertaken. There are other possible explanations for the mild phenotype. A cryptic rearrangement and low level mosaicism were excluded using locus specific probes and an extended examination of 60 metaphases. However, it is not possible to rule out epigenetic modification of the phenotype because of duplication elsewhere in the genome or that the phenotype is mild because the deletion is in a “protected” genomic region.

A meaningful correlation between this deletion and the clinical phenotype is not possible until further cases are described. It may then be possible to contribute further to the knowledge of the morbid anatomy of the human genome.

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