Potential mapping of corneal dermoids to Xq24-pter

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Editor—Corneal dermoids (CND) (MIM 304730) are rare, congenital, benign tumours involving the cornea. Histologically, the dermoids consist of a combination of ectodermal elements including keratinised epithelium, hair, and sebaceous glands and mesodermal elements including fibrous tissue, fat, and blood vessels in different proportions. The tumour is by definition a choristoma since it is histologically a normal tissue in an abnormal site. Clinically, these tumours appear as opacification of the cornea at birth and if untreated may result in blindness (fig 1). Treatment includes surgical removal of the tumour with corneal transplantation. When the tumours affect both eyes, surgical intervention is indicated within the first 3 months of life since untreated cases may be irreversible by that time.

Most CND cases are sporadic and unilateral. However, three hereditary forms associated with congenital dermoids involving the cornea have been described. The Goldenhar syndrome (oculoauriculovertebral dysplasia, MIM 164210) is a multiple anomaly syndrome that involves the eyes, ears, and the vertebra. The dermoids are mostly unilateral, originate in the corneal-scleral border, also known as the limbus, and rarely affect the centre of the cornea. Although most cases are sporadic, familial cases with an autosomal dominant mode of inheritance have been reported. The second form with hereditary dermoids of the cornea is ring dermoids (MIM 180550), an autosomal dominant condition with isolated dermoids at the periphery of the cornea at the limbus.

A third form of congenital dermoids involving the cornea was initially described by our institution in a family of Puerto-Rican ancestry. The three affected males had congenital, bilateral, and central corneal opacification at birth and no additional abnormalities. The described pattern of transmission was suggestive of X linked recessive inheritance. To date, this is the only family described with this inherited form of CND. Previous efforts to localise the gene on the X chromosome using RFLPs for linkage analysis gave a lod score of 2.4 at 0=0 with the DXS43pD2 probe at

Figure 1 Corneal dermoids as seen immediately after birth in subject IV.8.
Xp22.2. Here, we report the analysis of the extended family to establish linkage.

**Case reports**

Twenty three members of a four generation family were ascertained in our centre. Clinical data from the 15 subjects who participated in the previous linkage analysis were available to us but blood samples were available for only 10 out of the 15. II.2 and II.10 declined participation in the current study. In addition, we studied seven family members, including three affected males, not previously described (IV.2, IV.3, IV.5-IV.9). This study was approved by the Albert Einstein College of Medicine Institutional Review Board, in accordance with the guidelines of the Office for the Protection from Research Risks, and informed consent was obtained before participation (CCI 91-157).

All family members including carriers and unaffected members underwent full ophthalmic examination in our centre and ophthalmic records and histopathology slides of affected males were reviewed. We identified six males out of 23 family members who were affected with congenital corneal dermoids. The 17 unaffected family members did not exhibit any medical or ophthalmic condition that could be attributed to or associated with CND. The pattern of transmission is consistent with an X linked recessive mode of inheritance as indicated by the presence of only affected men, an absence of male to male transmission, and unaffected obligate carrier females (fig 2). In all cases the dermoids appeared as a bilateral, superficial, greyish layer with irregular, raised, whitish plaques and fine blood vessels that covered the centre of the cornea. The peripheral border of the cornea was intact in all cases. The ophthalmic examination of affected males was consistent with the previously described examination of subjects III.8, III.9, and IV.1. Briefly, ocular examinations showed normal adnexal structures, eyes were orthophoric to Hirschberg testing, and eye movements were conjugate and normal. Schiotz tonometry

Figure 2  Haplotype analysis of the four generation family segregating X linked CD. The at risk haplotype is blackened to show recombinations. Carrier females are indicated by a black dot within a circle and affected males are indicated by blackened squares. Unblackened squares and circles without a black dot denote unaffected males and females not known to be carriers, respectively. Asterisks show subjects who were analysed in the previous report but for whom blood samples were not available for current linkage analysis.
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probe which detects two reported Xp22.2 locus with the DXS43pD2 type analysis. We initially studied the previously endothelium, were intact in all cases.

inner layers, Descemet’s membrane and the these cases and in one additional case. The two epithelium was found to be keratinised in three cases and sebaceous glands were present in two cases and hair follicles and hair shafts were present in cases with sparing of the corneal periphery.

showed a highly vascularised, dense, and was consistent with a dermis-like tissue. It had four and five operations, respectively, and corneal transplant. Subjects IV.7 and III.8 had respectively were described as normal and clear. The because of the opacification, but intraoperative-ranged from 20 to 22 mmHg bilaterally. Ante-

Table 1 Two point lod scores between XLCD and markers flanking DXS43pD2 on Xp22.2

<table>
<thead>
<tr>
<th>Marker</th>
<th>Band*</th>
<th>Position (Mb)*</th>
<th>Female genetic map (cM)†</th>
<th>0.00</th>
<th>0.01</th>
<th>0.05</th>
<th>0.10</th>
<th>0.20</th>
<th>0.30</th>
<th>0.40</th>
<th>Zmax (θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXS986</td>
<td>p22.3</td>
<td>5.2</td>
<td>8.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>−4.60 (−1.96, −0.96, −0.17) 0.11, 0.14, 0.14 (0.40)</td>
</tr>
<tr>
<td>DXS237</td>
<td>p22.3</td>
<td>8.5</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>−5.62 (−2.90, −1.79, −0.80) 0.34, 0.01, −0.11 (0.40)</td>
</tr>
<tr>
<td>KAL1</td>
<td>p22.32</td>
<td>9.0</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>−5.13 (−2.44, −1.38, −0.49) 0.13, 0.00, 0.00 (0.00)</td>
</tr>
<tr>
<td>DXS8036</td>
<td>—</td>
<td>9.1</td>
<td>23.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>−2.68 (−1.30, −0.74, −0.24) 0.03, 0.04, 0.04 (0.40)</td>
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<tr>
<td>DXS987</td>
<td>p22</td>
<td>16.3</td>
<td>22.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>−2.91 (−0.99, −0.32, 0.10) 0.14, 0.07, 0.14 (0.30)</td>
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<tr>
<td>DXS207</td>
<td>p22.2</td>
<td>16.6</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>−7.24 (−3.85, −2.47, −1.22) −0.59, −0.22, −0.22 (0.40)</td>
</tr>
<tr>
<td>DXS43</td>
<td>p22.2</td>
<td>17.5</td>
<td>—</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>−7.30 (−3.96, −2.64, −1.51) −0.93, −0.44, −0.44 (0.40)</td>
</tr>
<tr>
<td>DXS43pD2</td>
<td>p22.2</td>
<td>17.5</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>−1.31 (−0.08, 0.30, 0.44) 0.31, 0.12, 0.44 (0.20)</td>
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<tr>
<td>DXS418</td>
<td>pter-p21</td>
<td>19.6</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>−7.48 (−4.04, −2.61, −1.27) −0.59, −0.20, −0.22 (0.40)</td>
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<tr>
<td>DXS1052</td>
<td>p22.1</td>
<td>24.7</td>
<td>32.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>−6.31 (−3.55, −2.40, −1.28) −0.67, −0.27, −0.27 (0.40)</td>
</tr>
</tbody>
</table>

*Obtained from Centre for Medical Genetics, Marshfield (Broman et al, 1996, http://www.marshmed.org/genetics/).
†Obtained from Centre for Medical Genetics, Marshfield (Broman et al, 1998, http://www.marshmed.org/genetics/).

Disease marker pairwise linkage analysis was performed and we genotyping results and slides were available to us and could be reviewed and confirmed. Multipoint linkage analysis was performed using only markers which are of known order and intermarker recombination fractions using data from the Marshfield genetic map‡ (http://www.marshmed.org/genetics/) using LINK-MAP from LINKAGE v 5.2.9-11. Because of computational limitations, a “shifting” three point analysis was performed.12 Analysis of the previously suggested DXS43pD2 probe showed two obligatory meiotic recombination events between the disease locus and the marker in subjects IV.3 and IV.8 not previously tested. Analysis of the additional nine markers flanking DXS43pD2 showed significantly negative lod scores at θ=0.00 and a negative pattern for linkage at Xp22.1-p22.3 (table 1). As disclosed from the new haplotypes, the two point linkage analysis excludes the postulated XLCD locus on Xp22 as the site for the XLCD gene in this family. Thereafter, a systematic genotype analysis was undertaken with 22 microsatellite markers, separated by 5 cM, spanning the X chromosome. The disease status in the X linked CND family came into phase as markers from the Xq24-qter region were examined (fig 2). As a two point linkage analysis gave positive lod scores of 2.9 for DXS102, DXS1232, and DXS8377 at a recombination fraction of θ=0.00 (table 2). The centromeric boundary was defined by the marker DXS1001 on Xq24 (approximately 45 cM from the telomere). No meiotic crossovers were observed with the 10 markers in the region DXS8057-DXS8377, a ~38 cM interval. In agreement with the two point linkage results, multipoint analysis gave a very flat lod score curve with a maximum of 2.92 over the non-recombinant interval between DXS1062/DXS8094 and DXS8028, a ~25 cM interval (fig 3).

Discussion
In conclusion, we mapped the gene for CND to chromosome Xq24-qter, within a ~45 cM region, by using haplotype and linkage analyses. Although CND was mapped to a large interval with six markers with lod scores of more than 2.8 at θ=0.00, our findings emphasise the caution that is required in interpreting linkage results in a single family. The
addition of new members to this family and their inclusion in the study showed two new recombinations in a locus previously suggested to be linked to the disorder.

The unique features of the corneal dermoids in this family were the bilaterality of the tumours and their localisation in the central matrix of the cornea while sparing the periphery of the cornea, Descemet's membrane, and the endothelial membrane. Since corneal dermoids are considered to be an aberrant development of ectopic tissue in the cornea, it can be speculated that it results from a mutation in a gene that has a role in the normal differentiation of the matrix component of the cornea. The interval found to be linked to CND is currently known to contain 119 genes of which 38 have protein products with unknown function or an abnormal function which has not been implicated in any known disorder. Examination of the known data regarding these genes failed to pinpoint any target gene that may have a role in corneal development. Since there are no additional informative recombinations in this interval, the candidate region cannot be further narrowed and only additional families with XLCD can assist in advancing mapping and identification of the XLCD gene.

Table 2  Two point lod scores between XLCD and markers on Xq21-qter

<table>
<thead>
<tr>
<th>Marker</th>
<th>Band*</th>
<th>Position (Mb)†</th>
<th>Female genetic map (cM)‡</th>
<th>Female lod score at 0=</th>
<th>Male lod score at 0=</th>
<th>Zmax (0)</th>
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<tbody>
<tr>
<td>DXS458</td>
<td>q21.2-23</td>
<td>137.7</td>
<td>1.31</td>
<td>1.63</td>
<td>1.65</td>
<td>1.37</td>
</tr>
<tr>
<td>DXS1200</td>
<td>q23</td>
<td>148.8</td>
<td>130.4</td>
<td>1.14</td>
<td>1.63</td>
<td>1.65</td>
</tr>
<tr>
<td>DXS1001</td>
<td>q24</td>
<td>150.6</td>
<td>130.4</td>
<td>1.14</td>
<td>1.63</td>
<td>1.65</td>
</tr>
<tr>
<td>DXS8057</td>
<td>q24</td>
<td>151.0</td>
<td>137.4</td>
<td>1.89</td>
<td>1.84</td>
<td>1.62</td>
</tr>
<tr>
<td>DXS1047</td>
<td>q26</td>
<td>154.1</td>
<td>144.8</td>
<td>2.13</td>
<td>2.08</td>
<td>1.86</td>
</tr>
<tr>
<td>DXS102</td>
<td>q26.3-27.1</td>
<td>154.7</td>
<td>148.8</td>
<td>1.90</td>
<td>1.85</td>
<td>1.62</td>
</tr>
<tr>
<td>DXS1232</td>
<td>q27.3</td>
<td>158.1</td>
<td>158.1</td>
<td>2.13</td>
<td>2.08</td>
<td>1.86</td>
</tr>
</tbody>
</table>

†Obtained from the Genome Data Base (http://gdbwww.gdb.org).
‡Obtained from Centre for Medical Genetics, Marshfield (Broman et al, 1998, http://www.marshmed.org/genetics/).

Figure 3  Multipoint analysis of a 70 cM region encompassing the X linked CD locus on Xq24-Xqter. The multipoint Zmax 2.92 was obtained with an interval between DXS1062 and DXS8028, a 25 cM interval. The physical distance of this interval is 3.7 Mb (The Genome Data Base http://gdbwww.gdb.org).


A D Paterson is supported by an MRC (Canada) Program Grant “The Centre for Applied Genomics”.

Satellites on the terminal short arm of chromosome 12 (12ps), inherited through several generations in three families: a new variant without phenotypic effect

Lionel Willatt, Andrew J Green, Dorothy Trump

EDITOR—Five of the human autosomes are acrocentric, chromosomes 13, 14, 15, 21, and 22, and are identified by the presence of satellite short arms. These short arms contain three bands, p11, p12, and p13, and are composed of repetitive DNA containing satellite repeats and copies of ribosomal RNA genes. Band p11, the pericentromeric region, is composed of several types of tandemly repeated DNA including satellite I, II, III, and IV, and β satellite DNA. Band p12, the satellite stalks, contains multiple copies of genes coding for ribosomal RNA and is known as the nucleolar organiser region (NOR) as the nucleolus is formed by an aggregation of ribosomal RNA. This can be recognised by staining with silver nitrate (AgNOR staining). Band p13 contains β satellite DNA and terminal telomeric sequences.

Loss or gain of the short arm of acrocentric chromosomes occurs without apparent phenotypic effect. For example, Robertsonian translocations occur when two acrocentric chromosomes are joined by centric fusion with the resulting loss of the short arm material and have no associated phenotype in this euchromatically balanced form. Chromosomal rearrangements involving the short arms of acrocentric chromosomes are a well known form of chromosomal variation. The most common variation results from rearrangements between the short arms of acrocentric chromosomes. Thus, the satellites of acrocentric chromosomes range in size from no satellites to double or triple satellites as shown by AgNOR staining. Translocations between the short arm of an acrocentric chromosome and the heterochromatic region of the long arm of the Y chromosome, resulting in acrocentric chromosomes with Y chromosome heterochromatin in place of satellites and satellited Y chromosomes, are also observed. More rarely, non-acrocentric chromosomes with terminal satellites have been described which arise from a translocation between the short arm of an acrocentric and the terminal region of another chromosome. Interstitial insertion of NORs from an acrocentric chromosome into another chromosome, giving rise to non-acrocentric chromosomes with interstitial satellites, is a rare form of chromosomal rearrangement without phenotypic effect. In contrast to these variant chromosomes, satellited non-acrocentric chromosomes resulting from an insertion or translocation between an acrocentric chromosome and another chromosome, in which there is loss of material from the non-acrocentric chromosome, are associated with an abnormal phenotype.

When a satellited non-acrocentric chromosome is observed it is essential to distinguish between these two possibilities.

We describe three pedigrees in which multiple family members have NORs at the telomeric region of the short arm of one chromosome 12 (12ps).

Case reports
In family 1, parental chromosome studies were undertaken following a stillbirth at 38 weeks. These studies showed a chromosome 12 with positively NOR staining satellite stalks at the end of the short arm (12ps) in the mother, who had an otherwise normal female chromosome complement. The paternal karyotype was normal. Family studies showed that this satellited chromosome had been inherited from her mother. Each of these women was well with no associated phenotype. There was no other significant family history (fig 1).

In family 2, chromosome analysis of an amniotic fluid sample received for maternal age showed a male karyotype with positively NOR staining satellite stalks at the end of the short arm of one chromosome 12 (12ps). Parental chromosome analysis showed that the 12ps chromosome was paternal in origin with no associated phenotype. The family history was unremarkable. The pregnancy proceeded to term and the child at the age of 3 years has no developmental problems (fig 1).

In family 3, a chromosome 22 (tuple 1) microdeletion was identified in a 3 month old child with tetralogy of Fallot and dysmorphic facies, consistent with DiGeorge/VCF syndrome. Parental chromosome studies showed that the microdeletion was de novo. However, the father was found to have one chromosome 12 with positively NOR staining satellite stalks at the end of the short arm (12ps) with no

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J Med Genet
2001;38:723–726

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