An autosomal recessive cone–rod dystrophy associated with amelogenesis imperfecta

M Michaelides, A Bloch-Zupan, G E Holder, D M Hunt, A T Moore

LETTER TO JMG

The cone–rod dystrophies (CORDs) are a clinically and genetically heterogeneous group of progressive retinal disorders. They have similarities to the rod–cone or retinitis pigmentosa-type dystrophies, but can usually be distinguished on the basis of clinical findings and electrophysiology. The CORDs usually present with cone dysfunction related symptoms, including photophobia, poor colour vision, and reduced central visual function, but with time, poor night vision develops, reflecting rod photoreceptor involvement.

The age of onset in the CORDs is variable, but most patients present in the first 2 decades of life. CORD is usually an isolated finding; less commonly it may be associated with other systemic abnormalities, including dental anomalies. The CORD syndromes in which the causative gene or chromosomal locus has been identified are shown in table 1.

The association of CORD with amelogenesis imperfecta (AI) has been reported only once previously, in a large consanguineous Arabic family from the Gaza strip. AI is an inherited group of disorders of tooth enamel deposition affecting enamel structure, composition, and thickness, and can be broadly divided into the primarily hypoplastic (quantitative defect with thin or missing areas of enamel but otherwise normal in structure) and hypomineralised/hypomaturation (qualitative defect of normal thickness enamel but poorly mineralised) variants. However, a new classification based on the molecular basis of the observed enamel phenotype will help to clarify the overlap in phenotypes that is commonly seen. The disorder in the Arabic family mapped to a 2 cM (5 Mb) region on chromosome 2q11, which includes the CNGA3 gene that encodes the α-subunit of the cGMP gated cation channel in cone photoreceptors. This represented a good candidate gene for this disorder because mutations in CNGA3 are associated with the cone dysfunction syndrome achromatopsia and with cone dystrophy. However, no disease causing sequence variants were identified in the family.

We have ascertained a second family with the syndrome CORD associated with AI and describe here the detailed ocular and dental phenotype. Screening of CNGA3 again failed to identify causative mutations, and we have demonstrated that CNGA3 is not expressed at the developmental stages of the mouse teeth studied. However, the segregation of an adjacent microsatellite marker is consistent with the causative gene residing in the same chromosomal region (2q) as identified for the Arabic family.

PATIENTS AND METHODS

A two generation Kosovan family with autosomal recessive CORD and amelogenesis imperfecta was ascertained (fig 1). After informed consent, a full ophthalmological and dental examination was performed, and blood samples were taken for DNA extraction and molecular genetic analysis. The protocol of the study adhered to the provisions of the Declaration of Helsinki.

Key points

- We report a second family with the autosomal recessive syndrome of cone–rod dystrophy and amelogenesis imperfecta, and have further characterised the ocular and dental phenotype.
- Affected individuals presented in the first few years of life with photophobia, pendular nystagmus, and reduced central vision. Night vision difficulties were reported by the end of the first decade.
- The progressive cone–rod dystrophy was associated with the hypoplastic/hypomineralised variant of amelogenesis imperfecta, an inherited group of disorders of tooth enamel deposition.
- Mutation screening of CNGA3, a previously identified candidate gene, failed to reveal any sequence changes capable of causing the disease.
- Segregation assessment of the adjacent microsatellite marker, D2S2187, provided evidence supporting disease association with this chromosomal region. However, study of CNGA3 and CNGB3 expression in developing teeth in mice failed to identify transcripts from either gene at the studied developmental stages, thereby providing further evidence to exclude CNGA3 as the causative gene.

Clinical assessment

Ocular

A full medical and ophthalmic history was obtained from the family and an ophthalmological examination performed. The affected siblings also underwent colour fundus photography and electrophysiological assessment that included an electro-oculogram, a flash electroretinogram (ERG), and a pattern ERG (PERG), according to the protocols recommended by the International Society for Clinical Electrophysiology of Vision. Detailed colour vision testing of the two affected brothers and their parents was performed, which included the use of the Hardy, Rand, and Rittler plates (American Optical Company, NY, USA), Farnsworth Munsell (FM) 100 hue test, Farnsworth D-15, the Mollon-Reffin (MR) minimal test, a computerised colour vision test, and anomaloscopy. The FM 100-hue, Farnsworth D-15, and the MR tests were all performed under CIE Standard Illuminant C from a MacBeth Easel lamp.

Abbreviations: AI, amelogenesis imperfecta; CORD, cone–rod dystrophy; ERG, electroretinogram; FM, Farnsworth Munsell test; MR, Mollon-Reffin test; PERG, pattern electroretinogram
Dental
The two affected brothers underwent a detailed dental assessment including radiographic examination (orthopantomograms). Primary teeth extracted from both brothers for clinical reasons were examined histologically.

CNGA3 mutation screening
Total genomic DNA was extracted from blood samples using a Nucleon® Biosciences kit. The coding sequences of CNGA3 were amplified by PCR in each individual using primer sequences and conditions as previously published.13 PCR were amplified by PCR in each individual using primer 2 10 pmol each of forward and reverse primers, 200 ng DNA, 1 U Bio . After resolution on a 1% (w/v) LMT agarose gel, products were excised and eluted. Direct sequencing of PCR products was carried out on an ABI 3100 Genetic Analyser using the original PCR primers in the sequencing reactions. The sequence was examined for alterations using Sequencing Analysis (ABI Prism™) and GeneWorks™ software.

Genotyping
Genotyping was carried out using the microsatellite marker D2S2187, which is located 0.1 Mb from CNGA3, with the forward PCR primer being fluoroscencently labelled. PCR reactions were carried in a 25 µl reaction volume, containing 125 ng DNA, 1×NH4 buffer (Bioline™), 1 mmol/l MgCl2, 200 µmol/l each dNTP, 10 pmol each of forward and reverse primers, 200 ng–1 µg DNA, 1 U BioTag. After resolution on a 1% (v/v) LMT agarose gel, products were excised and eluted. Direct sequencing of PCR products was carried out on an ABI 3100 Genetic Analyser using the original PCR primers in the sequencing reactions. The sequence was examined for alterations using Sequencing Analysis (ABI Prism™) and GeneWorks™ software.

RNA extraction and precipitation
Messenger RNA was extracted from the first molars and lower incisors of 19 (E19) and 21 day old (P2) CD1 mice using the QuickPrep® Micro mRNA Purification kit (Pharmacia Biotech). The dental tissue was homogenised in an extraction buffer containing a high concentration of guanidium thiocyanate. The extract was then diluted threefold with an elution buffer (10 mmol/l Tris-HCL pH 7.5 and 1 mmol/l EDTA) and centrifuged to produce a clear homogenate. The mRNA isolation was achieved by passing the homogenate through an oligo(dT)-cellulose pellic, which was then washed several times in high and low salt buffers before the mRNA was eluted at 65°C in 0.4 ml of elution buffer.

In order to precipitate the mRNA, a 1/10 volume of potassium acetate solution (2.5 mol/l), 10 µl of a glycogen solution (5–10 mg/ml), and 1 ml of 95% (v/v) ethanol were added, followed by overnight storage at ~20°C. Precipitated mRNA was collected the following day by centrifugation, dried, and dissolved in 50 µl of elution buffer.

RT-PCR
cDNA synthesis from an mRNA template was achieved by RT-PCR with the Superscript First Strand Synthesis System (GibcoBRL). The mRNA was targeted with an oligo(dT)12–18 primer (0.5 µg), and cDNA synthesis was performed by the Superscript II RT enzyme (50 U). RNase H was added to remove the RNA, leaving only cDNA available as a template for subsequent PCR reactions.

CNGA3 and CNGB3 expression studies
cDNA synthesised from the E19 and P2 murine dental tissue was used as a template in 50 µl PCR reactions using primers designed against murine CNGA3 and CNGB3 sequences (table 2). As a positive control, primers designed against the murine housekeeping gene, GAPDH, were used with both 19 and 21 day tooth cDNA. The efficacy of the designed CNGA3 and CNGB3 primers was tested on murine whole eye cDNA.

RESULTS
The syndrome is present in the two generation Kosovan family as shown in fig 1.

---

**Table 1** Syndromic cone–rod dystrophies (CORDs) with identified genes and known chromosomal loci

<table>
<thead>
<tr>
<th>Syndrome/CORD; OMIM number</th>
<th>Mode of inheritance</th>
<th>Chromosome locus</th>
<th>Mutated gene</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORD &amp; amelogenesis imperfecta; 217080</td>
<td>AR</td>
<td>2q11</td>
<td>Nil</td>
<td>1</td>
</tr>
<tr>
<td>CORD &amp; spinoocerebellar ataxia type 7; 164500</td>
<td>AD</td>
<td>3p12−13</td>
<td>SCA7</td>
<td>2</td>
</tr>
<tr>
<td>CORD &amp; NF1; 136550</td>
<td>AD</td>
<td>17q</td>
<td>NF1</td>
<td>3</td>
</tr>
<tr>
<td>CORD &amp; Bardet-Biedl syndrome; 209900, 606151, 600151, 600374, 603650, 607590</td>
<td>AR</td>
<td>11q13</td>
<td>BB51</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16q21</td>
<td>BB52</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3p13−p12</td>
<td>Nil</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15q22.3–q23</td>
<td>BB54</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2p11</td>
<td>BB56</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4q27</td>
<td>BB57</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2p13</td>
<td>AAMS1</td>
<td>11</td>
</tr>
<tr>
<td>CORD &amp; Alström syndrome; 203800</td>
<td>AR</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AD, autosomal dominant; AR, autosomal recessive; NI, not identified; Ref., reference.
470 Letter to JMG

Ocular phenotype

The two affected brothers presented in the first few years of life with nystagmus, marked photophobia, and reduced visual acuity. Both brothers complained of progressive deterioration of central vision with later development of difficulties with night vision. When examined at 8 and 10 years old respectively, both had a visual acuity of 3/60 in each eye and had fine pendular nystagmus. There was no strabismus. Fundus examination revealed bilateral macular atrophy and pigmentation (fig 2). Both brothers were hypermetropic and astigmatic; RE +6.50/-2.0 x 12, LE +6.0/−2.0 x 170 and RE +4.50/-1.75 x 10, LE +4.50/-1.5 x 170. Flash ERG testing revealed no detectable photopic cone responses. Rod function was markedly abnormal (fig 3). Two ERGs performed 4 years apart were suggestive of progressive deterioration of retinal function. Detailed colour vision testing failed to demonstrate any residual colour vision.

Their parents and unaffected siblings were also assessed. They were asymptomatic and had a normal ocular clinical examination.

Dental phenotype

Both brothers had the inherited enamel defect, amelogenesis imperfecta. The 8 year old boy was in the mixed dentition phase (fig 4A, B). The primary dentition was almost worn out, especially in the molar sector. The teeth were discoloured to yellow/brown. Small inter-proximal carious lesions were noted on the mesial and distal aspects of 55, 54, 64, 65, 75, 74, 85, and 84. The first permanent molars were yellow, partially erupted, and severely dystrophic, showing almost no enamel protective layer. The erupting lower first permanent incisors (41, 31) were whiter in colour, suggesting the presence of a thin enamel layer. The occlusion was a Class I molar relationship with no anterior or posterior crossbites and no sign of crowding.

The 10 year old boy was also in the mixed dentition stage although he displayed an almost complete permanent dentition apart from the upper primary canines (53, 63) (fig 4D, E). The teeth looked dysplastic and yellow/brown with almost no visible enamel. The tooth surface was rough and pitted. The lower permanent incisors were slightly whiter and the incisal edge displayed some wear (fig 4E). The dentition was spaced, especially in the upper arch. Anterior crowding was visible.

Radiographic examination (fig 4C, F) revealed teeth with no differential contrast between the putative enamel and dentine layers. This, together with the teeth coronal morphology, suggested an almost total lack of normal enamel or the presence of only a thin residual hypomineralised enamel layer. The younger brother’s primary molars displayed narrowing and obliteration of the pulp chambers (fig 4C). The primary molars and canines had an absence of enamel, with small inter-proximal radiolucencies corresponding to carious lesions on 55, 54, 64, 65, 75, 74, 84, and 85. The first primary molars had short roots, and the first permanent molars lacked enamel and were taurodontic. The complete permanent dentition was developing apart from the third molars. The coronal structure of the developing teeth appeared abnormal. The non-erupted permanent tooth germs displayed a similar pattern, demonstrating that the lack of enamel may be a primary defect and that the enamel did not wear off after eruption. An upper anterior occlusal radiograph was taken to assess the development of 11 and 21, which had failed to erupt. The root formation was noted to be immature and the crowns to have a bulbous appearance. The elder brother’s teeth demonstrated almost no enamel (fig 4F). The premolars appeared smaller and tubular with no visible limit between the crown and the root. The upper molars were taurodontic. The lower first permanent molars displayed long, thin roots.

Table 2: Primers for amplification of murine CNGA3 and CNGB3

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5’–3’)</th>
<th>Tm, C</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNGA3 F</td>
<td>AGTCTCTATAATGCTTGTC</td>
<td>54</td>
<td>480</td>
</tr>
<tr>
<td>CNGA3 R</td>
<td>GGAGATGCTGCTGACTAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNGB3 F</td>
<td>AGATGCGACGACAATCTAC</td>
<td>52</td>
<td>480</td>
</tr>
<tr>
<td>CNGB3 R</td>
<td>ACTGTATACCATATGATTGCA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Primary teeth extracted for clinical reasons were examined histopathologically. Analysis of the lower second right primary incisor (82) of the 8 year old boy revealed a normal deciduous tooth appearance with enamel of relatively normal thickness and normal root structure. There was evidence of attrition. On decalcified sections, the dentine was essentially normal with no evidence of residual enamel matrix. However, microscopic histology of the 10 year old boy’s primary canine revealed that the enamel showed areas of hypoplasia with thinning and irregular root structure. There was no evidence of hypomineralisation on decalcified sections.

These features, when taken together, are those of a hypoplastic/hypomineralised variant of AI, and demonstrate that the defect may not be homogeneous in all sectors of the primary dentition. It is possible that the same applies for the permanent dentition.

CNGA3 mutation screening and segregation of the chromosome 2q11 region

Screening of CNGA3 failed to identify any disease causing sequence variants. An SNP in exon 2b that encoded a Met112Ile substitution was identified, but was found to be informative only in determining one of the parental disease

|Figure 2| Fundus photographs showing bilateral macular retinal pigment epithelial mottling and atrophy.|
associated alleles and not both (fig 1). However, the pattern of segregation does rule out homozygosity for a large deletion that includes the CNGA3 gene. The microsatellite marker D2S2187, which is located adjacent to the CNGA3 gene, was therefore used to determine whether the region of chromosome 2q11 that contains CNGA3 segregates with the CORD/AI phenotype. The three alleles present in this family provide evidence in support of an association between the disease and this chromosomal region (fig 1).

**CNGA3 and CNGB3 expression in developing mouse teeth**

Study of CNGA3 and CNGB3 expression in developing teeth in E19 (birth) and P2 (21 days post coitum) CD1 mice failed to identify transcripts from either gene (fig 5). E19 and P2 mice were chosen because enamel secretion/mineralisation would be expected to occur at these stages of development. If the cation channel genes had a role in dental mineralisation or enamel formation, it would seem likely that their transcripts would be detectable at these stages. The murine primers designed for CNGA3 and CNGB3 were validated by demonstrating that a PCR product of the correct size was generated using murine whole eye cDNA as the template, and a positive control in the form of the murine housekeeping gene, GAPDH, was used to check that cDNA had been successfully generated from these dental tissues. As shown in fig 5, neither of the channel genes appears to be expressed in teeth.

**DISCUSSION**

We have described a family with the autosomal recessive syndrome CORD associated with AI. The retinal dystrophy was of early onset, presenting in the first few years of life with photophobia and nystagmus. Visual acuity was severely reduced to 3/60 in childhood and there was complete absence...
of colour vision. Electrophysiological testing revealed undetectable cone ERGs and profoundly abnormal rod responses. The dental phenotype co-segregating with CORD was that of hypoplastic/hypomineralised AI.

The only previous description of this syndrome is in a large Arabic family with 29 affected members across 7 generations. The phenotype reported for that family was similar to that in the present study. The only significant ocular difference was that night blindness was not reported in the Arabic family and that scotopic ERGs were only mildly reduced in the youngest subject tested (12 years old), whereas the two brothers in our family reported early onset of night blindness and their rod ERGs were profoundly abnormal.

The type of AI present was, however, different, with a hypomineralised variant identified in the Arabic family and a hypoplastic/hypomineralised type in our family. AI is a collective term for a number of conditions with abnormal enamel formation, and precise clinical diagnosis can be difficult. The differences in the findings in the two families may not therefore be significant. The precise delineation of the various forms of AI may need to await the identification of the causative genes.

Linkage analysis performed in the original Arabic family established a lod score of 7.03 for the microsatellite marker D2S2187 on chromosome 2q11, with a disease interval encompassing the cation channel gene, CNGA3. This is the first report of AI associated with this region of chromosome 2. Subsequent mutation screening of CNGA3 failed to identify a disease causing sequence change. However, CNGA3 remained an attractive positional candidate gene, as linkage data in the present study are consistent with a 2q11 chromosomal location for the disorder in our family. Additionally, with the molecular mechanisms underlying enamel formation currently being poorly understood, a gene involved in cation transfer may well be involved in the mineralisation process that underpins enamel deposition. However, we were also unable to find a disease causing

---

**Figure 4** Amelogenesis imperfecta phenotype. (A, B) The 8 year old boy in the mixed dentition phase. Severe signs of wear of the primary dentition are present, especially in the molar sector, with marked yellow/brown discoloration throughout. Dental caries are present. The first permanent molars are yellow, partially erupted, and severely dysplastic with almost no enamel protective layer. (D, E) The 10 year old proband was also in the mixed dentition stage. The teeth appear dysplastic and yellow/brown with almost no visible enamel. The tooth surface is rough and pitted. (C, F) Radiographic examination (orthopantomograms) showing teeth with no differential contrast between the putative enamel and dentine layers, suggesting an almost total lack of enamel, or the presence of only a thin residual hypomineralised enamel layer.

**Figure 5** Gel photographs (left, E19; right, P2) showing that no PCR product was present for either CNGA3 or CNGB3 when using cDNA synthesised from developing mouse teeth as template, but that it was possible to obtain a PCR product with GAPDH primers (murine housekeeping gene). Lower bands in each lane are primer dimers.
mutation in CNGA3, and analysis of an SNP in CNGA3 that segregates in our family also rules out the presence of a large deletion that includes the CNGA3 gene. Finally, the absence of expression of CNGA3 (and the related CNGB3 gene) in developing teeth in E19 and P2 CD1 mice argues against the involvement of CNGA3 in the disorder. It is thus unlikely that CNGA3 is involved in either the AI or CORD syndromes associated with this disease. An alternative explanation is that the CORD/AI phenotype is caused by a mutation in an adjacent gene.

Other candidate genes in the 2q11 region include INPP4A (Hs.32944), which encodes inositol polyphosphate-4-phosphatase type I, a 107 kDa protein, with a pattern of expression that includes the brain, retina, retinal pigment epithelium, and choroid. A null mutation in the mouse orthologue inpp4a in weeble mouse mutants results in severe locomotor instability and significant neuronal loss in the cerebellum and in the hippocampus. Neither the retinal nor the dental phenotypes of these mice have been described, but it is of interest that the substrates of INPP4A are intermediates in a pathway affecting intracellular Ca2+ release,22,23 and are also involved in cell cycle regulation. The only gene in the region that is specifically expressed in the retina is L102 (Hs.436468), which encodes a poorly characterised lysosome. None is documented as having expression in teeth. Identification of the gene(s) responsible for the CORD/AI syndrome will help shed further light upon the mechanisms of enamel formation and retinal dystrophy.

ACKNOWLEDGEMENTS

The work was supported by a grant from the Guide Dogs for the Blind Association. We are also grateful to the patients who kindly agreed to take part in this study and to Drs P Speight and P Crawford for helpful discussion. We would like to thank S Dunkley and A Johnson for their involvement in the continuing dental care.

Authors’ affiliations

M Michaelides, D M Hunt, A T Moore, Institute of Ophthalmology, University College London, 11–43 Bath Street, London EC1V 9EL, UK
M Michaelides, A T Moore, G E Holder, Moorfields Eye Hospital, City Road, London EC2V 2PD, UK
A Bloch-Zupan, Department of Paediatric Dentistry, Eastman Dental Institute for Oral Health Care Sciences, University College London, 256 Gray’s Inn Road, London WC1X 8LD, UK
A Bloch-Zupan, Institute of Child Health, Developmental Biology Unit and Great Ormond Street Hospital for Children NHS Trust, 30 Guilford Street, London WC1N 1EH, UK

Conflict of interest: none declared

Correspondence to: Professor A T Moore, Institute of Ophthalmology, University College London, 11–43 Bath Street, London EC1V 9EL, UK; tony.moore@ucl.ac.uk

Revised version received 5 December 2003
Accepted for publication 8 December 2003

REFERENCES

19 Mollon JD, Raffin JF. A computer-controlled colour vision test that combines the principles of Chichet and of Stilling. J Physiol 1989;414:51.

www.jmedgenet.com
An autosomal recessive cone–rod dystrophy associated with amelogenesis imperfecta

M Michaelides, A Bloch-Zupan, G E Holder, D M Hunt and A T Moore

J Med Genet 2004 41: 468-473
doi: 10.1136/jmg.2003.015792

Updated information and services can be found at:
http://jmg.bmj.com/content/41/6/468

These include:

References
This article cites 22 articles, 3 of which you can access for free at:
http://jmg.bmj.com/content/41/6/468#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Eye Diseases (298)
- Hereditary eye disease (92)
- Epidemiology (630)
- Ethics (220)
- Molecular genetics (1254)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/