Letters to the Editor

Dutch patients with glycogen storage disease type II show common ancestry for the 525delT and del exon 18 mutations

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Editor—Glycogen storage disease type II (GSD II) is an autosomal recessive lysosomal storage disorder caused by deficiency of acid α-glucosidase. The enzyme deficiency results in intralysosomal accumulation of glycogen in skeletal muscle and in other tissues. There are early and late onset phenotypes which differ with respect to age at onset, extent of organ involvement, and clinical course of the disease.1 The genotype frequency of GSD II was recently shown to be 1 in 40 000 by mutation screening in the general population, which is higher than previously estimated.2 3 Over 40 different mutations in the acid α-glucosidase (GAA) gene have been reported.4 Most mutations are rare and have been found in only a few patients. However, some mutations have been reported in several unrelated patients with defined ethnic origins. The C1935A transversion, frequently found in Chinese patients with infantile GSD II, appears to originate from a common founder.5 Other frequent mutations include the R854X mutation in Afro-Americans,6 the 2741AG→CAGG insertion in Turkish patients,7 and the G925A mutation in European patients.8 It remains to be determined whether these frequent mutations represent common descent or result from independent recombination. The IVS1(−13T→G) mutation is the most frequent mutation in late onset GSD II patients from different ethnic origins.9 10

In The Netherlands, most late onset GSD II patients carry the IVS1(−13T→G) mutation in combination with either the 525delT or the del exon 18 mutation, whereas infantile GSD II patients often show homozygosity or compound heterozygosity for the 525delT and the del exon 18 mutations.11 The latter mutations are fully deleterious and are associated with complete loss of enzyme activity.12 13 The deletion of exon 18 extends from IVS17 to IVS18 and includes the coding sequence of exon 18. Analysis of the deletion junction showed a direct eight nucleotide repeat sequence flanking the deletion, with one direct repeat included in the deletion and the second direct repeat at the deletion junction.14 15 This repeat sequence could be instrumental in the mutation event. So far, the mutation has not been reported in patients of non-white origin. The 525delT mutation has also not been reported in non-white patients, and is relatively rare in “non-Dutch” patients.16 In order to determine whether the 525delT and del exon 18 mutations represent founder events or independent, de novo mutations, we constructed haplotypes using four single nucleotide polymorphisms (SNPs) in the GAA gene. We used a set of 28 unrelated GSD II patients to determine the extent of haplotype sharing between the individual patients carrying identical mutations. The patient population included 26 white Dutch patients and their parents from 26 families with infantile GSD II, and three “non-Dutch” patients and their parents from two families with adult GSD II. All patients carried at least one frequent mutation (525delT or del exon 18) and had deficient GAA activity, measured in fibroblasts and leukocytes. Genomic DNA was extracted from cultured skin fibroblasts and from peripheral blood cells using standard procedures.17 Mutation analysis was performed as described previously.18 We analysed four intragenic single nucleotide polymorphisms (SNPs) by PCR amplification, followed by digestion of the PCR product with the appropriate restriction enzyme (table 1). To amplify exons 3, 8, 11, and 17, information was obtained from Martiniuk et al.19 Fragments were electrophoresed on a 2% agarose gel. Genotyping parents of GSD II patients assigned the phase of the alleles. The order of SNPs and mutations was as follows: 525delT - exon 3 SNP - exon 8 SNP - exon 11 SNP - exon 17 SNP - del exon 18.

We estimated allele frequencies by direct counting of chromosomes. It has been shown in several studies12 that inclusion of genotypes from incomplete families, or the inclusion of reconstructed genotypes, may introduce serious bias into the estimation of allele and haplotype frequencies. Therefore, in the analysis of linkage disequilibrium and the estimation of
haplotype frequencies, only families for which DNA was available for genotyping from the patient and from both parents were included. Genotypes for a given polymorphic marker in a given family were only included in the statistical analysis if completely unambiguous results had been obtained for that marker in all available DNA samples in that family. As a result of this rigorous constraint, the total number of scorable haplotypes was not identical for all marker combinations. The statistical significance of allelic association between various polymorphisms on wild type chromosomes was assessed using Fisher’s exact test. Haplotype frequencies were determined using the EM algorithm, as implemented in the EH program.20 Table 2 summarises the frequencies of the alleles observed for the SNPs in wild type chromosomes (n=52) and the panel of 525delT chromosomes (n=24) and del exon 18 (n=14) chromosomes.

### Wild type chromosomes

The chromosomes that were not transmitted by parents to their affected children were considered as wild type chromosomes. A pairwise analysis of SNPs on non-transmitted (wild type) chromosomes showed close to significant evidence for linkage disequilibrium between two pairs of adjacent polymorphisms: exon 3 and exon 8 SNPs (p≈0.06, with complete absence of the major-minor haplotype), exon 8 and exon 11 SNPs (p≈0.09, with complete absence of the minor-minor haplotype), but no disequilibrium for one pair, exon 11 and exon 17 SNPs (p≈0.52).

In the estimation of haplotype frequencies for the combination of the exon 3, exon 8, and exon 11 SNPs, only four of the eight possible haplotypes were observed (table 3), with highly significant statistical evidence for linkage disequilibrium (p≈0.0003), but no obvious shared haplotypes on the remaining mutant chromosomes (table 3). These data are consistent with a common founder for each of the two common mutations separately.

### Origin of 525delT and del exon 18 chromosomes

Table 4 shows extended haplotypes of chromosomes bearing the 525delT and del exon 18 mutations, including the exon 17 SNP. This list also includes haplotypes from patients that could be unequivocally reconstructed from incomplete families. Such haplotypes were not included in the statistical analysis of core haplotypes because the inclusion of reconstructed haplotypes introduces bias.

Although wild type chromosomes did not show linkage disequilibrium between the exon 11 and exon 17 SNP, all of the 525delT chromosomes shared the exon 3 (minor) - exon 8 (major) - exon 11 (minor) - exon 17 (minor) haplotype. These data further support common ancestry for the 525delT mutation. The majority of the del exon 18 chromosomes share the exon 3 (minor) - exon 8 (major) - exon 11 (major) - exon 17 (minor) haplotype. The data in table 3 suggest the presence of only one haplotype for the del exon 18 chromosomes. While data from unequivocable scoring chromosomes were consistent with the presence of one core haplotype, the additional chromosomes listed in table 4 showed two other haplotypes. Three chromosomes which differ for the intragenic haplotype were excluded from the statistical analysis owing to incompleteness of the families. These data further support common ancestry for the majority of the del exon 18 chromosomes.

In the present study, we performed haplotype analysis in GSD II patients with the 525delT and del exon 18 mutations. The results show that both the 525delT and del...
exon 18 mutations in Dutch GSD II patients originate from common founders. This conclusion is mainly based on the observation of strong allelic association between SNPs within the GAA gene and the two mutations (table 3). We selected, in our initial analysis, a subset of three SNPs which showed significant evidence for linkage disequilibrium with each other, identifying a total of four core haplotypes. Both the 525delT and the del exon 18 mutations were in complete association with one of these core haplotypes. In further analyses we included one additional SNP to construct extended haplotypes. The 525delT mutation has never been reported in non-white patients and is relatively rare outside The Netherlands. This cannot be excluded that other, non-Dutch, white patients carry the same haplotype, since Dutch immigrants may have introduced the mutation into Canada and the USA, for instance. It will be interesting to determine whether patients with a 525delT mutation observed outside The Netherlands share the same haplotype.

Although the majority of the del exon 18 chromosomes shared an identical haplotype, three of the chromosomes carried a different haplotype (table 4). The presence of at least three different haplotypes with an identical mutation suggests that the repeat sequence could have been instrumental in several independent mutational events. Remarkably, the del exon 18 mutation is rarely observed outside The Netherlands. It will be interesting to investigate the repeat sequence in other ethnic groups, with further studies in non-white GSD II patients.

We thank Hans Kristian Ploos van Amstel for help in the preparations for this study, and Nicole Werrinkamp for technical assistance. We are grateful to Wim J Kleijer from the Department of Clinical Genetics, Erasmus University Rotterdam, for providing the cell lines of GSD II families.

Recurrent mutations in the deafness gene \(GJB2\) (connexin 26) in British Asian families

Sarah Rickard, David P Kelsell, Tony Sirimana, Kaukab Rajput, Beegee MacArdle, Maria Bitner-Glindzicz

Editor—Mutations in \(GJB2\), the connexin 26 (Cx26) gene, are thought to account for over 50% of autosomal recessive, non-syndromic, congenital deafness, the most common form of genetic deafness\(^1\) and 10–30% of sporadic cases\(^2\). Over 90 recessive mutations in the \(GJB2\) gene have been reported since it was originally described\(^3\) (the connexin 26 (\(GJB2\)) deafness homepage at http://www.iro.es/cx26deaf.html). The most common mutation is a deletion of a guanuinucleotide at position 30–35 (35delG), accounting for approximately 30–63% of mutations in white populations with a carrier frequency of 1:31 in Mediterranean populations\(^4,5\). However, the 35delG mutation is present at a lower prevalence in different ethnic groups\(^6,8\) with other mutations occurring at a higher prevalence, such as 167delT in the Jewish population\(^9–12\).

Both the high carrier frequency of \(GJB2\) mutations and the prevalence of non-35delG mutations in non-white populations implies that mutations other than 35delG may be more common in non-white ethnic minorities who have settled in the UK, particularly those in which consanguinity is prevalent.

In order to examine strategies suitable for sensitive, medium throughpout mutation detection in \(GJB2\), we used denaturing high performance liquid chromatography (DHPLC) to screen for mutations in a cohort of 51 multi-ethnic patients with non-syndromic deafness who presented at our centre for genetic counselling. We found that DHPLC detected all the control mutations in the sample and that no mutations were identified by sequencing that were not detected by DHPLC. Three mutations, W24X, W77X, and Q124X, found in Indian, Pakistani, and Bangladeshi families in this study, have been previously observed in families from the Indian subcontinent, suggesting that they may be common mutations in these ethnic groups.

Methods and results

Fifty one subjects with non-syndromic hearing loss were ascertained through genetic counselling that no mutations were identified by sequencing that were not detected by DHPLC. Three mutations, W24X, W77X, and Q124X, found in Indian, Pakistani, and Bangladeshi families in this study, have been previously observed in families from the Indian subcontinent, suggesting that they may be common mutations in these ethnic groups.

Table 1

<table>
<thead>
<tr>
<th>Mutation</th>
<th>No of controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>35delG/35delG</td>
<td>3</td>
</tr>
<tr>
<td>35delG/wild type</td>
<td>2</td>
</tr>
<tr>
<td>167delT/167delT</td>
<td>1</td>
</tr>
<tr>
<td>167delT/wild type</td>
<td>1</td>
</tr>
<tr>
<td>V27I/E114G</td>
<td>1</td>
</tr>
<tr>
<td>35delG/313–326 14bp deletion</td>
<td>2</td>
</tr>
<tr>
<td>313–326 14bp deletion/wild type</td>
<td>2</td>
</tr>
</tbody>
</table>

...
two minutes, and so on in 5°C decrements until 25°C was reached. Five µl of each sample was mixed with 5 µl of normal control PCR product (“spiked”) and also subjected to heteroduplex formation. A wild type control was included in each run. Spiked and unspiked DHPLC analysis was carried out for the first and second overlapping fragments, giving a total of four runs for each sample. All samples were directly sequenced fragments, giving a total of four runs for each sample. Spiked and unspiked DHPLC analysis was carried out for the first and second overlapping fragments, giving a total of four runs for each sample. All samples were directly sequenced, using BigDye™ Terminator Cycle Sequencing kit (PE Applied Biosystems) and run on an ABI 377 DNA sequencer. PCR amplification used primers Cx263F (5’-CTGGGCAATGCGTTAAACTG GGGTCCTGTGTTGTGCATTC-3’) and the products were purified using Microspin S400 columns (Amersham Pharma-cia).

Fifty one samples were tested, of which 12 had known nucleotide changes (table 1). DHPLC analysis of the two overlapping fragments of the gene detected all 12 positive control base changes shown in table 1 in their relevant amplicons, as well as eight additional altered elution peaks in cases with no known mutation. The results of sequencing these eight cases can be seen in table 2.

One of these changes was identified in a person from a large Sri Lankan family and contained an unusual double nucleotide substitution, c→t at 493, R165W, on one allele, and a previously described sequence variant g→a at 457, V153I, on both alleles.

The two W77X heterozygotes were the consanguineous parents of an affected child, who was subsequently confirmed to be a W77X homozygote by restriction enzyme digestion. No mutant homozygotes were detected in any of the unspiked samples.

Discussion
Severe to profound congenital deafness is a common genetic disorder affecting 1:1000 births. With an increasing demand for GJB2 mutation detection by audiological physicians, geneticists, and families, a rapid and accurate screening service for the variety of mutations prevalent in our multi-ethnic society will be essential in regional DNA diagnostic laboratories. In this cohort of multi-ethnic deaf subjects, DHPLC identified the 12 known mutations among the samples and also detected sequence changes in eight cases where no mutation had previously been identified. No other nucleotide variants were found upon subsequent sequence analysis of each sample. Our screening strategy would not have detected the IVS+1g→a mutation, which occurs at the donor splice site in the first intron, between exon 1 (non-coding) and exon 2, which contains all of the coding sequence of the gene, as this would be in a separate PCR amplicon. As expected in a recessive disorder, no homozygote mutations were identified in samples from this cohort that were not spiked with wild type DNA, as no heteroduplexes were formed.

Recent comparisons of SSCP versus DHPLC in other genes have shown DHPLC to be superior in almost every study, with mutation detection rates of 92-100%. Moreover, it has many advantages over conventional gel based mutation detection techniques, as it is easy to use, gives consistent results, and allows analysis and storage of data on computer. With its microtitre plate based technology, which can be combined with a PCR robot, there is also the potential to allow higher throughput screening. This may become necessary given the prevalence of congenital deafness in the population and the increasing awareness of the role of GJB2 as an aetiological factor by families and those caring for large numbers of deaf children in primary care.

As with other disorders such as cystic fibrosis, the prevalence of a common mutation (35delG) decreases significantly outside the white population. Approximately half the white subjects screened were already known to be 35delG negative, and DHPLC yielded only two new mutation positives (one 35delG homozygote and one 35delG/W24X compound heterozygote), both in cases which had not previously been tested for 35delG. However, the remainder of the new mutations identified were in patients from the Indian subcontinent, and none of these mutations were 35delG.

Examination of the previously undetected mutations that DHPLC identified, with regard to ethnic origins of the patients, has yielded interesting data. The W77X truncating mutation was detected in two families, one a Pakistani family in which the proband was a W77X homozygote and in another Bangladeshi subject, who was also homozygous for the mutation. This mutation has been described previously in Pakistani and Indian kindreds.

Table 2 Results of spiked DHPLC and sequence analysis in cases with no previously identified mutation

<table>
<thead>
<tr>
<th>No</th>
<th>1st fragment (DHPLC)</th>
<th>2nd fragment (DHPLC)</th>
<th>Mutation</th>
<th>Effect</th>
<th>Genotype</th>
<th>Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Shifted peak</td>
<td>Normal</td>
<td>Deletion of G at 30–35*</td>
<td>35delG</td>
<td>35delG/35delG</td>
<td>White</td>
</tr>
<tr>
<td>2</td>
<td>Shifted peak</td>
<td>Normal</td>
<td>G→A at 71*</td>
<td>W24X</td>
<td>W24X/wild type</td>
<td>Indian</td>
</tr>
<tr>
<td>3</td>
<td>Shifted peak</td>
<td>Normal</td>
<td>Deletion of G at 30–35*</td>
<td>35delG</td>
<td>35delG/W24X</td>
<td>White</td>
</tr>
<tr>
<td>4</td>
<td>Shifted peak</td>
<td>Normal</td>
<td>G→A at 71*</td>
<td>W24X</td>
<td>W24X/wild type</td>
<td>Pakistani</td>
</tr>
<tr>
<td>5</td>
<td>Shifted peak</td>
<td>Normal</td>
<td>G→A at 231*</td>
<td>W77X</td>
<td>W77X/wild type</td>
<td>Bangladeshi</td>
</tr>
<tr>
<td>6</td>
<td>Shifted peak</td>
<td>Normal</td>
<td>G→A at 231*</td>
<td>W77X</td>
<td>W77X/wild type</td>
<td>Bangladeshi</td>
</tr>
<tr>
<td>7</td>
<td>Normal</td>
<td>Shifted peak</td>
<td>C→T at 370†</td>
<td>Q124X</td>
<td>Q124X/Q124X</td>
<td>Indian</td>
</tr>
<tr>
<td>8</td>
<td>Normal</td>
<td>Shifted peak</td>
<td>C→T at 493†</td>
<td>R165W</td>
<td>R165W (het)/V153 (hom)</td>
<td>Sri Lankan</td>
</tr>
</tbody>
</table>

*Mutations that occur in the first PCR fragment.
†Mutations in the second fragment.
suggesting that it may be common in the Indian subcontinent. We also identified two hearing impaired sibs from India who were heterozygous for the W24X truncating mutation, which has also been described in Pakistani and Indian families. A second mutation in the GJB2 coding region has not been identified in these sisters by DHPLC or by sequencing. It is possible that they have an unidentified mutation in the untranslated regions or promoter of the gene, or even in another deafness gene. Finally, we have identified a homozygous Q124X mutation in a person of Indian origin, which has also been described in a patient from the same ethnic background. The discovery of these previously reported mutations suggests that a number of common mutations may be enriched in families from the Indian subcontinent, although without population studies it is not possible to be sure whether the prevalence in this ethnic group is statistically higher. Haplotyping of DNA from different families with these mutations may indicate whether they are likely to be founder mutations or not.

Our DHPLC and sequence analysis has shown an unusual combination of sequence variants in a large Sri Lankan pedigree. The proband had mild/moderate hearing loss and was shown to have R165W amino acid variant on one chromosome and V153I on both chromosomes. His mildly affected father was shown to be heterozygous for both changes on the same allele. It is possible that the R165W variant is a mild dominant mutation in this family. R165W has not been previously reported as a disease causing mutation and was not detected in forty normal controls. The arginine residue at position 165 is conserved in the highly homologous human Cx30, 31, and 32 genes, and also in the mouse Gjb2 gene, and occurs in the second extracellular domain of the protein. V153I has been reported in a heterozygous state in hearing subjects (Hilbert et al. The connexin 26 (GJB2) deafness homepage at http://www.iro.es/cx26deaf.html) and we have detected it heterozygously in two out of 186 normal controls. Further investigation has now shown that a profoundly deaf woman from this ethnic group, who married into the family, is homozygous for V153I. However, we have found V153I to be segregating within this community, which operates a dowry and caste system, and we cannot be certain that the variant is the cause of her deafness and not a homozygous non-pathogenic polymorphism. Valine to isoleucine is a conservative amino acid change. V27I and V37I are other amino acid changes involving a valine to isoleucine substitution and both have been identified heterozygously in normal controls. V27I was found to be enriched in the normal Japanese population (39%), suggesting that it occurs too frequently to be a deafness causing mutation, whereas V37I is thought to be pathogenic as it has been found on both alleles of a deaf patient. However, it is extremely difficult to determine the effect of these three sequence variants when all occur in the normal population. Further functional and genetic studies are under way in this family, in order to determine the pathogenicity of both V153I and R165W.

In the UK, many deaf children attend primary care based clinics under the care of community paediatricians and audiologists. With completion of the first draft of the sequence of the Human Genome, awareness of the role of genetics in hearing loss is becoming more widely appreciated throughout the health care system. Thus, the demand for molecular diagnosis will increase. Genetic services will need to respond by providing rapid, sensitive, semiautomated mutation screening of the GJB2 gene.

Research at the Institute of Child Health and Great Ormond Street Hospital for Children NHS Trust benefits from R&D funding received from the NHS Executive.

A region of homozgyosity within 22q11.2 associated with congenital heart disease: recessive DiGeorge/velocardiofacial syndrome?

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EDITORS—DiGeorge syndrome (DGS, MIM 188440) and velocardiofacial syndrome (VCFS, MIM 192430) are associated with interstitial deletions of chromosome 22q11.2 and are considered to be phenotypic variations of the same underlying genetic defect. Studies of patients with diagnoses of DGS or VCFS have estimated that between 68% and 88% have 22q11.2 microdeletions detectable by fluorescence in situ hybridisation (FISH). Most of the deletions arise de novo, but data collected from a large group of patients estimated that 28% of deletions are inherited. Of subjects with a deletion, 90% have the same approximately 3 Mb region deleted, 7% have a nested 1.5 Mb deletion, and in other rare cases, unique deletions and translocations have been detected.

The common clinical features associated with DGS/VCFS are congenital heart malformation, abnormal facies, thymic hypoplasia, cleft palate, and hypocalcaemia. However, despite the vast majority of patients diagnosed with DGS/VCFS having the same region of 22q11.2 deleted, there is wide phenotypic variability. The combination of symptoms can be so severe that the patient dies in the neonatal period. Information about deletion status was not available at the time of corrective surgery. Of particular interest was a male child born to consanguineous parents, who was found to have truncus arteriosus type II and interrupted aortic arch type B. His calcium status was normal, facial appearance was not abnormal, and although total T cell numbers were within normal ranges, CD8+ T cell numbers were low. A diagnosis of VCFS was made.

Methods

Blood was collected at surgery when the child was 7 days old. Deletion analysis was performed by FISH on metaphase chromosomes from peripheral blood lymphocytes using probe D22S75 (N25, Oncor). Two signals were obtained and the child was deemed not to harbour a 22q11.2 deletion. The child died aged 4 months. A year later, a female child was born to the same family. Her diagnosis was truncated arteriosus type II, but without an interrupted aortic arch. She was noted to have an abnormal facial appearance, with a small mouth, simple, cupped, low set ears, and sagging cheeks. Calcium status was normal and a diagnosis of VCFS was made. Blood was collected at corrective surgery when the child was 20 days old. FISH showed that there was no detectable deletion at 22q11.2. Parental metaphase chromosomes were also examined by FISH and no deletions were detected. Since DGS has also been associated with deletions of 10p13-14, chromosomes from both sibs and their parents were tested with probes SD10p1 and SD10p49 (kindly provided by Professor Peter Scambler). Again, signals were detected on both copies of chromosome 10 in all cells of both sibs and their parents.

Since conventional FISH probes had not detected deletions at 22q11.2 or 10p13-14, it
was possible that one of the parents carried a microdeletion that was passed on to the affected children. Also, the pedigree of this family showed that there was a relatively high degree of consanguinity (fig 1). We genotyped the family for a series of polymorphic markers across the DiGeorge chromosomal region (DGCR) in order to identify whether one of the parents carried a small deletion that had not been detected by routine FISH, or whether there was a region of homozygosity at 22q11.2 in this consanguineous family that might be associated with CHD.

The initial genotyping was informative for all markers tested (fig 1). The two affected sibs, VI.2 and VI.3, were found to be homozygous for three markers, NLJH1, D22S941, and D22S944. This region of homozygosity maps within both the commonly deleted 3 Mb region and the 1.5 Mb nested deletion found in a minority of DGS/VCFS patients. The region of homozygosity segregated with the presence of congenital heart defects associated with DGS/VCFS. Since the parents were heterozygous for all markers from D22S1638 to D22S264, there was no evidence to suggest that a microdeletion, undetectable by FISH, was carried on one of the parental chromosomes. To confirm that there was no microdeletion on chromosome 10, the affected sibs, the unaffected older sib, their parents, and paternal grandparents were also genotyped for markers D10S189, D10S547, and D10S191 on 10p13-14. The parents were informative for all three markers, and the affected sibs were discordant for all three loci (data not shown).

Homozgyosity for the region that includes NLJH1, D22S941, and D22S944 is one interpretation of these data. However, there has been a previous report of DGS/VCFS in a sib pair as a result of germline mosaicism in the maternal chromosomes\(^{15}\) so we determined whether there might be hemizygosity at these loci in the affected sibs. Five FISH probes were generated from BAC clone 77h2 (AC000052), cosmid 49c12 (AC000079), cosmid 81h (AC000086), cosmid 91c (AC000091), and PAC p158119 (AC000547). 77h2 maps between D22S427 and D22S420, and p158119 maps distal to COMT, both regions for which the affected sibs are heterozygous. 49c12, 81h, and 91c map within the apparently homozogous region.\(^{16}\) Signals were detected on both copies of chromosome 22 in the young affected sib for all five probes, ruling out germline mosaicism as the mode of transmission in this family (data not shown).

Allele frequencies were estimated from the six chromosomes of IV.8, IV.1, and V.1 (fig 1), who are all related, but do not appear to share any haplotypes. Linkage analysis performed on the haplotypes shown in fig 1 resulted in a multipoint lod score of 1.84. Ordinarily, a lod score of 3 is considered necessary to prove linkage at a given locus. However, where the link between locus and phenotype has been established beyond doubt in previous analyses using other families, there is said to be a prior probability of linkage which lowers the required threshold for proof in new families with the same phenotype. Further, this result was not obtained after testing many loci throughout the genome and selecting the most significant, but was the result of segregation analysis at the only two known DGS/VCFS loci. These data therefore provide strong evidence of recessively inherited DGS/VCFS in this family.

A fourth child was born to the family (VI.4, fig 1) after the analysis had been done for the other members. Genotyping showed that the child shared the extended haplotypes from D22S427 to D22S425 seen in the affected sibs.

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**Figure 1** Pedigree of the family with features of DGS/VCFS. The boxed area delineates the region of interest within the DGCR, which is coloured grey for VI.2, VI.3, and VI.4 where the region is homozygous.

\(^{15}\) PCR primers for the marker are: forward, 5'-GTAATCCTGGGCA TC TATC-3', reverse, 5'-TTCCAGTCTTT GGCTATTAC-3'.
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Chest x-ray and echocardiogram were performed at 6 months and the aortic arch was found to be left sided. The facial appearance was assessed as normal by a clinical geneticist. However, we were not able to rule out an aberrant right subclavian artery. At 6 months, the child was clinically well, but too young for us to assess many of the other possible features of DGS/VCFS, such as hypernasal speech, learning difficulties, or psychiatric illness. Ethical approval and parental consent had been obtained for chest x-ray, echocardiogram, and physical examination only, and so we were not able to assess immune cell numbers. The decision of the parents was that, unless clinically necessary, there should be no further investigations of the youngest child, and so we are currently not able to perform a more in depth evaluation.

Discussion

At first analysis, it might appear that the birth of the fourth child confounds our hypothesis that the region of homozygosity is associated with a DGS/VCFS phenotype. However, data from both mouse models and studies of DGS/VCFS patients show that even when the deletion is present, phenotypic variation is broad and penetrance can be incomplete. Studies have suggested that, despite the wide phenotypic variation, all subjects with a 22q11.2 deletion have some feature of DGS/VCFS. However, such studies are performed on the basis that all subjects included in the study carry the deletion, and testing for the deletion will have been as a result of the presence of some feature of DGS/VCFS. It remains a possibility that there are perfectly healthy subjects with undetected 22q11.2 deletions in the general population, but to test such a hypothesis would require screening an extremely large cohort of people with no features of DGS/VCFS for deletions of 22q11.2.

The region syntenic to human 22q11.2 is found on mouse chromosome 16 and studies have shown a high degree of gene conservation, although with some changes in gene order. Recently, engineered mouse models have begun to dissect out the genes that might be important in DGS/VCFS. Mice heterozygous for a deletion encompassing most of the region homologous to the human 22q11.2 DGS/VCFS deletion were generated. A total of 30% of heterozygous embryos and 18% of adult heterozygous mice presented with cardiovascular abnormalities most frequently found in DGS/VCFS patients. Furthermore, the defects seen in the mice heterozygous for the deletion could be overcome by genetically complementing the deletion, supporting the idea that haploinsufficiency of a gene or genes within the deleted region is responsible for the phenotype. Mice carrying smaller deletions within the mouse DGCR have also been generated, but these show none of the cardiovascular abnormalities seen in DGS/VCFS.

Taken together, data from the mouse models help narrow the region in which candidate genes for DGS/VCFS are likely to reside to that bounded by Comt proximally and Ufd1l distally. The homologous region on human chromosome 22q11.2 contains the same genes in the same order, but with the orientation reversed. This overlaps the region for which the two affected children in this family are homozygous. The maximum region of homozygosity is from D22S1638 proximally to COMT distally, and is approximately 1 Mb; the minimum region is from NLJH1 proximally to D22S944 distally and is approximately 570 kb.

Current evidence supports the notion that when one copy of 22q11.2 is deleted, the DGS/VCFS phenotype occurs as the result of haploinsufficiency of a gene or genes that have been deleted. It is possible that in this family the associated haplotype carries a mutation that leads to a minor reduction in the product of a gene or genes in the region. Such a mutation might then have no discernible effect in heterozygotes, but homozygosity could lead to functional haploinsufficiency.

It is of particular interest that in the mouse model where cardiovascular abnormalities were found and in which there would have been a common genetic background, there was phenotypic variability and incomplete penetrance. Phenotypic variation in DGS/VCFS is well documented and in familial cases where the affected members carry identical 22q11.2 deletions, phenotypic variation can be wide. Moreover, there are now several published reports of monozygotic twins with the deletion who are discordant, particularly for congenital heart disease.

Further mouse models have recently been developed, refining the genes that are important in developing DGS/VCFS, which implicate Tbx1 in the aetiology of the condition, particularly the cardiovascular malformations. Our data are consistent with these models, as the human homologue, Tbx1, is within the region for which the affected members of this family are homozygous.

In summary, we present evidence from a consanguineous family with two children presenting with conotruncal cardiac defects and features of DGS/VCFS and a region of homozygosity at 22q11.2. Further analysis of the homozygous region may facilitate the identification of the genes that are involved in cardiac morphogenesis.

We are grateful to the family for their participation and continued interest in the study. We wish to thank Professor Peter Scambler for constructive discussions and Professor Chris Inghelbrecht for advice and critically reading the manuscript. Chromosome 22 clones for FISH analysis were kindly provided by the Clone Resources Group, The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK (clonerequest@sanger.ac.uk). JH is a Wellcome Trust Research Career Development Fellow.

High frequency of the ApoB-100 R3500Q mutation in Bulgarian hypercholesterolaemic subjects

Anelia Horvath, Alexey Savov, Stephen Kirov, Elina Karshelova, Iva Paskaleva, Assen Goudev, Varban Ganev

Editor: One of the most common single site mutations in the human ApoB gene, R3500Q, results in mild to severe hypercholesterolaemia and an increased risk for early onset atherosclerosis. Intensive mutation screening studies of the ApoB gene in subjects with hypercholesterolaemia have identified a few less frequent variants, associated with an even milder phenotype and localised within a region coding for the ApoB-100 receptor binding domain. It is widely accepted that the average population frequency of the R3500Q mutation is about 1:500–1:700 among white populations. However, results from population studies, as well as data from studies of high risk groups in Europe, show that frequencies vary

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to a large extent among population groups, ranging between 1:717 and 1:1250.5 The simultaneous occurrence of the underlying G to A substitution and a rare ApoB haplotype across different ethnic groups favours the hypothesis of a common origin of the R3500Q mutation and its further migration spread.7 Population groups with the highest frequencies are clustered in central Europe, and the mutation frequency decreases as one moves east, north, and south west,5 8–22 becoming extremely rare in Finland, southern Italy, and Spain.23–25 There is a lack of information about the mutation prevalence in the south east of central Europe, with the exception of one study on hypercholesterolaemic subjects from the fringes of Europe (Turkey) that failed to detect the R3500Q mutation.26 This lack of information about a sizeable region of Europe flanked by populations with high (Austria26 and Hungary27) and extremely low prevalence (Turkey28) leaves open the question of a frequency gradient to the south east.

This study of the occurrence of the R3500Q mutation and its associated haplotype(s) in subjects with hypercholesterolaemia from Bulgaria aimed to obtain data from a hitherto unexplored region of Europe and, thereby, to shed additional light on the mutation’s spread and common origin. Also, mutation screening of a region coding for the ApoB-100 receptor binding domain between amino acid residues 3448 and 3562, which harbours other reported (R3500W2 and R3531C3) and possibly new ApoB-100 mutations, was undertaken.

Material and methods

One hundred and thirty unrelated subjects (53 females and 77 males) with hypercholesterolaemia (plasma total cholesterol above 7.0 mmol/l) were studied. Two pedigrees (10 family members) of carriers of the R3500Q mutation were identified among the group of 126 hypercholesterolaemic subjects from the non-carrier group (table 1). The individual values of total cholesterol, LDL cholesterol, and ApoB-100 of the R3500Q mutation carriers ranged from 6.11 to 10.90 mmol/l, from 4.44 to 9.22 mmol/l, and from 1.41 to 2.03 g/l, respectively, showing broad inter- and intrafamilial variability. Similar findings have been observed in samples of hypercholesterolaemic subjects from other European populations and are attributed to differences in environmental factors, diet, and gene-gene interactions. It is worth noting that some of the mutation carriers have phenotypes indistinguishable from those of healthy, normolipidaemic, non-carriers of the same gender and age by their forties, meaning that symptomatic identification of the R3500Q mutation in this group of subjects has not yet occurred.

Two tailed test was applied and p values <0.05 were considered significant for rejection of the null hypothesis.

DNA was extracted by standard techniques27 from 10 ml whole venous blood collected in EDTA anticoagulated Vacutainer tubes (Sherwood Medical, Germany) and stored at 4°C until analysis. Detection of the R3500Q mutation was done by competitive allele specific PCR.28 The allele frequency was estimated using the “gene counting” method. Screening of the ApoB region (nucleotides 10551-10895) coding for a part of the ApoB-100 receptor binding domain was done by single strand conformation analysis (SSCA). Amplicons were obtained by PCR with oligonucleotides under conditions used by Tybjærg-Hansen et al24 and were separated using a procedure described elsewhere.25 Five polymorphisms within the region of ApoB were analysed. Ins/Del in the promoter region (sp24/27), XbaI (codon 2488), MspI (codon 3611), EcoRI (codon 4154), and 3VNTRs were analysed as described previously,24–25 and their cosegregation with the R3500Q mutation was followed in the available pedigrees.

Results

Four of the 130 unrelated hypercholesterolaemic subjects were found to be carriers of the R3500Q mutation (0.031, 95% CI 0.01-0.082). Three additional carriers were identified among the 10 studied members of the two pedigrees.

The comparison of laboratory parameters of the seven R3500Q mutation carriers identified in this study (four probands and three family members) with the group of 126 hypercholesterolaemic non-carriers showed that the carrier group as a whole was not significantly different from the non-carrier group (table 1). The individual values of total cholesterol, LDL cholesterol, and ApoB-100 of the R3500Q mutation carriers ranged from 6.11 to 10.90 mmol/l, from 4.44 to 9.22 mmol/l, and from 1.41 to 2.03 g/l, respectively, showing broad inter- and intrafamilial variability. Similar findings have been observed in samples of hypercholesterolaemic subjects from other European populations and are attributed to differences in environmental factors, diet, and gene-gene interactions. It is worth noting that some of the mutation carriers have phenotypes indistinguishable from those of healthy, normolipidaemic, non-carriers of the same gender and age by their forties, meaning that symptomatic identification of the R3500Q mutation in this population group was not yet observed.

Table 1  Laboratory variables (average (SD)) for the R3500Q mutation carriers (n=7) and comparison with unrelated hypercholesterolaemic non-carriers (n=126)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age (y)</th>
<th>Total cholesterol (mmol/l)</th>
<th>HDL cholesterol (mmol/l)</th>
<th>Triglycerides (mmol/l)</th>
<th>LDL cholesterol (mmol/l)</th>
<th>ApoB (g/l)</th>
<th>ApoA1 (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carriers</td>
<td>42.6 (12.1)</td>
<td>8.18 (1.64)</td>
<td>1.30 (0.25)</td>
<td>1.47 (0.68)</td>
<td>6.22 (1.63)</td>
<td>1.41 (0.28)</td>
<td>1.45 (0.27)</td>
</tr>
<tr>
<td>Non-carriers</td>
<td>50.6 (10.7)</td>
<td>8.84 (1.87)</td>
<td>1.16 (0.38)</td>
<td>1.92 (0.83)</td>
<td>6.61 (1.98)</td>
<td>1.78 (0.32)</td>
<td>1.57 (0.39)</td>
</tr>
<tr>
<td>t value</td>
<td>1.71*</td>
<td>1.03*</td>
<td>1.39*</td>
<td>1.68*</td>
<td>0.92*</td>
<td>1.55*</td>
<td>1.11*</td>
</tr>
</tbody>
</table>

*p value >0.05.
subgroup, although highly desirable, would require wide scale screening.

Mutations in the receptor binding domain resulting in mild to moderate hypercholesterolaemia have been reported in some other populations with a much lower frequency than the R3500Q mutation. Our screening of the ApoB region (nucleotides 10 551-10 895), coding for a part of the APOB-100 receptor binding domain, showed two distinct mobility patterns (results not shown). Four of the samples coinciding with the heterozygous samples for the R3500Q mutation, as shown by competitive allele specific PCR analysis, showed additional sharp and clearly separated bands in comparison with the remaining 126 identical patterns. Thus, other mutations in the screened region, apart from R3500Q, were excluded in this sample of Bulgarian hypercholesterolaemic subjects by SSCA. Therefore, we conclude that other mutations in this region are rare causative factors for hypercholesterolaemia in Bulgaria.

Analysis of the five polymorphisms within the region of ApoB-100 in the two pedigrees showed unequivocal linkage of the R3500Q mutation with a haplotype Ins, XbaI-, MspI+, EcoRI-, 49 3'VNTR (I, X-, M+, R-, 49 3'VNTR). The results from one of the two pedigrees are shown in fig I. The association of the mutation with the same haplotype could not be ruled out by the observed genotypes of the other two unrelated carriers with hypercholesterolaemia, who did not have families available for analysis. The association of the R3500Q mutation in the Bulgarian population with the same rare haplotype, which is reported to be associated with the mutation almost exclusively across different ethnic groups, supports the hypothesis of a common origin of the mutation.

**Discussion**

In our sample of unrelated hypercholesterolaemic subjects from Bulgaria, the R3500Q mutation accounts for 0.99-8.17% (95% CI) of the cases with hypercholesterolaemia (defined as total cholesterol >7.0 mmol/l), and represents the most common single gene defect resulting in hypercholesterolaemia identified so far in Bulgaria. Population studies and estimations based on extrapolation from the prevalence of the R3500Q mutation among hypercholesterolaemic subjects show a nearly 18-fold difference in its frequency in different populations. It is at V34 suggested that the R3500Q mutation originated some 6000-7000 years ago. Miserez and Muller estimated that the mutation originated in the Celtic population, settled in central Europe, and spread across Europe. Data on mutation frequencies reviewed in this study, together with the mutation's high prevalence in populations from eastern Europe, lead us to assume that clear distribution gradients can be tracked from central Europe in all directions except to the south east, for which continuous data are available for Hungary only. Very recently, Myant et al. estimated that the mutation spread has reached the hitherto unstudied populations of people with total cholesterol above 7.0 mmol/l.
mmol/l (6.34%) among an unselected group of 4800 Bulgarians (N Vassilevski, Countrywide Integrated Noncommunicable Diseases Intervention - CINDI - Program, personal communication), and the frequency of the R3500Q mutation in our group, one can estimate a prevalence of 1:451 in adult Bulgarians. This places the Bulgarian population among the European populations with a medium prevalence of the mutation. Our findings also suggest that along with its spread in other European areas, the mutation’s expansion has moved to the south east and involved the Balkans. Given that Bulgaria is situated in the heart of the Balkans, occurrence of the R3500Q mutation might be expected in other neighbouring populations; this requires further studies on other populations from the region.

In conclusion, this is the first study showing occurrence of the R3500Q mutation in south eastern Europe. The mutation is linked to the same rare haplotype as in the other populations; this supports the hypothesis of its common origin and suggests that along with its spread from central Europe to other European areas, the mutation’s expansion has moved to the south east.

This work was supported by the Medical Research Council at the Medical University of Sofia (grant 024/2000).


A community based study on intentions regarding predictive testing for hereditary breast cancer

M Welkenhuysen, G Evers-Kiebooms, M Decruyenaere, E Claes, L Denayer

EDITOR—The recent developments in human genetics have led to the availability of predictive DNA tests for the hereditary subgroups of some cancers. During the past five years, genetic testing for mutations in the \( BRCA1 \) and \( BRCA2 \) genes, predisposing to hereditary breast/ovarian cancer (HBOC), has entered clinical practice. Several genetic centres/hospitals offer predictive testing for HBOC to women with a family history of the disease. Women who carry a HBOC mutation might decide on regular breast screening to increase the chances of early detection of the disease. Alternatively, they might opt for prophylactic surgery to reduce their breast/ovarian cancer risk as much as possible. In addition to the uncertainties involved in these management options, the ambiguity because of the incomplete and variable penetrance of the \( BRCA1/BRCA2 \) mutations has to be dealt with as well; recent estimations of the cumulative breast cancer risk for female \( BRCA1/BRCA2 \) mutation carriers vary between 50% and 85%. Alternatively, the absence of a \( BRCA1/BRCA2 \) mutation in affected family members does not eliminate the risk of developing breast cancer. It merely reduces the risk to the level in the general population, which is about 10% in the industrialised world. Despite these uncertainties, the high frequency of breast cancer in the population may trigger questions about and interest in the predictive test for breast cancer. Also, media attention may play an important role by creating high hopes, as well as an increased awareness or misconceptions of the personal and population risk for breast cancer. Commercial companies may try to encourage testing for \( BRCA1/BRCA2 \) mutations by selling tests directly to physicians and/or the public. On the other hand, public concern, pessimism, or fear about the new genetic technology may discourage interest in applications like predictive genetic testing for HBOC, although such a negative effect regarding medical interventions has not been observed. Most studies among first degree or more distant relatives of breast/ovarian cancer patients report high levels of interest (between 80% and 95%) in predictive testing for HBOC. The interest of women without a family history of breast cancer is usually less pronounced, although it is still quite high (ranging from 45% to 90%, but mostly between 60% and 75%). This lower level of interest is in line with the positive relationship between the extent of the family history and the level of interest observed in a number of studies. Examples of other possible correlates of the interest in predictive testing for HBOC that have been investigated in the above mentioned studies are sociodemographic variables like education and age, emotional variables like cancer worry and depression, awareness measures, and control related variables like the belief that breast cancer is curable and that regular mammograms give a feeling of control, as well as control related personality and coping styles. Several studies paid attention to one or more components of the Health Belief Model to explain intentions or interest regarding predictive testing for HBOC. According to the Health Belief Model, a specific health behaviour is influenced by (1) the perceived susceptibility to or the perceived risk of the health threat, (2) the perceived severity of the health threat, (3) the perceived benefits of engaging in the health behaviour, and (4) the perceived costs or barriers to...
performing the health behaviour. The Health Belief Model predicts that the higher the perceived susceptibility to and the perceived severity of a health threat, the more a person will engage in health behaviour. With regard to the benefits and costs components, the Health Belief Model argues that people who perceive more benefits are more motivated to perform the health behaviour, while the reverse is true for people who perceive more costs. The perceived risk component has been studied frequently, as well as the perceived benefits and costs of having a predictive test for HBOC or reasons for and against having such a test. However, the perceived seriousness of hereditary breast cancer has (almost) never been included as a possible determinant of interest in a predictive test for HBOC.

The aim of the present study is twofold: first, to investigate the intentions to have a predictive test for hereditary breast cancer (HBC) among Flemish women from the general population who were not selected for a family history of breast cancer or for breast related health problems; secondly, to examine which factors influenced the reported interest in this unselected group, with special attention to the four components of the Health Belief Model, as well as a general attitudinal variable concerning the development of a predictive genetic test for HBC. The latter factor was included since the public perception of the applications of the new genetic technology may be an important mediator of personal intentions.

Methods

PROCEDURE

The participants in our study were students at the Institute for Family Sciences, a Flemish adult education institute in Brussels providing a three year programme with courses on family relations, psychology, health, and social care. During their lunch break, they were asked to complete a questionnaire on perceptions of breast cancer in general, as well as on sociodemographic and background variables (Questionnaire 1). After handing in the questionnaire, the students received a text with information on hereditary breast cancer (HBC), its mode of inheritance, the associated risks, the possibilities and limitations of the predictive test for HBC as well as on the management options (four pages). The following problems involving the predictive test for HBC were addressed: the incomplete penetrance of the HBC mutations, the genetic heterogeneity of breast cancer, the possibility of an inconclusive test result, the fact that non-carriers of a HBC mutation can still get breast cancer, and the residual breast cancer risk after prophylactic mastectomy. The text was accompanied by a questionnaire (Questionnaire 2) which the students were invited to complete and return. Ovarian cancer was not dealt with in the text or questionnaire. Should the survey have elicited questions or problems involving breast cancer, the opportunity was provided to consult the researcher (MW or MD, psychologists).

MEASUREMENTS

The following sample characteristics were measured in Questionnaire 1 by multiple choice or open ended questions: gender, age, marital status, number of children, and level of education. Additionally, we asked whether breast cancer had occurred in the family and, if so, which family member(s) (had) suffered from the disease (open ended questions). The remaining questions were all included in Questionnaire 2.

The dependent variable in our study was the personal intention to have a predictive test for HBC. This was measured by a multiple choice question (“In case breast cancer occurs in your family, would you ask for a predictive genetic test to determine if you carry a breast cancer mutation?”) with four response alternatives (definitely not, probably not, probably, definitely). The participants were invited to explain their answer (open ended question). For the analysis of the relationship with other variables, the intention to have a predictive test for HBC was recoded as a dichotomous variable. The “definitely not” and “probably not” answers were collapsed into a single category of negative intentions or uninterest; the “probably” and “definitely” answers were grouped into a single category of positive intentions or interest. The independent variables were the following.

(1) The participants’ awareness of the subject of our study; this was checked by means of the following questions: “Had you heard about hereditary breast cancer before reading the text?”, “Had you heard about the predictive genetic test for hereditary breast cancer before reading the text?”, “Have you ever asked a medical doctor for information about hereditary breast cancer and/or the predictive test?" (yes/no answers). The sum of positive answers was the awareness score.

(2) The general attitude towards the development of a predictive test for HBC: this was assessed by four bipolar five point rating scales, with scores ranging from 1 to 5. Higher scores corresponded to a more positive attitude. The following pairs of adjectives were used: important/unimportant, acceptable/unacceptable, needless/essential, good for humanity/bad for humanity. The internal consistency among the four scales was good (Cronbach α=0.88). The sum of the four individual scale scores divided by the number of scale items represented the general attitude score. When one (or more) of the individual scale scores was missing, the general attitude score was not calculated.

(3) The components of the Health Belief Model. (A) The measurement of the susceptibility and seriousness components was based on two breast cancer specific instruments designed by Champion for her research on the Health Belief Model. The instruments consist of six and 12 statements respectively with which one can agree or disagree on a five point Likert scale, for example, “I am likely to get breast cancer in the future” for the perceived susceptibility scale, and “I am afraid
Table 1 Intention to have a predictive test for HBC, awareness of HBC and the predictive test, general attitude toward the development of a predictive test for HBC, perceived susceptibility to HBC, and perceived seriousness of HBC

<table>
<thead>
<tr>
<th>Intention: “In case breast cancer occurs in your family, would you ask for a predictive genetic test to determine if you carry a breast cancer mutation?”</th>
<th>(n=318)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definitely not</td>
<td>8%</td>
</tr>
<tr>
<td>Probably not</td>
<td>26%</td>
</tr>
<tr>
<td>Probably</td>
<td>45%</td>
</tr>
<tr>
<td>Definitely</td>
<td>21%</td>
</tr>
<tr>
<td>Awareness (% yes)</td>
<td></td>
</tr>
<tr>
<td>Heard about HBC</td>
<td>79% (n=325)</td>
</tr>
<tr>
<td>Heard about the predictive test</td>
<td>19% (n=324)</td>
</tr>
<tr>
<td>Asked a medical doctor for information</td>
<td>7% (n=329)</td>
</tr>
</tbody>
</table>

Table 2 Mean importance ratings of the arguments for and against having a predictive test for HBC (7 point scales; the arguments are listed according to the mean rating of importance)

<table>
<thead>
<tr>
<th>Arguments for:</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early detection of breast cancer increases the chances of a cure</td>
<td>5.9 (SD=1.59, n=309)</td>
</tr>
<tr>
<td>To learn if you (or your parents) have an increased risk</td>
<td>5.7 (SD=1.62, n=317)</td>
</tr>
<tr>
<td>To take precautions to reduce the breast cancer risk</td>
<td>5.7 (SD=1.69, n=317)</td>
</tr>
<tr>
<td>Because a treatment for breast cancer exists</td>
<td>5.3 (SD=1.74, n=317)</td>
</tr>
<tr>
<td>To have certainty</td>
<td>5.2 (SD=1.80, n=316)</td>
</tr>
<tr>
<td>To be released from the uncertainty</td>
<td>4.7 (SD=2.05, n=313)</td>
</tr>
<tr>
<td>When the test exists, it is self-evident to have it</td>
<td>3.8 (SD=2.00, n=316)</td>
</tr>
</tbody>
</table>

Arguments against:

<table>
<thead>
<tr>
<th>Arguments against:</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I could not cope living with an unfavourable test result</td>
<td>4.3 (SD=1.92, n=323)</td>
</tr>
<tr>
<td>I am happier not knowing if I have a breast cancer mutation</td>
<td>3.9 (SD=2.03, n=321)</td>
</tr>
<tr>
<td>The decisions in my life do not depend on such a test</td>
<td>3.9 (SD=1.85, n=319)</td>
</tr>
<tr>
<td>I do not worry much about breast cancer, it is one of many risks</td>
<td>3.7 (SD=1.78, n=317)</td>
</tr>
<tr>
<td>I do not believe in preventive measures</td>
<td>3.1 (SD=2.00, n=319)</td>
</tr>
<tr>
<td>Because I worry about the impact of the test result on the relationship with my (future) partner</td>
<td>3.0 (SD=1.92, n=321)</td>
</tr>
<tr>
<td>Because I am afraid that the test result might have a negative influence on my studies or my career</td>
<td>3.0 (SD=1.81, n=319)</td>
</tr>
<tr>
<td>Because I worry about the reactions of my partner if I were a carrier</td>
<td>2.8 (SD=1.85, n=323)</td>
</tr>
</tbody>
</table>

correlational analysis were used when categori- cal or continuous measures were involved, respectively. To compare mean scale scores, t tests were used. For the multivariate analysis of the relationship between the dependent variable and the set of sociodemographic variables and independent variables, stepwise logistic regression was performed. For all the statistical analyses, the SAS System software was used.

SAMPLE

Since the proportion of male participants was small (about 10%), only the results of the female participants were included in the analyses. About 70% (n=332) of the 471 women who filled in Questionnaire 1 and who subsequently received the informative text as well as the questionnaire on HBC and the predictive test returned a completed questionnaire (Questionnaire 2). After exclusion of three women who have or have had cancer, the sample consisted of 329 women between 19 and 65 years old (mean age=37.9 years). Most of them were married (69%), 15% had a partner, 8% were single, and the remainder were divorced (7%) or widowed (1%). The majority had chil- dren (85%, the mean number of children was 2.2). Before starting the programme at the Institute for Family Sciences, a minority of the sample (4%) had obtained a university degree, 34% had completed another type of higher education, 36% had had a general education in secondary school, 18% had had a technical education, and the remaining 8% had had a vocational training. Twenty-one percent of the sample (n=68) reported that breast cancer (had) occurred in the family.

Results

DESCRIPTIVE DATA FOR THE TOTAL SAMPLE

Table 1 presents the data for the dependent variable and most independent variables for the total sample. The intentions to have a predictive test for HBC were mainly positive. Awareness of HBC was moderate, but only one fifth of the sample had heard about the predictive test for HBC and less than 10% had asked a medical doctor for information about these issues. As expected, the latter was more likely when breast cancer occurred in the family (χ²=22.40, p<0.01). The results of the three questions added up to the following awareness scores: 21% answered no to all three questions and got score 0; 57% answered yes to one question and obtained score 1; 19% obtained score 2 (two affirmative answers); 3% answered yes to all three questions and got score 3. The general attitude toward the development of a predictive test for HBC was favourable; the mean ratings for each of the four items were high, resulting in a mean general attitude score of 4.3. The mean perceived susceptibility score and the mean perceived seriousness score in our unselected sample were low to moderate. The mean importance ratings for the individual arguments for and against predictive testing for HBC are shown in table 2. The means for the benefits and the barriers subscales were 4.9 (SD=1.30) and 3.4 (SD=1.21), respectively. A t test showed that
Table 3  Intercorrelations (r; Pearson coefficients) between the intention to have a predictive test for HBC (dichotomised variable), the sociodemographic variables, and the independent variables. The significant relations are in bold; the corresponding p values are indicated below the table.

<table>
<thead>
<tr>
<th>Variables</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
<th>(5)</th>
<th>(6)</th>
<th>(7)</th>
<th>(8)</th>
<th>(9)</th>
<th>(10)</th>
<th>(11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Intention</td>
<td>−0.03</td>
<td>−0.17</td>
<td>−0.05</td>
<td>−0.35</td>
<td>0.00</td>
<td>0.47</td>
<td>−0.15</td>
<td>0.07</td>
<td>0.58</td>
<td>−0.24</td>
</tr>
<tr>
<td>(2) Age</td>
<td>0.52</td>
<td>0.06</td>
<td>−0.03</td>
<td>−0.13</td>
<td>−0.01</td>
<td>0.08</td>
<td>−0.11</td>
<td>−0.01</td>
<td>0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>(3) Number of children</td>
<td>0.06</td>
<td>0.07</td>
<td>−0.01</td>
<td>−0.01</td>
<td>−0.08</td>
<td>−0.09</td>
<td>0.06</td>
<td>0.01</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>(4) Education</td>
<td>−0.04</td>
<td>−0.09</td>
<td>−0.01</td>
<td>−0.04</td>
<td>0.04</td>
<td>−0.01</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(5) Breast cancer in the family</td>
<td>0.18</td>
<td>−0.21</td>
<td>0.35</td>
<td>0.03</td>
<td>−0.30</td>
<td>0.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6) Awareness</td>
<td>0.00</td>
<td>0.06</td>
<td>−0.02</td>
<td>−0.02</td>
<td>−0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(7) General attitude score</td>
<td>−0.13</td>
<td>0.00</td>
<td>0.61</td>
<td>−0.28</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(8) Perceived susceptibility score</td>
<td>0.31</td>
<td>−0.14</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>(9) Perceived seriousness score</td>
<td>0.10</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(10) Benefits score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(11) Barriers score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

p<0.001 for 0.14 < |r| < 0.21, p<0.001 for 0.20 < |r| < 0.24, p<0.0001 for |r| > 0.23.

The mean for the arguments in favour of testing was significantly higher than the mean for the arguments against (t=12.74, p<0.001).

VARIABLES ASSOCIATED WITH THE INTENTION TO HAVE A PREDICTIVE TEST FOR HBC IN THE TOTAL SAMPLE

Table 3 shows the univariate Pearson intercorrelations in the total sample between the dichotomised intention and the sociodemographic and independent variables. A chi-square test indicated that the dichotomised intention to have a predictive test for HBC was not associated with marital status ($\chi^2=4.45, p=0.62$). To identify factors having an independent association with the intention regarding the predictive test for HBC, a multivariate stepwise logistic regression analysis was performed, modelling uninterest. The results of the logistic regression in table 4 show that the perceived susceptibility score, which had an unexpected negative univariate association with the dichotomised intention (table 3), was not retained as a predictor in the regression. The benefits score was the first to enter the logistic regression with an odds ratio of 0.34. This means that when the benefits score increased by one unit, the odds to report a negative intention regarding the predictive test for HBC decreased by a factor of 0.34. Similarly, the odds ratio of 0.52 for the general attitude score indicates that with each increase in the attitude score, the odds to express uninterest decreased by a factor of 0.52. In other words, women who rated the arguments for a predictive test for HBC as more important (the benefits score) and women with a more positive attitude toward the development of such a test (the general attitude score) were less likely to report uninterest in the test. Higher odds of a negative intention were associated with a higher number of children and with the occurrence of breast cancer in the family. The value for the latter variable was quite high; the women in our sample who have (had) a relative with breast cancer had 3.61 higher odds of being uninterested in the predictive test for HBC than the women without a relative with the disease. The observation that women with breast cancer in their family were more doubtful about the predictive test is an unexpected and intriguing finding which was analysed further.

EXPLORE THE INFLUENCE OF THE OCCURRENCE OF BREAST CANCER IN THE FAMILY ON THE INTENTION TO HAVE THE PREDICTIVE TEST FOR HBC

To explore the influence of the occurrence of breast cancer in the family on the intention to have the predictive test for HBC, the sample was divided into two subgroups: a subgroup of women who have (had) at least one relative with breast cancer (the BC group, n=68) and a subgroup of women who have (had) no relatives with the disease (the No BC group, n=261). The results for the intention to have a predictive test for HBC for the separate subgroups are shown in table 5. Uninterest in a predictive test for HBC was observed much more often in the BC group (66% versus 25% in the No BC group, $\chi^2=40.72, p=0.001$). The spontaneous explanations for the reported intention that were given most frequently in both subgroups are also shown in table 5. The explanations involving preventive measures, knowledge, or certainty were more likely to be given by the No BC group, whereas the BC group was more inclined to use explanations referring to emotions like anxiety or to the emotional burden of being a carrier. Further, the following significant relationships with the occurrence of breast cancer in the family were observed (table 3, fifth row): the BC group had a more negative attitude towards the development of such a test than the No BC group (the mean awareness scores were 1.3 and 1.0 respectively; the mean general attitude scores were 4.0 and 4.4 respectively). The mean importance ratings of the arguments against were the same in the two subgroups (3.7 and 3.4 respectively). Subsequent within
Table 5 | Intention to have a predictive test for HBC: results for the group of women with breast cancer in the family (the BC group, n=68) versus the group of women without breast cancer in the family (No BC group, n=261)

<table>
<thead>
<tr>
<th></th>
<th>BC group (n=67)</th>
<th>No BC group (n=251)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intention: &quot;In case breast cancer occurs in your family, would you ask for a predictive genetic test to determine if you carry a breast cancer mutation?&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definitely not</td>
<td>18%</td>
<td>5%</td>
</tr>
<tr>
<td>Probably not</td>
<td>48%</td>
<td>20%</td>
</tr>
<tr>
<td>Probably</td>
<td>23%</td>
<td>50%</td>
</tr>
<tr>
<td>Definitely</td>
<td>9%</td>
<td>25%</td>
</tr>
<tr>
<td>Spontaneous explanations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevention, early detection, examinations (n=54)</td>
<td>12%</td>
<td>25%*</td>
</tr>
<tr>
<td>Having certainty (n=52)</td>
<td>10%</td>
<td>25%*</td>
</tr>
<tr>
<td>Too scary, burden, too difficult to cope with (n=51)</td>
<td>41%</td>
<td>16%**</td>
</tr>
<tr>
<td>Knowing the risks for my children (n=26)</td>
<td>2%</td>
<td>13%*</td>
</tr>
</tbody>
</table>

*χ² test, p<0.05. **χ² test, p<0.001.

subgroup comparisons of the mean importance ratings show that the arguments for the predictive test were judged as more important than the arguments against in the No BC group (5.1>3.4, t=14.24, p<0.0001), while there was no difference in the BC group (4.1 and 3.7, t=1.54, p=0.13).

Separate stepwise logistic regression analyses explored whether the set of variables explaining a negative intention differed between the BC group and the No BC group. The results of these analyses are presented in table 6. In both subsamples, the benefits score was the first variable to enter the logistic regression; with each increase in the benefits score, the odds to express uninterest decreased by a factor of 0.40 in the No BC group and by a factor of 0.07 in the BC group, indicating that the explanatory value of the benefits score was higher in the No BC group. Age entered as an additional explanatory variable in the BC group; for each increase in age by one year, the odds to report a negative intention increased by a factor of 1.20. In the No BC group, the general attitude score and the number of children had an additional explanatory value; women without relatives with breast cancer were less likely to report uninterest in the predictive test for HBC when they had a more positive attitude towards the development of such a test, while they were more likely to report uninterest when they had more children.

Discussion

At first glance, our results confirm the strong interest in the predictive test for HBC found in most other studies. In the present study, the general level of interest was higher than the level observed in a study on the predictive test for Huntington’s disease among a comparable community sample,37 in which about half the women expressed interest in the predictive test for this neurogenetic disease. This is not surprising given the crucial differences between breast cancer and Huntington’s disease, for instance regarding the familiarity with the disorder and the possibilities for risk reduction and treatment. Also consistent with previous research,20 15 our sample considered the benefits of the predictive test for HBC on the whole as more important than its costs, and the possibilities of decreasing the breast cancer risk and to learn about one’s children’s risk were valuable arguments for testing. However, the contrast between women with versus without breast cancer in the family (the BC group versus the No BC group) forces a shift from this general level of discussion to a more specific one. The women in our sample who are related to a breast cancer patient (the BC group) were much less interested in the predictive test for HBC; a negative intention was expressed by two thirds of the BC group. This lack of interest is not only much larger than in previous studies among high risk women,13–15 20 but also more pronounced than in other studies among women with breast cancer in the family who have no increased risk for the disease.19 28 51 The discrepancy in our findings might be explained by cultural differences in dealing with risks and preventive options in the context of breast cancer or by specific characteristics of our sample. Being students at the Institute for Family Sciences, they might have been more interested in the relational and/or psychological issues in this context, rather than in technical information. Additionally, the kind of information that we provided on the predictive test for HBC, especially on the uncertainties and limitations involved, could have led to more reluctance for testing.39 Another explanation could be the method of recruitment of the study sample; our findings are based on a community sample without selection for a family history of breast cancer or breast related problems, while this kind of selection did take place in most other studies. The question about having a predictive test for HBC probably seems less hypothetical or unexpected in such selected samples, which might have resulted in less reluctant reactions than in our BC group. This may especially be the case when comparing with studies conducted on high risk families in a hereditary cancer registry and/or as part of...
a thorough research protocol. However, since the information on the number of relatives with breast cancer and/or the degree of kinship in our BC group was not always complete and could not be confirmed, more detailed comparisons with other studies are difficult.

Despite its lack of specification, the occurrence of breast cancer in the family had a strong independent association with uninterest in the predictive test for HBC. As shown by logistic regression analysis, women in the BC group were more than three times more likely to express uninterest in the test. To our knowledge, our study is the first to show such a strong negative relation between breast cancer in the family and interest in the predictive test. Shiloh et al. observed a higher proportion of extreme rejection of the predictive test among at risk relatives of breast cancer patients (although their mean reported intention did not differ from the mean in the group of relatives without an increased risk). The greater uninterest among women who have breast cancer in the family in our study might have been the result of a stronger avoidance reaction in the BC group; because of their emotional involvement with breast cancer in the family and/or their higher perceived susceptibility to the disease, these women want to avoid the threatening possibility of detecting that they are carrying a HBC mutation. The stronger emotional involvement in the BC group is corroborated by the greater tendency in this group to give emotional explanations for the intention regarding predictive testing than in the No BC group. The operation of self-protective avoidance tendencies among at risk relatives of breast cancer patients was also suggested by Shiloh et al. and is in line with the idea that too much fear can lead to inaction (see also the conflict theory of Janis and Mann). This idea receives further support from the negative univariate association between perceived susceptibility to breast cancer and the intention to have a predictive test for HBC observed in our study. Obviously, this contrasts with the predictions of the Health Belief Model. However, although the Health Belief Model has proven to be a valuable framework for understanding and predicting reactions to health threats, it has to be recognised that it is a rational, cognitive model which pays insufficient attention to emotional mechanisms. The importance of emotions in intentions to take a genetic test is illustrated in a study by Wroe et al.; they found that emotional reasons were reported more frequently by people who had already actively thought about having a specific genetic test than by students for whom the test was hypothetical. Audrain et al. and Evers-Kiebooms et al. also stress the role of emotions in decisions about genetic testing. An example of an emotional factor that could have played a mediating role in not wanting a predictive test for HBC is self-blame or guilt about passing the disease onto the children. This might have especially been the case in our sample of students in Family Sciences, for whom family relations are likely to be of particular interest. Indirect support for this possibility stems from the logistic regression analyses on the total sample and on the No BC group; a higher number of children was significantly associated with higher odds of not wanting the test. However, this kind of independent association remained absent in the BC group.

An alternative explanation for the reaction of the BC group in our study against the predictive test is a more rational one. The experience with breast cancer in the family might have led to more awareness concerning the lack of effective preventive and/or therapeutic measures for the disease than in the No BC group, thereby reducing the perceived benefits of having a predictive test for HBC and/or increasing the perceived barriers or costs in the BC group. As such, the uninterest in the predictive test among the women in the BC group might have been the result of a rational decision based on the weighing up of their perception of costs and benefits. A similar systematic and rational process in the No BC group, with a different perception of barriers and benefits owing to the absence of familial experience with breast cancer, might have resulted in interest in the test. The differential perception of barriers and benefits of the predictive test for HBC according to the occurrence of breast cancer in the family is indeed supported by our findings: while the No BC group judged the benefits as more important than the barriers (a pattern that also emerged in previous studies), the dominance of benefits disappeared in the BC group. On the other hand, as far as the explanatory value of these two Health Belief Model components is concerned, the results of the logistic regression analyses did not differ according to the occurrence of breast cancer in the family. In the BC group as well as in the No BC group, the perceived benefits were the first to enter the regression, while the perceived barriers remained absent. The failure of the perceived barriers component to enter the logistic regression can partly be explained by its substantial correlation with the other components of the Health Belief Model (table 3). However, this also suggests that the intentions in the No-BC group as well as in the BC group, despite the equivalence in the importance ratings of benefits and barriers in the latter group, were disproportionally affected by the perception of the benefits of the predictive test for HBC. A similar result was obtained by Lerman et al. in their multivariate analysis of BRCA1 test use. This underlines that providing comprehensive information on the complexities, the limitations, and the uncertainties involved in predictive testing for HBC (as well as on its benefits) is crucial for genetic counselling as well as public education purposes. Moreover, research has indicated that the intentions regarding genetic testing are not only influenced by the content of the information that is given, but also by the focus of attention. Therefore, it is important to encourage and help people to consider all aspects of the test and all of its possible consequences, negative as well as positive ones. In a genetic counselling context, it was furthermore

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shown by Lerman et al.\(^1\) that a personalised approach extending beyond the standardised provision of information and giving room to the discussion of emotions can modify the evaluation of benefits and costs of the predictive test for HBC, perhaps through the more thorough processing of the information provided. Indeed, genetic counsellors should be sensitive to interpersonal differences in emotional and informational needs and priorities as well as in the diversity of reasons for predictive test requests.\(^2\) For instance, older women might be more concerned about their children's risks or about age related health problems than about their own genetic susceptibility for breast cancer. It is also found that older women in the BC group tend to be more reluctant about a predictive test for HBC for themselves is illustrative in this context. General concerns about the development of a predictive test for HBC as measured by the general attitude score seem not to be a priority in the BC group. Only for women who have no relatives with breast cancer does such a general measure play a role in their intention to have a test. When breast cancer does occur in the family, the influence of the general attitude score seems to be outweighed by the more personalised and instrumental measure concerning the perceived benefits of the predictive test. A similar finding concerning the influence of general attitudes towards health and medical testing has been reported by Shiloh et al.\(^3\)

Overall, the results of our study show that the perceived benefits component is the most powerful Health Belief Model component in explaining interest in the predictive test for HBC. However, the absence of an independent association with perceived seriousness, perceived susceptibility, and perceived barriers does not mean that these factors are not important. They can influence other variables in the chain of the cognitive-emotional decision making process about the predictive test for HBC. Awareness of the possibility of their mediating role, as well as of the potential influence of emotions and/or more general concerns about the applications of the new genetic technology, is important for health care providers at different levels: at the level of genetic counseling, to facilitate free and informed decisions about predictive testing for HBC and at the level of community and clinical education programmes, to design targeted information addressing the specific needs and concerns of women at different risk levels. Although past experience with predictive testing for Huntington's disease\(^4\) and with carrier testing for cystic fibrosis\(^5\) has shown that reported interest in genetic testing cannot be equated with actual uptake, the results of our study and previous research on the predictive test for HBC are valuable in helping to reach these goals.

Ocular malformations, postaxial polydactyly, and delayed intramembranous ossification: a new autosomal dominant condition

Donna M Martin, Jerome L Gorski

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Case reports

CASE 1

A 5 month old boy was referred for evaluation of multiple congenital anomalies including skeletal and unilateral ocular anomalies. He was born weighing 4167 g at 39 weeks' gestation to a 27 year old, gravida 2, para 1 mother. His father was 32 years old. No intrauterine exposure to alcohol or other teratogens was reported. He was diagnosed in the immediate postnatal period with a markedly enlarged right globe, opacification of the cornea and lens, and bilateral postaxial polydactyly. At 1 day of age, increased anterior chamber pressure was noted, and he underwent surgery for congenital glaucoma at the age of 1 week with revision at 1 month. Repeated examinations of the left eye showed no increased pressure and no abnormalities of the lens, iris, or retina. Within the first month of life, he underwent bilateral ligation of the supernumerary digits.

Physical examination at the age of 5 months showed growth parameters on the 50th centile:
weight 7.34 kg, length 66.5 cm, and OFC 44.1 cm. Cranial examination was remarkable for a large 7 × 7 cm anterior fontanelle. The posterior fontanelle and a third fontanelle were open at 1 × 1 cm. The right globe was much larger than the left, with opacity of the right cornea and lens. Facial features were notable for a high, prominent forehead, shallow nasal bridge, downward slanting palpebral fissures, bilateral epicanthic folds, and mild micrognathia. Ear helices were slightly overfolded and prominent. No dental hypoplasia or enamel defects were noted. A left accessory nipple was present. The clavicles were normal with no hypoplasia. Examination of the extremities showed no distal digital or radial abnormalities. Chromosome analysis of peripheral blood leucocytes showed a normal 46,XY karyotype. Re-evaluation at 18 months showed unchanged ocular and facial features (fig 1A). His parents were non-consanguineous; a family history of ocular and skeletal anomalies was present in his father (case 2) and brother (case 3).

CASE 2
The 31 year old father of case 1, also examined in our clinic, had a past medical history notable only for ocular and skeletal abnormalities. He was noted in infancy to have left sided scleralisation of the cornea, microphthalmia, and eccentric placement of the pupil. Ophthalmological evaluation confirmed these findings and showed left sided staphylomatous retinal coloboma and microcornea. He had a history of delayed anterior fontanelle closure in early childhood, but no palpable cranial abnormalities or persistence of the fontanelles on physical examination. Mild facial dysmorphism was present, including a prominent nasal bridge, downward slanting palpebral fissures, mild bilateral ptosis, and micrognathia (fig 1B). Dentition was normal. His ears were normally placed, with overfolded helices and a preauricular tag in the right pretragal region. The clavicles and chest were normal. A small scar at the lateral aspect of the left fifth digit was noted, consistent with surgical ligation of non-osseous postaxial polydactyly. He also had broad fingertips, prominent finger pads, and shallow nails, with no evidence of radial abnormalities or distal digital hypoplasia.

CASE 3
The proband’s 2½ year old brother was born by normal vaginal birth, weighing 3884 g, at 39 weeks’ gestation to a 25 year old primigravida, whose pregnancy was uncomplicated by exposure or illnesses. Postaxial polydactyly of the left hand was noted at delivery and treated by ligation. He also had left internal tibial torsion, left talipes equinovarus, and mild metatarsus adductus of the left lower extremity requiring surgical repair at 5 months, followed by immobilisation. Other medical problems developed in the first two years, including admission to hospital for bronchodilator treatment for reactive airway disease. Bilateral tympanostomy tube placement and right inguinal herniorrhaphy were performed at 15 and 17 months, respectively. At 18 months, persistence of the anterior fontanelle was noted with normal thyroid function tests. Repeated auditory examinations, growth, and development were normal.

Examination in our clinic at the age of 2½ years showed normal growth parameters on the 50th-75th centile: weight 13.5 kg, height 94.6 cm, and OFC 50 cm. His anterior fontanelle was open 2 × 2 cm, with a palpable third fontanelle. The posterior fontanelle was closed. Mild facial dysmorphism was noted, including prominence of the nasal bridge, downward slanting palpebral fissures, and bilateral epicanthic folds (fig 1C). Dentition was normal. No clavicle abnormalities were noted. He had an accessory nipple over the abdomen in the left nipple line. Examination of the extremities showed surgical scars over the lateral aspect of

Figure 1 Photograph of (A) the proband at 18 months, showing right sided corneal clouding, downward slanting palpebral fissures, and mild micrognathia, (B) the 31 year old father, showing left corneal clouding and microphthalmia, downward slanting palpebral fissures, and mild micrognathia, and (C) the 2½ year old brother, showing facial features including downward slanting palpebral fissures and prominent nasal bridge.
the left fifth digit at the first metacarpophalangeal joint and over the lateral aspect of the left foot. The left lower extremity exhibited marked internal tibial torsion, with mild metatarsus adductus. The fingernails were mildly short, but no digital hypoplasia or radial anomalies were present. Physical examination was otherwise normal. Skull radiograph confirmed persistence of the anterior fontanelle and no wormian bones or abnormal falcine calcification (fig 2). Skeletal survey showed no shortened or abnormally formed bony elements. The family pedigree indicated ocular and skeletal anomalies in the patient’s father and 5 month old brother (fig 3). By history, no other family members were affected.

Discussion

We describe a family of three affected males with microphthalmia and related ocular abnormalities, postaxial polydactyly, delayed intramembranous ossification of the skull, minor facial dysmorphic features, and accessory nipples. Characterisation of the ocular abnormalities showed anterior and posterior ocular defects, varying from unilateral congenital glaucoma in the 5 month old boy to unilateral microcornea, retinal coloboma, and microphthalmia in the father. Skeletal abnormalities included non-osseous postaxial polydactyly of the upper extremities and delayed fontanelle closure in all three affected males, and left sided talipes equinovarus and tibial torsion in the 2½ year old boy. Growth and development were normal, and karyotype analysis showed normal chromosomes. This unique collection of features appears to be segregating in the family by male to male transmission, suggesting autosomal dominant inheritance.

The eye defects in this family include anterior segment anomalies (glaucoma and cataracts), posterior segment defects (retinal coloboma), and a combination of both (microphthalmia). Several different embryological germ layers contribute to the development of anterior and posterior ocular structures. Neural crest cells and ectoderm form the anterior chamber and lens placode, respectively, whereas neuroectoderm gives rise to the optic vesicles and, along with mesoderm, participates in optic fissure closure. The embryological heterogeneity of congenital ocular defects is thought to contribute to the wide variability in phenotype commonly seen among subjects within the same family. The absence of ocular defects in the 2½ year old boy suggests incomplete penetrance, and also raises the possibility that the eye and skeletal defects may be segregating independently. It is interesting to note that both the father and his 5 month old son exhibited unilateral ocular defects. In one recent study, unilateral defects were present in 40% (52 of 131) patients with anophthalmia or microphthalmia. The same study also found that bilateral and unilateral ocular malformations are similarly associated with other congenital anomalies, making laterality an unhelpful predictor of other organ system defects.

In addition to ocular defects, affected family members uniformly had upper extremity non-osseous postaxial polydactyly and the 2½ year old boy had unilateral talipes equinovarus and tibial torsion. Polydactyly is a common congenital anomaly of endochondral bone formation that occurs in isolation or with syndromes and chromosomal abnormalities. Normal karyotype studies in this family ruled out an inherited chromosomal rearrangement. A recent study of associated anomalies in 5927 subjects with polydactyly indicated that postaxial polydactyly is significantly associated with syndactyly and negatively associated with limb deficiencies and deformities. The same study...
also concluded that polydactylies are rarely associated with other congenital anomalies except in recognisable syndromes.

Comparison of the clinical features present in this family showed minimal overlap with six other known genetic conditions, particularly in the type and extent of ocular and distal limb anomalies; however, each of these previously recognised conditions included other major diagnostic features not present in this family (table 1). Of the autosomal dominant conditions associated with microphthalmia or related ocular malformations and distal limb anomalies, none is reported to have delayed intramembranous ossification, a prominent finding in this family. In acroreno-ocular syndrome (OMIM 102490), distal limb defects are typically preaxial and renal abnormalities are prominent.1 4 Apical dystrophy (OMIM 120400), or coloboma of the macula with type B brachydactyly, involves absence of the distal phalanges, different from the non-osseous polydactyly present in this family.5 14 Although oculodentodigital dysplasia (ODD, OMIM 164200) shares several features with this family, including microphthalmia, microcornea, glaucoma, and minor postaxial digital anomalies, the typical dental abnormalities of ODD (hypoplastic enamel and small teeth) were absent in this family, and the family had features not seen in ODD, including accessory nipples and delayed intramembranous ossification.11 15 Some cases of Pallister-Hall syndrome (PHS, OMIM 146510) have microphthalmia and postaxial polydactyly, but the major features of PHS (hypothalamic hamartoblastoma, hypopituitarism, and imperforate anus) were not present in this family.11 14 Kenny-Caffey syndrome (OMIM 127000), characterised by dwarfism, cortical thickening of tubular bones, and transient hypocalcaemia, has been reported with delayed intramembranous ossification and hyperopia, but does not include distal limb anomalies.15 16 The distinctive facial features and micrognathia present in acrofacial dysostosis type 1 (OMIM 154400) were absent in the family.17

Delayed intramembranous ossification may be clinically under-reported, since it resolves with age and often causes no serious medical problems; however, the lack of syndromic associations with this condition are striking. Delayed anterior fontanelle closure results from abnormalities in intramembranous ossification of the frontal and parietal bones of the calvaria. Delayed fontanelle closure can also, like ocular and distal limb defects, occur in isolation or as part of other conditions. Skeletal survey in the 2½ year old male showed normal mineralisation and no bony anomalies to suggest underlying metabolic disease. Delayed intramembranous ossification has been reported in one family with a familial translocation (2;3)(p15;q12).18 19 Delayed intramembranous ossification also occurs in cleidocranial dysplasia, a dominantly inherited skeletal dysplasia characterised by hypoplastic clavicles, large fontanelles, dental anomalies, and delayed skeletal development.20 The absence of clavicle and dental anomalies in this family makes this diagnosis unlikely.

Based on this comparison with other genetic conditions (table 1), we conclude that this family is segregating a trait that adversely affects the eye and distal limb strongly suggests that this family is segregating a newly recognised condition.

### Table 1 Comparison of autosomal dominant conditions with ocular and skeletal anomalies

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Present cases</th>
<th>Acroreno-ocular syndrome</th>
<th>Macular coloboma with type B brachydactyly</th>
<th>Oculodentodigital dysplasia</th>
<th>Pallister-Hall syndrome</th>
<th>Kenny-Caffey syndrome</th>
<th>Acrofacial dysostosis type 1, Nager type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microphthalmia or coloboma</td>
<td>2/3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Hyperopia</td>
<td>-</td>
</tr>
<tr>
<td>Postaxial polydactyly</td>
<td>2/3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Delayed intramembranous ossification</td>
<td>3/3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Accessory nipples</td>
<td>2/3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Talipes equinovarus</td>
<td>1/3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Thumb and radial defects</td>
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<td>+</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>Renal anomalies</td>
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</tr>
<tr>
<td>Other</td>
<td>0/3</td>
<td>*</td>
<td>†</td>
<td>‡</td>
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*Preaxial polydactyly.
†Deafness, upper extremity 4, 5 syndactyly.
‡Preaxial polydactyly, upper extremity 4, 5 syndactyly, dental hypoplasia, spastic paraparesis, hypotelorism.
§Hypocalcaemia, hypophosphataemia, short stature, macrocephaly, micro-orchidism.
**Distinctive facies, micrognathia.

- Ocular and skeletal malformations often occur together as part of multiple anomaly syndromes.
- We present a father and two sons with a constellation of anomalies including microphthalmia and related ocular malformations, delayed intramembranous ossification, and postaxial polydactyly. Clinical evaluation showed no chromosomal rearrangements or metabolic disorders. The physical features displayed in affected family members were compared to the clinical features of known inherited conditions associated with ocular, limb, and skeletal malformations.
- The male to male transmission in this family suggests that this multiple congenital anomaly syndrome has autosomal dominant inheritance. A comparison of the clinical features segregating in this family to features comprising autosomal dominant conditions affecting the eye and distal limb strongly suggests that this family is segregating a newly recognised condition.
Recessively inherited lower incisor hypodontia

Sinikka Pirinen, Anu Kentala, Pekka Nieminen, Teppo Varilo, Irma Theesfeld, Sirpa Arte

Hypodontia, congenitally missing teeth, is common in modern man. The teeth most often missing in populations of European origin are the upper lateral incisors and second premolars. The condition is known to have a strong genetic component. Among present two located genes in humans, MSXI and PAX9, are known to cause missing permanent teeth. Mutations in MSXI also are known to cause the condition. Several experimental and clinical studies indicate that other genetic components are involved. Hypodontia is also often seen in syndromes, particularly in those which present with other ectodermal abnormalities, and in non-syndromic patients with cleft lip/palate. The population prevalence of the common incisor-premolar hypodontia (IPH, MIM 106600) is 8-10% in healthy European children. Some or all third molar teeth are missing in one-fifth of the population, and missing third molars are seen in varying combinations in IPH patients and/or family members. Family studies also indicate that peg shaped upper lateral incisors, impacted canines, rotated bicuspids, and short root anomalies are caused by the same genetic components that cause missing incisors and premolars. The condition is inherited as an autosomal dominant trait with reduced penetrance and is mostly restricted to the permanent dentition. When a large number of teeth (>6) are congenitally missing, the term used is oligodontia (MIM 6044625). The prevalence of oligodontia in European populations is estimated at 0.08%, but this figure also includes syndromic patients. We describe 37 Finnish families from 34 families with several lower incisors and upper lateral permanent incisors congenitally missing. In half of the patients, the corresponding deciduous teeth had either been missing or peg
An atopic condition had been diagnosed in two thirds of the patients. Occurrence of the trait within the families followed an autosomal recessive mode of inheritance. We have called the condition Recessive Incisor Hypodontia (RIH) and suggest that it belongs to the Finnish Disease Heritage, the enrichment of some 40 rare disorders.

**Methods**

Ten patients with missing lower and upper lateral incisors were first seen at the Hypodontia Unit of the Department of Pedodontics and Orthodontics, University of Helsinki over the past five years. Their parents did not display similar hypodontia, but sometimes had one congenitally missing permanent tooth. In two families, the condition was seen in sibs. In order to discover the prevalence of this condition in Finland, a questionnaire was sent to all active orthodontists and municipal health centres in the country (362 letters). We received radiographs of 220 patients and 65 patients selected from these were further studied after giving their consent.

Inclusion criteria were three or four congenitally missing lower incisors, at least two congenitally missing or peg shaped lower permanent incisors together with missing upper lateral incisors, preceded by agenesis of at least one lower deciduous incisor, and a pedigree consistent with an autosomal recessive mode of inheritance. Exclusion of obvious dominant oligodontia, anhidrotic ectodermal dysplasia (EDA, also carriers), and incontinentia pigmenti (IP) was attempted by careful clinical examination, anamnestic information, and pedigree analysis. The final sample consisted of 37 patients from 34 families. Three pairs of sibs were included (fig 1).

The patients, parents, some grandparents, and sibs were examined radiographically and interviewed, and facial and oral photographs were taken. Alginate impressions were made of all cooperative patients. Dental age was calculated from radiographs by the age medians for tooth formation and standard deviations (SD) for dental ages.

Parents and sibs showing dental anomalies such as hypodontia of one or two teeth, peg shaped teeth, retained cuspids or taurodontic teeth are shown in fig 1. The ancestors were traced back to 1850 from local church registries using the names, dates, and birth places of parents. Microfilm copies in The National Archives of Finland were used for earlier periods. To analyse the mode of inheritance, the ratio of affected to healthy sibs was corrected for the absence of healthy sibships born to two heterozygous parents. For DNA analysis, samples of venous blood were taken from patients, grandparents, parents, and sibs. The study was approved by the Ethics Committee of the Institute of Dentistry, University of Helsinki.

**Results**

All 37 patients were under 22. Their clinical characteristics are presented in table 1 with typical panoramic radiographs in fig 2. Nine of the patients had no permanent lower incisors, two of them also lacked the corresponding deciduous teeth. In seven, the upper permanent lateral incisors were also missing and in five the deciduous upper lateral incisors as well. In one, the upper central incisors were peg shaped with an anomalous structure (fig 2B). In four patients, three missing lower permanent incisors were evident. Of these 13 severely...
affected patients, 10 were boys. The remaining 24 had a varying combination of missing or peg shaped lower and upper permanent and deciduous incisors. A deciduous tooth/teeth was missing in 19/36 (53%). In addition to incisors, other permanent teeth were also missing (fig 3). Mean dental age based on tooth formation was slightly delayed (SDS 0.8). Taurodontism was noted in the molar teeth of 16/26 patients (62%).

Photographs of the younger patients are shown in fig 4. Hair, nails, eyelashes, eyebrows, and perspiration were normal. Heights and weights were also normal for age. A large proportion of the patients reported allergies (62%), such as atopic skin (52%) and asthma (43%), diagnosed by a doctor.

The male/female ratio of the patients was 19/18 (1.05). The proportion of affected sibs was calculated from 30 sibships with 76 children. The apparent proportion of affected sibs was 0.43. After mathematical correction for the absence of healthy sibships born of two heterozygous parents, using the a priori correction of truncate complete ascertainment, the proportion was 0.22. Pedigrees of 31 families were traced back at least to the fifth generation, and, when possible, to the late 17th century (10 generations). In two families, the parents of the proband had a common ancestor six and seven generations back (fig 5), but no other family linkages between the families were found. A map of Finland, where the birth places of the great grandparents of the patients are marked, is shown in fig 6. In the case of 23/31 index cases, the maternal and paternal ancestors originated from the same rural area.

Minor dental anomalies in the form of a missing upper lateral incisor or a missing third molar was seen in 41% of the parents and in 27% of the healthy sibs (fig 1). Both parents and sibs reported allergies (46% and 40% respectively) and skin problems (46% and 35% respectively). Ten of the 29 examined mothers (34%), eleven of the 22 fathers (50%), six of the 22 healthy sisters (27%), and six of the 20 healthy brothers (30%) are shown in the pedigrees on the basis of their minor dental anomaly (fig 1).

Discussion
Our attempt at a nationwide ascertainment resulted in a response to more than half of our questionnaires, but because the oldest patient was only 22, it is likely that we only found a proportion of the total number of RIH patients in Finland. By using strict criteria in an effort not to overdiagnose, some of the cases reported to us and some of the mildly affected sibs were perhaps misclassified.
There are many published reports of conditions with missing lower incisors. In Japan the prevalence of tooth agenesis is of the same order as in Europeans, but the lower lateral incisor is the most commonly missing tooth.\(^\text{21}\) Witkop syndrome is an autosomal dominant condition with missing lower incisors and dysmorphic nails.\(^\text{22}\) In a patient from Minnesota,\(^\text{23}\) dentition and a face very similar to that of our patients can be seen. First cousins of Egyptian origin, born of consanguineous marriages, with absent or conical lower deciduous incisors and thin hair and finger nails, with cleft palate in one and a branchial cyst in the other, were described by Fried,\(^\text{24}\) who suggested autosomal recessive inheritance. A similar patient from Turkey, a child of first cousins, has also been described.\(^\text{25}\) The Norwegian sibs reported\(^\text{26}\) could also well have the autosomal recessive condition described here. A brother and sister from Lebanon with only a few permanent teeth and thin nails were recently reported\(^\text{27}\) with the suggestion that they may have the condition described by Fried.\(^\text{24}\) The pattern of missing teeth, including deciduous teeth, can also be similar in incontinentia pigmenti\(^\text{28}\) and in Kabuki syndrome.\(^\text{29}\)

Congenitally missing deciduous teeth, as seen in 53% of the present patients, is not common in IPH. The prevalence figures are commonly close to 0.5%\(^\text{30–32}\) and, interestingly, are higher (0.9%) in Finland.\(^\text{33}\) All these figures may also include children with syndromic hypodontia or oligodontia. The genetics of hypodontia in the deciduous dentition has not been systematically studied, but has been assumed to be a symptom of IPH, as the corresponding permanent tooth is also usually missing. Detailed descriptions of the distribution of missing teeth in hypodontia patients are rare. However, in two Scandinavian studies, the pattern of missing lower and upper lateral incisors typical of RIH is evident.\(^\text{16,34}\) This also suggests that the condition also exists in other populations but has perhaps been overlooked because of overlapping symptoms with IPH. Retarded dental development and short rooted (taurodontic) molar tooth form are characteristic of hypodontia and oligodontia and were clearly seen in the condition described here.

The number of cases of atopic diseases, commonly seen in our patients and also reported by the family members, exceeds the reported population prevalences\(^\text{35}\) and is an interesting finding in the present context. Of the similar published conditions, only the Norwegian report mentions asthma as a symptom of the patient. Recently, a distinct anhidrotic ectodermal syndrome with missing teeth and immunological abnormalities, EDA-ID, has been described. The condition seems to be caused by impaired NF-kB signalling.\(^\text{36}\)

In recessively inherited conditions heterozygous manifestations may occur. Here, minor dental anomalies were noted in less than half of
recessive mode of inheritance, characterised by missing deciduous and permanent incisors, and an increased inclination to eczema and asthma. We have named the condition Recessive Incisor Hypodontia (RIH). The patients resemble reported patients from consanguinous marriages from various parts of the world. Attempts to clarify the molecular pathology of this condition are at present being carried out and the results will be of interest in developmental studies and in the study of molecular mechanisms of atopic diseases.

We warmly thank our patients and the numerous families for making this study possible. We thank dentists throughout Finland, in particular Professor Satu Alaluusua for informing us about patients with missing lower incisors. We thank Professors Reijo Norio and Leena Palotie-Peltonen for valuable advice and support. The study was supported by the Foundation of Paediatric Research, the Ulla Hjelt Fund, and the Academy of Finland.

Table 1 Candidate loci for congenital diaphragmatic hernia

<table>
<thead>
<tr>
<th>Locus</th>
<th>Reported anomaly</th>
<th>Other phenotypic features</th>
<th>Reference</th>
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<tr>
<td>1q</td>
<td>Del(1)(q32q42)de novo</td>
<td>Low set ears, neck webbing, hypospadias, undescended testes</td>
<td>17, 26</td>
</tr>
<tr>
<td>3q</td>
<td>Mosaic del(3)(q11.1q13.2)de novo</td>
<td>Bilateral euvchomenous</td>
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<tr>
<td>4p</td>
<td>Del(4)(p16)de novo</td>
<td>Wolf-Hirschhorn syndrome, Dandy-Walker malformation, AVSD</td>
<td>6, 66</td>
</tr>
<tr>
<td>8p</td>
<td>Del(8)(p23.1)de novo</td>
<td>Facial dysmorphism, Delichocephy</td>
<td>18</td>
</tr>
<tr>
<td>8q</td>
<td>t(8;13)(q22.3q22)mat</td>
<td>Tricuspid stenosis, T21q32 mosaic</td>
<td>7</td>
</tr>
<tr>
<td>12p</td>
<td>Mosaic tetrasomy 12p</td>
<td>No</td>
<td>7</td>
</tr>
<tr>
<td>15q</td>
<td>t(5;15)(p15;q21.3)mat</td>
<td>Facial dysmorphism,Palster-Tillian syndrome</td>
<td>19, 20</td>
</tr>
<tr>
<td></td>
<td>t(15;17)(q24.3;q23.3)mat</td>
<td>Balanced</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>del(15)(q24qter)</td>
<td>No</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>t(1;5)(q14;q21.2)de novo</td>
<td>Fallot's tetralogy</td>
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Editor—Congenital diaphragmatic hernia (CDH) is seen in 1/2000 to 1/5000 fetuses and liveborn infants.\(^1\)\(^2\) Around 60% of fetuses diagnosed by antenatal ultrasound scanning at 20 weeks’ gestation die in utero and the mortality rate in those surviving to term remains 30-50%. Coexistent major structural malformations are seen in a large proportion of cases, the commonest in liveborn infants being cardiac anomalies and neural tube defects.\(^3\) The genetic contribution to the aetiology of CDH is poorly understood. Although no large scale, population based, offspring recurrence study exists, familial clustering of CDH has been attributed to polygenic inheritance, which predicts an offspring recurrence risk of 1-2%. Familial congenital diaphragmatic hernia is, however, well described with autosomal dominant inheritance in most reported families, although no linkage studies have been performed. Candidate genes may therefore be localised by studying the large proportion of patients with CDH and an underlying chromosome abnormality. Autosomal trisomies, typically of chromosomes 13, 18, and 21, account for many of these cases. More complex structural rearrangements have also been reported in some series, although many of these cases have additional organ malformations.\(^4\)\(^5\) However, a number of de novo structural anomalies associated with CDH have been documented, defining candidate loci for future study; these are summarised in table 1.

Case report

We report a male infant with CDH associated with a proximal deletion of the long arm of chromosome 3 in mosaic form. Clinical genetic evaluation was sought during the third pregnancy of a 29 year old female and a 32 year old male. She had previously had two healthy children and there was no history of pregnancy loss. Fetal ultrasound examination at 22 weeks’ gestation showed a large, left sided diaphragmatic hernia with mediastinal shift but no hydrops. No other structural abnormality was seen. A placental biopsy was taken for cytogenetic analysis. Both direct and long term preparations showed an abnormal mosaic male karyotype with an additional, unidentified small chromosome in approximately 50% of cells examined. Analysis of cultured amnioocytes confirmed the marker chromosome in 50% of cells. Fluorescence in situ hybridisation (FISH) studies indicated that the marker was derived from the centromeric region of chromosome 3 (fig 1A) and contained euchromatic material. The karyotype was assigned as...
47,XY,+mar.ish der(3)(D3Z1+)/46,XY and was interpreted as mosaic partial trisomy 3. Polyhydramnios developed from 30 weeks' gestation but remained stable with no hydrops. A male infant was delivered at term by elective caesarian section for breech presentation. He required ventilation from birth. A chest x-ray confirmed a large, left sided congenital diaphragmatic hernia with extreme pulmonary hypoplasia. Echocardiography showed a grossly dilated right ventricle with high right sided pressures creating a right-left shunt through a patent ductus arteriosus. Surgical repair of the diaphragmatic hernia could not be contemplated at any stage owing to the child’s unstable clinical state. Despite full supportive treatment, his condition deteriorated and he died on his second day of life following withdrawal of intensive care. Necropsy showed a non-dysmorphic male infant with the following growth parameters: head circumference 36.5 cm (75th-91st centile), height 53 cm (75th centile), and weight 4200 g (91st centile). The right hemidiaphragm was present and the right lung was trilobed, hypoplastic, and compressed. The left hemidiaphragm was absent and the chest contained a diaphragmatic hernia consisting of stomach, pancreas, duodenum, small bowel, a large segment of colon, the left lobe of the liver, and the spleen. The left lung was twisted on its pedicle and was extremely hypoplastic. Cardiac situs was normal but the mediastinal contents were displaced to the right. Cardiac anatomy was normal and there was a wide patent ductus arteriosus. Gross brain anatomy was normal; histological examination showed evidence of early global hypoxic/ischaemic damage and mild brain stem atrophy around an enlarged cerebral aqueduct. There were no other significant histological findings.

Postnatal cytogenetic analysis of peripheral lymphocytes enabled further characterisation of the abnormal mosaic karyotype detected antenatally. Two cell populations were present in approximately equal proportions. The first contained 46 chromosomes with an interstitial deletion of a proximal segment of the long arm of chromosome 3 extending into the subcentromeric region and resulting in monosomy of 3q11.1 to 3q13.2. The second cell population contained 47 chromosomes with the same deleted chromosome 3 and a small additional ring chromosome identical to the marker chromosome detected antenatally and composed of pericentromeric chromosome 3 material (fig 1B). The karyotype was reassigned as 46,XY,del(3)(q11.1q13.2)/47,XY,del(3)(q11.2q13.2),+r(3). Parental karyotypes were normal.

**Discussion**

The proximal breakpoint for the deletion reported here was located within the centromeric region of chromosome 3, to which the FISH probe D3Z1 hybridises, and the deleted segment contained both euchromatin and heterochromatin. It is likely, therefore, that the ring chromosome present in 50% of this infant’s lymphocytes was composed of deleted material from proximal 3q and the cell population containing this ring was therefore effectively “balanced”. The second cell population was haploinsufficient for the chromosomal

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Figure 1 (A) Image of FISH studies performed on cultured amniocytes showing hybridisation of the probe D3Z1 to the centromeric regions of both chromosomes 3 and the marker chromosome (arrows). (B) Partial karyotype from a chromosomally “balanced” postnatal peripheral lymphocyte showing both an intact and deleted chromosome 3 and a ring chromosome derived from the deleted segment.
region 3q11.1-3q13.2. In both cell populations, disruption of a gene at the distal breakpoint cannot be excluded.

Reports of interstitial deletion of the long arm of chromosome 3 are rare, with deletions involving band 2 being more commonly reported. The small number of case reports detail variable multiple congenital abnormalities and neurodevelopmental delay. Deletions involving band 3q23 are, however, associated with features of the blepharophimosis–ptosis–epicanthus inversus syndrome (BPES), an autosomal dominant disorder which maps to this region.1–15 Few subjects with proximal 3q deletions involving band 1 have been described; of those published, none had CDH and, although no clear phenotype has emerged, a small number share features such as absent corpus callosum, hypotonia, and severe neurodevelopmental delay.

The deletion in our patient is just proximal to that found in a fetus reported by Wolstenholme et al.10 The fetus was ascertained antenatally following detection of a large diaphragmatic hernia on ultrasound scanning. Fetal necropsy confirmed a left sided diaphragmatic hernia and showed facial features consistent with BPES. The fetal karyotype showed an interstitial deletion of the long arm of chromosome 3: 46,XY,del(3)(q21q23). This mapped the BPES locus more accurately to 3q23 but the significance of the diaphragmatic hernia was unclear. The finding of diaphragmatic hernias in both cases is therefore of interest, although difficult to explain since the deletion intervals do not appear to overlap. A gene involved in the development of the diaphragm may map to proximal 3q and may, for example, be susceptible to position effect.

We propose that the congenital diaphragmatic hernia in our case is a direct consequence of mosaic proximal 3q deletion.

A further locus on distal 3q is suggested by the occurrence of CDH in severe de Lange syndrome, whose critical region appears to lie at 3q26.3, and the similarity between mild de Lange syndrome and the dup(3q) syndrome.16–20 However, CDH has not been reported in dup(3q) syndrome and the relationship between these syndromes has yet to be determined.

Proximal 3q and the loci detailed in table 1 therefore provide a focus for future molecular genetic studies of familial CDH. This case also clearly underlines the importance of postnatal confirmation of all karyotypic abnormalities detected antenatally.

The authors wish to thank Dr S Vadeyar in the Department of Fetalmaternal Medicine, Queen’s Medical Centre, Nottingham for referring this case. Dr J Grant, Department of Neonatal Intensive Care, was responsible for postnatal supportive care. Necropsy was performed by Dr C J H Padfield in the Department of Pathology, Queen’s Medical Centre, Nottingham. We thank Dr R Osrart, Mr A Wilkinson, and Mrs C Cooper from the Prenatal Division, Cytogenetics Service, Nottingham City Hospital for their prenatal diagnostic work and for providing illustrations for this report.

A supernumerary marker chromosome with a neocentromere derived from 5p14→pter

Barbara Fritz, Ilona Dietze, Annelise Wandall, Müchever Aslan, Angela Schmidt, Evelyn Kattner, Robin Schwerdtfeger, Ursula Friedrich

EDITOR—Supernumerary marker chromosomes (SMCs) comprise a heterogeneous group of structurally arranged chromosomes. SMCs are found in approximately 0.14–0.72/1000 newborns and they may be associated with developmental abnormalities and malformations. The great variability of clinical symptoms in patients with SMCs is the result of the difference in the genetic content of the marker. The phenotypic consequences of SMCs are difficult to predict, especially if a de novo marker is detected prenatally. Therefore, the precise identification of a marker chromosome is of essential importance in genetic counselling. Earlier figures based on the results of a large prenatal multicentre study using conventional methods suggested a risk for an abnormal phenotype of 13% for SMCs. Combining FISH data and conventional analyses, the estimated risk for an abnormal phenotype turned out to be twice as high, approximately 28% for nonacrocentric autosomal SMCs and about 7% for acrocentric autosomal SMCs. An even more accurate method of identification of the chromosomal origin of supernumerary marker chromosomes is FISH with microdissection probes and reverse painting, allowing a definite delineation of phenotype-karyotype correlations.

In recent years, a novel class of mitotically stable human marker chromosomes that are devoid of alpha satellite DNA has been identified. These alphoid markers have been shown to contain functional centromeres outside the normal centromere domain, which are called neocentromeres. Marker chromosomes derived from chromosome 5 are rare and a marker chromosome 5 with a neocentromere has not been reported so far.

We describe a patient with an inverted duplication of the distal part of the short arm of chromosome 5 and the formation of a neocentromere leading to a supernumerary marker chromosome. The comparison of the clinical findings of this patient with a tetrasomy of distal 5p with similar cases previously described suggests a gene dosage effect of this chromosome segment. Absence of centromeric specific sequences in the marker and a weak reaction with anticientromere antibodies indicates the formation of a neocentromere between bands 5p14 and 5p15 and therefore the first alphoid SMC described that originates from chromosome 5.

Case report

This girl is the first child of healthy, non-consanguineous parents. The mother was 21 and the father 25 years old at her birth. Prenatal ultrasound showed intrauterine growth retardation with an abdominal circumference below the mean. The fetus showed severe microretrognathia (fig 1) and unilateral foot deformity on ultrasound. A transabdominal chorionic villous biopsy, performed at 25 weeks of gestation, showed an aberrant 47,XX,+?C karyotype in three metaphases analysed from direct villi preparations. G banded chromosome preparations from an amniocyte sample showed an unbalanced female karyotype in the fetus with 47,XX,+mar. The supernumerary marker was detected in all 19 metaphases analysed. Normal parental karyotypes indicated a de novo origin of the marker in the fetus.

The parents decided to continue the pregnancy. Delivery was induced at gestational week 37 (+5) because of cessation of intrauterine growth. The girl weighed 1890 g (<3rd centile) and was 43 cm long (3rd centile). The head circumference was 31.5 cm (3rd centile). The Apgar score was 1/6/7. She had respiratory difficulties and was ventilated for four days. She had microretrognathia and a cleft hard and soft palate (Pierre-Robin anomaly). Spontaneous motor activity was markedly reduced with generalised muscular hypotonia initially. Muscle tone, however, turned out to be unstable and progressed gradually to hypertonia. She developed contractures of her fingers, elbows, and feet.

At the age of 4½ months, the girl was 58 cm long, weighed 3700 g, and OFC was 37.5 cm. All values were below the 3rd centile. The girl was severely dystrophic and continued to be so at a recent follow up at 8 months. Psychomotor development was delayed. She smiled reactively, but had no head control. She required tube feeding because of dysphagia owing to her...
cleft palate and generalised muscular hypotonia. Craniofacial features included microcephaly, prominent forehead with narrow temples, telecanthus, slight protrusion of the bulb, upward slanting, narrow palpebral fissures, a short nose with a depressed nasal bridge, prominent philtrum, small mouth with a thin upper lip, cleft hard and soft palate, severe microretrognathia, and large, thin ears (fig 2). There were no signs of auditory or visual impairment. Radiography showed thoracic asymmetry, a high diaphragm, and a left convex scoliosis of the thoracic spine. Echocardiography identified an atrial septal defect and a hypoplastic right pulmonary artery. Ultrasound examination of the brain showed enlarged lateral ventricles and gyral flattening. She had had two epileptic fits previously (see Note added in proof). Abdominal ultrasound investigations were normal. There was no family history of congenital malformations.

Methods
Prenatal cytogenetic analysis was performed on short term chorionic villi cultures and amniocyte cultures. Chromosomes from peripheral blood were examined at birth and at the age of 6 months. Metaphase chromosomes were analysed by standard trypsin-Giemsa banding (GTG), quinacrine banding (QFQ), C banding (CGB), and NOR staining. In both parents, chromosomes from lymphocyte cultures were examined. To determine the origin of the marker chromosome microdissection, DOP amplification, biotin labelling of the probe, and reverse painting were performed according to the protocol of Friedrich et al.10 To evaluate the construction of the supernumerary marker chromosome, eight polymorphic microsatellites localised to 5pter-5p13 (D5S392, D5S406, D5S1953, D5S208, D5S486, D5S1473, D5S419, D5S426) were analysed by PCR amplification of genomic DNA from the parents and DNA from the patient’s placenta. PCR primers were obtained from MWG-Biotech AG (Ebersberg, Germany). DNA was amplified in a total volume of 25 µl using standard conditions and a “high touch down protocol” with annealing temperatures of 63°C.14 The products were separated on 8% polyacrylamide gels and visualised by silver staining. Dosage differences between alleles indicated the involvement of the respective locus in the triplication.

Results
Prenatal chromosome analysis of amniocyte cultures and postnatal studies in peripheral blood lymphocyte cultures showed 47,XX chromosomes and a non-satellited, C band negative marker of unknown origin in 19 and 100 cells studied respectively. The slightly asymmetrical marker was approximately the size of a chromosome 20. Chromosome analysis of the parents showed normal karyotypes, which indicated a de novo origin of the additional material in the fetus.

In order to determine the origin of the marker, reverse painting was performed (fig 3A). Hybridisation with the microdissection library onto the patient’s metaphases gave signals covering the whole marker chromosome and the distal short arm of chromosomes 5.
No centromeric region was labelled with this probe and no hybridisation signals were seen on other chromosomes (fig 3B). The mitotic stability of the marker chromosome in the patient’s cells pointed to the presence of a functional centromere. Hybridisation with a pan alpha satellite centromere probe at low stringency conditions showed signals at the centromeres of all chromosomes except the marker (fig 3C). Neither FISH with a probe specific to the centromeres of chromosomes 1, 5, and 19 (fig 3D) nor PRINS with a pericentromeric satellite III probe yielded detectable centromeric signals (not shown). Telomere and subtelomere specific sequences could be found at both ends of the marker chromosome (fig 3E). There was, however, a weak reaction with antibodies directed against centromere specific proteins (fig 4). Thus, the mitotic stability of the C band negative marker chromosome is the result of the formation of a neocentromere. FISH and G banding studies thus characterised the marker as an inverted duplication of 5p14→pter. The appearance of the marker was not totally symmetrical. A primary constriction at the presumed neocentromere was seen on the border of band 5p14 to 5p15.1 (fig 5). The complementary deletion of chromosome 5 has not been recovered. The patient has, therefore, partial tetrasomy of the distal part of chromosome 5p, and the karyotype is 47,XX,+inv dup (5)(pter→p14::p14[neocen]→pter).

For determination of the parental origin of the de novo marker, eight different DNA polymorphisms were tested on genomic DNA. While most of the markers were uninformative, loci D5S1473 and D5S426 showed evidence of an increase in paternal dosage indicating paternal origin of the marker chromosome (fig 6).

Discussion
A partial tetrasomy of 5p14→pter was found in a newborn girl with congenital anomalies. It was the result of a presumably mitotically stable, supernumerary marker chromosome. The identification of the marker was achieved using chromosomal microdissection, generating a painting probe via DOP-PCR and reverse
painting. FISH performed with a probe from the most distal 5p region showed that the marker was an inverted duplication. So far, there has been only one report of tetrasomy 5p of almost the same region (5p14→p15.3) as in our patient. This patient had a triplication on one chromosome 5 and showed comparable clinical manifestations. In these two patients the most prominent symptoms were severe hypotrophy of the newborn, hypertonia, failure to thrive, brain abnormalities and seizures, a distinct facial appearance (in particular, significant microretrognathia), cardiovascular malformations, and flexion contractures of different joints.

Tetrasomy of the whole short arm of chromosome 5 has been described previously in three patients. These patients, however, were mosaics for an isochromosome 5p. With the exception of similar facial dysmorphic signs, they did not appear to be severely affected, although larger regions of chromosome 5 were included in the tetrasomy (table 1). This observation could be explained by the presence of mosaicism.

Duplications leading to complete trisomy of 5p14→pter cause mild clinical symptoms. The main characteristics are short stature and psychomotor retardation indicating that the phenotypic severity might depend on specific regions of the duplicated material. Duplications of the whole of 5p (p11→pter) as well as partial proximal duplications affecting at least band 5p13 are associated with a more severe phenotype including macro- and dolichocephaly, facial anomalies with microretrognathia and large, simple ears, additional eye anomalies, abnormalities of the central nervous system, congenital heart defects, and renal and intestinal malformations. Newborn infants who survived showed moderate to severe mental retardation and nearly half of the patients developed infantile seizures. The critical region of 5p associated with a severe phenotype was, therefore, proposed to be proximal to band p14.

The distinct phenotypes of the two patients with distal tetrasomy, however, seem to be more compatible with the phenotypes of patients with complete trisomy of the whole short arm of chromosome 5 (table 1). Symptomatic findings were microcephaly, hypertonia, and contractures in the two patients with tetrasomy 5p14→pter. In these patients, the most proximal breakpoint of the marker chromosome was estimated to be at 5p14. It may be that for the clinical picture a gene dosage effect is of more significance than the breakpoint involved leading to trisomy or tetrasomy. Deregulated expression of genes can be caused by amplification of regulatory DNA regions. Interestingly, a gene for topoisomerase related function protein 4 (TRF-4) required for sister chromatid cohesion and mitotic chromosome condensation resides in 5p14, which is frequently amplified in various tumours.

Reviewing known genes in 5pter→5p14 shows that the expression pattern of different genes like cadherin 10 or adenylcyclase 2 is largely brain specific and they may be involved in human brain disorders.

Mitotically stable, analphoid markers were first described by Callan et al and Crolla et al and at least 40 such markers have been registered. Chromosomal rearrangements with neocentromeres derived from chromosome 5 have not been reported so far. However,
the characteristics of other analphoid markers are similar to our patient's marker. Inversion duplications are the most common type in that a duplication of a relatively small distal subfragment is seen. As in 21 of 40 cases, the karyotype in our patient is normal except for the supernumerary chromosome giving rise to partial tetrasomy for the duplicated portion. The neocentromere does not occur at the inversion breakpoint, making the appearance of the marker asymmetrical. When examined with molecular techniques, no neocentromere has been shown to localise to the inversion breakpoint. In most cases with tetrasomy, the marker is present in the mosaic state. In our patient the marker was identified in two different tissues, in all 19 amniocytes and in 100 metaphases from peripheral blood. However, the question arises whether the marker will be lost over time. Focusing on markers with neocentromeres, Reddy et al reported the development of mosaicism as a result of age, thus suggesting mitotic instability of these markers. In vitro results indicated that markers with neocentromeres are stable in short term lymphocyte cultures, while they are less stable in long term lymphoblast and fibroblast cultures. Mitotic instability has long been known from alphoid markers and even inherited markers may appear as mosaics. In a follow up of children with supernumerary marker chromosomes, Gravholt and Friedrich pointed to the fact that mosaics change constantly under the assumption that markers containing ribosomal DNA proliferate, whereas those lacking ribosomal DNA are selected against and disappear.

DNA polymorphism studies disclosed the paternal origin of the marker and duplication of identical paternal alleles at polymorphic loci suggested a mitotic origin, as already described for other examples of analphoid marker chromosomes. Deriving from a single parental chromosome, the resulting marker chromosome would, therefore, be identical in their primary DNA sequences in both chromosome arms. This suggests a priori that both arms carry the same putative latent centromeric site. The mechanism of activation or inactivation of a neocentromeric site remains unknown, but the situation parallels that found in dicentric chromosomes, in which only one centromere remains active. Spreading of an epigenetic state in cis might be responsible. Generally, the interaction between centromeric DNA and proteins seems to be more complex than previously thought. As shown by Gimelli et al recently, the same alpha satellite DNA sequence could either organise an active centromere or in other situations bind many

### Table 1 Clinical features in patients with complete trisomy 5p and tetrasomy 5p

<table>
<thead>
<tr>
<th></th>
<th>Complete trisomy 5p (p11–pter) (n=8*)</th>
<th>Mosaic tetrasomy (5)(p11→pter)</th>
<th>Tetrasomy (5)(p14→pter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>28–47 (mean 29.5)</td>
<td>29 40 31</td>
<td>28 25</td>
</tr>
<tr>
<td>Paternal age</td>
<td>26–35 (mean 29.8)</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td>At term 6</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>4F/4M</td>
<td>F/F</td>
<td></td>
</tr>
<tr>
<td>Birth weight</td>
<td>1275–3000</td>
<td>2670 2550 3040</td>
<td>2900 1890</td>
</tr>
<tr>
<td>Birth length</td>
<td>44–55</td>
<td>46</td>
<td>52.5 43</td>
</tr>
<tr>
<td>OFC at birth</td>
<td>32.5–39</td>
<td>36.5</td>
<td>32.5 31.5</td>
</tr>
<tr>
<td>Macro/microcephaly</td>
<td>8/8 macro</td>
<td>Macro</td>
<td>Micro Micro</td>
</tr>
<tr>
<td>Ventriculomegaly</td>
<td>5/8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Supraorbital edges</td>
<td>1/8 depressed</td>
<td>Depressed</td>
<td>Depressed</td>
</tr>
<tr>
<td>Hypertelorism</td>
<td>5/8</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Epicanthus</td>
<td>5/8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Upper palpebral thy fisses</td>
<td>5/8 + +</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Eye abnormalities</td>
<td>1/8</td>
<td></td>
<td>Coloboma</td>
</tr>
<tr>
<td>Depressed nasal bridge/short nose</td>
<td>7/8 + +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Midspace hypoplasia</td>
<td>4/8 +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Philtrum</td>
<td>5/8 long</td>
<td>Long</td>
<td>Short Short</td>
</tr>
<tr>
<td>Macroglossia</td>
<td>4/8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palate</td>
<td>2/8 high</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Microtrematodacty</td>
<td>6/8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dysplastic ears</td>
<td>8/8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Prematurely pits</td>
<td>0/8</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Short neck/redundant skin</td>
<td>7/8 +</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Chondactyly</td>
<td>1/8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proximal implanted toes</td>
<td>1/8 +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Club feet</td>
<td>5/8</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Congenital heart defect</td>
<td>6/8 +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>High diaphragm</td>
<td>1/8</td>
<td>+</td>
<td>+ (right side)</td>
</tr>
<tr>
<td>Respiratory difficulties</td>
<td>6/8 +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Recurrent infections</td>
<td>2/8 +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>4/8 +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Postnatal growth failure</td>
<td>3/8 +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Muscle tone</td>
<td>8/8 hypo</td>
<td>Hypo</td>
<td>Hypo Hyper</td>
</tr>
<tr>
<td>Flexions contractures</td>
<td>0/8 +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Psychomotor retardation</td>
<td>5/8 +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Seizures/abnormal EEG</td>
<td>4/8 +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Early death</td>
<td>4/8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Others</td>
<td>Genital anomalies, larynx</td>
<td>Gut malformation, dysplastic kidneys</td>
<td>Thoracospinal scoliosis</td>
</tr>
</tbody>
</table>

*Only cases with complete trisomy 5p without involving another chromosome aberration were considered, six cases reviewed by Lorda-Sanchez et al, one by Reichenbach et al, and one by Velagaleti et al.*
fewer proteins, thus forming an inactive centromere. In our patient, FISH analyses with a chromosome 5 specific alpha satellite probe, a pan alpha satellite probe, and a satellite III probe failed to detect any common centromeric or pericentromeric sequences on the inv dup(5) marker, thus indicating the presence of a neocentromere. Immunofluorescence with antibodies against centromere proteins confirmed the presence of a functional centromere at the constricted arm of the inv dup(5p) chromosome. A neocentromere is a newly derived functional centromere formed outside the normal centromere domain. Neocentromeres have been observed most often in chromosomes 5, 6, 7, 12, 16, 18, and 19. Many theories have been put forward to explain their origin. The most convincing one is the presence of many different centromere competent sites within the human genome, which by epigenetic modification can be modified into functional centromeres. Most probably, the crucial point is not the DNA base sequence as such but rather the conformation assumed by the DNA. Based on ideas from previous publications, Koch has elaborated the hypothesis that double dyad symmetries of a particular size as well as short, conserved base motifs adjacent to the dyad symmetries are common in alpha and non-alpha satellite DNA and may define the mitotically functional human centromere. Further studies will disclose which mechanisms involved in the formation of a neocentromere are valid. This type of marker may therefore be useful in delineating the key components of the functioning centromere.

We report on a 9 month old girl with congenital anomalies, dysmorphic features, seizures, and psychomotor retardation who had a prenatally diagnosed supernumerary marker chromosome. Chromosomal microdissection and reverse painting disclosed the 5p origin of the marker.

The marker chromosome reacted weakly with anticientromere antibodies but it was C band negative and devoid of detectable alpha satellite or pericentromeric satellite III sequences suggesting the formation of a neocentromere in a previously undescribed chromosomal region at 5p14.

The karyotype of the patient was 47,XX,+inv dup(5)(pter→p14:p14→[neocen]→pter), resulting in tetrasomy of distal 5p. Paternal origin of the de novo marker was shown by microsatellite analysis.

The patient had some but not all manifestations of the dup(5p) syndrome including severe microretrognathia and large ears, eye anomalies, abnormalities of the central nervous system, congenital heart defects, and respiratory difficulties.

Note added in proof
At 17 months, the girl had developed recurrent seizures, the EEG being severely abnormal, like the chaotic picture of a hypsarrhythmia.

The microdissection method was established during a guest professorship of Dr Friedrich at the University of Marburg. We are grateful to Evelyn Winkler for excellent technical assistance. This work was supported by P E Kempeß Stiftung Marburg. We are particularly grateful to the parents of our patient for their cooperation.

A case of Roberts syndrome described in 1737

A W Bates

EDITOR—In 1735 Johanna Sophia Schmied, from the village of Taucha near Leipzig, gave birth to a stillborn child with multiple abnormalities, described at the time as a “very rare” monster. The case was reported by a local physician, Gottlieb Friderici, in a tract, *Monstrum humanum rarissimum*, published in Leipzig two years later. Friderici performed a necropsy and published his findings along with a case history of the pregnancy and two detailed plates engraved by a local draughtsman “from life”. The mother was aged 28 years, of short stature and slender, with a “choleric-melancholic” temperament. She had been married to a “hunchback” for 10 years, and they had three other children, all “free of imperfections”; the fourth child is that described by Friderici. “Halfway” though the pregnancy, the fetal movements were felt very faintly and the uterus was not thereafter seen to increase in size, whereas her husband recalled that in the previous pregnancies her belly had grown normally. The baby was stillborn after a labour of seven hours.

A large anterior encephalocele was present. Friderici remarked that, although the appearance resembled hydrocephalus, the protuberance contained cerebral matter. The frontal bone was very abnormal to the bridge of the nose. The nose was “vestigial”, but the nostrils were patent, and a probe inserted into the oral cavity passed through a fissure in the palatal bone. The mouth was “lipless”, the eyes protruded, and the orbits were shallow. A tiny external auditory meatus was found, but the pinnae were absent. The legs, like the forearms, were “simple”, composed of only one bone. There were pterygia in the popliteum (M in fig 1), the groin, and running from the mouth to the upper thorax. The digits of the feet were distorted but all digits were present. The fingernails “resembled those of an animal”.

All ribs were present. In the engraving the thorax appears deformed, though this may be because the illustration was made from the reconstructed body after necropsy. The pleural cavities and pericardium contained “thin fluid”. The liver appeared unusually large and the kidneys were unequal in size. Two small intra-abdominal testes were located. No external genitalia were identified. Quantities of meconium were passed via the anus.

The specimen was brought to Friderici and examined within a few hours of delivery. Fig 1 was printed life sized and was hand coloured. The crown-rump length of the figure is 20 cm and the foot length 4.5 cm; these dimensions correspond to a gestational age of some 24 weeks, compatible with the history, and suggesting that it was drawn in the correct proportions. The head appears microcephalic, though this may be because of the encephalocele. The upper limbs show marked shortening, and were likened by Friderici to the wing of a chicken without the feathers. The combination of anterior encephalocele, microcephaly, shallow orbits, cleft palate, marked micrognathia, hypomelia of the upper limbs, single forearm and leg bones (most probably absence of the radius and fibula, though fusion is possible), and flexion contractures is consistent with a severe lethal form of Roberts-SC phocomelia syndrome (MIM 268300).

The inheritance of this condition is autosomal recessive with great variability of expression; the largest review has shown that it is more often sporadic than hereditary. Consanguinity is not discussed in the account but the physical descriptions of the parents as of short stature and a “hunchback” do not rule out their having had minor dysmorphic features. It is too early in gestation to assess cryptorchidism, and growth retardation cannot be assessed owing to probable intrauterine death.
and microcephaly. Encephalocele is an uncommon feature, seen only in severe forms of Roberts syndrome, but the frontal location is typical. The presence of pleural and pericardial effusions raises the possibility of a cardiovascular or renal anomaly but there is no description of the anatomy of the heart or kidneys. The gall bladder and spleen were not described.

The differential diagnosis includes Bartsocas-Papas syndrome and acrofacial dysostosis, particularly Nager type. Some features, such as popliteal and other pterygia and absent penis and pinnae, are suggestive of Bartsocas-Papas syndrome, but the encephalocele, absent limb bones, the absence of syndactyly, and the well formed digits (a second plate in the original

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**Figure 1** Engraving of the external features of the fetus, from Friderici, 1737 (courtesy of Leipzig Universitätsbibliothek).
description shows well formed nails on both hands and feet) are not features that would be expected in this syndrome. Severe Nager acrofacial dysostosis can present with similar features, but encephalocoele and pterygia would not be expected. The most probable diagnosis is that of Roberts syndrome, though there are some unusual features, such as the prominent pterygia and absent external genitalia.

Roberts and SC phocomelia syndrome are generally regarded as the same nosological entity though the absence of cleft palate in the SC syndrome may be a difference. Roberts syndrome has been interpreted as a human mitotic mutation syndrome that leads to a wide spectrum of secondary developmental defects. The phenotype is highly variable. The eponymous description of the syndrome was in 1919, though Mayer’s case of 1829 was recognised as Roberts syndrome by Van den Berg and Francke, and a fetus described by Virchow in 1898 has recently been interpreted as Roberts syndrome after re-examination of the specimen. A case has also been identified as Roberts syndrome after re-examination of the specimen. Van den Berg DJ, Francke U. Roberts syndrome: a review of 100 cases and a new rating system for severity. Am J Med Genet 1993;47:1104-123.

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9 Roberts JB. A child with double cleft lip and palate, protruision of the intermaxillary portion of the upper jaw and imperfect development of the bones of the four extremities. Ann Surg 1919;70:252-3.
12 Bouchard P. Infante monstroso Lugduni in viam publicam die V. Martii A. MDCLXXI. exposito. Miscellanea Curiosa 1672:3-14-16.

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A region of homozygosity within 22q11.2 associated with congenital heart disease: recessive DiGeorge/velocardiofacial syndrome?

Judy Henwood, Chris Pickard, Jack P Leek, Christopher P Bennett, Yanick J Crow, John D R Thompson, Mushtaq Ahmed, Kevin G Watterson, Jonathan M Parsons, Emma Roberts and Nicholas J Lench

J Med Genet 2001 38: 533-536
doi: 10.1136/jmg.38.8.533

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