Idiopathic hypoparathyroidism in two patients with 22q11 microdeletion

Recently, Scire et al. reported the presence of haplinsufficiency in chromosome 22q11 in two patients with hypoparathyroidism. We wish to report two further cases in which we analysed 22q11 deletion by FISH.

The two patients were ascertained through their idiopathic hypoparathyroidism. The table shows their main clinical features and laboratory findings. All probes used for FISH were cosmid clones. D0832 was kindly provided by S Halford (Institute of Child Health, London, UK). Cos71 was a cosmid clone separated from a microdissected chromosome 22q11 (Kurashashi et al., submitted). CH-KAD26 and CHKAD9 were provided by the

Clinical and laboratory findings in present cases

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>F</td>
</tr>
<tr>
<td>Age</td>
<td>18 y</td>
</tr>
<tr>
<td>Hypocalcemia in 1st year</td>
<td>+</td>
</tr>
<tr>
<td>PTH level during hypocalcemia</td>
<td>Not detected</td>
</tr>
<tr>
<td>Ellsworth-Howard test</td>
<td>Normal *</td>
</tr>
<tr>
<td>Learning disabilities</td>
<td>+</td>
</tr>
<tr>
<td>Cleft palate</td>
<td>-</td>
</tr>
<tr>
<td>Congenital heart disease</td>
<td>+</td>
</tr>
<tr>
<td>T cell impairment</td>
<td>-</td>
</tr>
<tr>
<td>Nasal speech</td>
<td>+</td>
</tr>
<tr>
<td>Abnormal face</td>
<td>-</td>
</tr>
<tr>
<td>Psychiatric problem</td>
<td>-</td>
</tr>
<tr>
<td>Intracranial calcification</td>
<td>+</td>
</tr>
</tbody>
</table>

* Normal response to exogenous PTH. This finding is compatible with idiopathic hypoparathyroidism.
† She attends special class for mental retardation.
‡ Low response to concanavalin A and phytohaemagglutinin.

Japanese Cancer Research Resources Bank. The order of the chromosome 22q11 markers is tel-cos71-CH-KAD26-D0832-cen (Kurashashi et al., submitted). A distal marker, CH-KAD9 assigned to 22q13.3, was used as a control probe to identify the chromosome 22 homologues. D0832, cos71, and CH-KAD26 were deleted in our patients. These findings are consistent with the presence of a 22q11 deletion.

The acronym CATCH 22 has been proposed to designate the variable expression of Cardiac anomaly, Abnormal facies, Hypocalcemia, T-cell impairment, and Cleft palate, and Hypoparathyroidism, resulting from 22q11 deletion. The 22q11 deletion has been widely analysed in patients with conotruncal cardiac defect but not in patients with idiopathic hypoparathyroidism. We used three cosmid probes for the detection of haplinsufficiency of 22q11. All probes were used were deleted in all our patients. These findings suggested that some cases with idiopathic hypoparathyroidism are a part of the CATCH 22 syndrome. Isolated idiopathic hypoparathyroidism (without cardiac defect and thymic defect) occurs in 22q11 haploinsufficiency and one end of the spectrum of the CATCH 22 syndrome.

However, the reason for the clinical heterogeneity in CATCH 22 is not clear. Aetiological causes may occur because of teratogenic exposure (alcohol, maternal diabetes, retinoids). Therefore, an environmental factor may also play a role in the heterogeneity of clinical expression of the CATCH 22 syndrome. Also the parental origin of 22q11 deletion may influence the clinical phenotype and also to examine the clinical and molecular correlations.

Prototype sequence clues within the Fanconi anaemia group C gene

Fanconi anaemia (FA) is a genetically heterogeneous disease with variable clinical manifestations that include congenital abnormalities, pancytopenia, and propensity to neoplasia. Recently, FA has received widespread attention as a potential candidate for gene therapy. The complementary group C complementing sequence (FAC) has been described and 15% of FA patients harbour mutations in FAN. However, the function of the predicted FAC protein remains unknown. As a matter of didactic exercise we have examined the FAC sequence by eye, both at the DNA and protein level. We have found two motifs that might provide clues for the elusive function of the FAC gene. At the DNA level, there is a p53 binding site consensus sequence near the 3’ end of the gene that contains only a single mismatch (table A). Experimental evidence shows that a 5’ to 3’ orientation of the consensus sequence, such as observed in the FAC gene, is less effective, but does not prevent p53 binding. The location of a p53 binding site other than within the 5’ promoter region is somewhat unusual, but there is a precedent in the mdm2 gene. FAC cells show chromosomal instability and a defective cell cycle, both of which could derive from impairment of some p53 mediated function.

At the protein level, there is evidence for two motifs that might function as classical leucine zippers (table B). There are seven instead of six amino acids between one of the leucine repeats, and there are occasional substitutions of leucine by valine, threonine, and isoleucine, but computer modelling predicts a typical alpha helical structure for these.
repeats. The second of the zipper motifs is preceded both by a helix-turn-helix region (with the crucial glyline at position 208) and a distinctly basic domain (position 160 to 176). The picture that emerges (figure) is that of a prototype nuclear protein designed to interact both with DNA and proteins. The strong conservation between the murine and human motifs (table) argues for their functional significance: the conservation amounts to 100% for the p53 binding site motif (78% in the remainder of the sequence), and to 90% for the amino acid residues within the leucine zipper domains.

The apparent contradiction between nuclear and subsequent activation of the protein can be reconciled if one assumes that the inactive region of the protein is located in the cytoplasm, and that transfer into the nucleus and subsequent activation occurs only in response to DNA damage.

WOLFGANG LIEBTRAU
MANFRED BÜHNER
HOLGER HOEHN
Institut für Humangenetik,
Biozentrum, University of Würzburg,
Am Hubland, 97074 Würzburg,
Germany.


The COX8 gene is not the disease gene of the CMH4 locus in familial hypertrophic cardiomyopathy

Familial hypertrophic cardiomyopathy (FHC) is an autosomal dominant disorder characterised by ventricular hypertrophy which mainly affects the interventricular septum and causes severe myocardial and myocardial disarray. The gene implicated in the CMH4 locus is not yet known. We attempted to assess whether the gene COX8, which is localised at 11q12-q13 and encodes the subunit VIII of the cytochrome c oxidase (COX),1 is the CMH4 disease gene. COX is the terminal enzyme of the mitochondrial respiratory chain and participates in the production of ATP through oxidative phosphorylation. Therefore, an alteration of a gene encoding one of the COX subunits might modify ATP production in the cell, leading to a compensatory myocardial hypertrophy. Furthermore, several deficiencies in COX activity have been described in hypertrophic cardiomyopathies.7-10 All these characteristics made the COX8 gene a good candidate for the CMH4 locus, and analyses were performed on family 714 in which this locus was described.

Since the genomic structure of the COX8 gene is not known, Southern blot analysis was carried out, as described in Schwartz et al.,11 in a search for deletions or insertions or both, using COX8 cDNA as specific probe.12 The DNA of 60 people, 11 of whom were affected, was analysed after digestion with 12 restriction enzymes (BamHI, BclI, BglII, EcoRI, EcoRV, HindIII, Hind, MspI, PstI, Ps1I, Ral, and TaqI). The results showed no difference between the DNA of affected and healthy people as regards the length of the DNA fragments shown (data not shown), indicating that there were no major modifications in the genomic structure of the COX8 gene. The next step was to look for alterations in the mRNA. Since the COX8 gene is ubiquitously transcribed,13 northern blot analyses were performed, using 10 μg of total RNA purified from lymphoblastoid cell lines of four affected and four healthy people, and COX8 cDNA was used as specific probe.14 These analyses showed no differences between the length of COX8 mRNA in healthy and affected members of the family. Individual COX8 mRNAs were further quantified, using 18S RNA as internal probe. The ratios of COX8 mRNA/18S RNA were as follows: for the affected subjects, 0-62, 0-69, 0-63, and 0-54 respectively, and for the healthy subjects, 0-55, 0-61, 0-66, and 0-70. These results showed no significant differences in mRNA levels. Consequently, the absence of major alterations in COX8 transcripts enabled us to exclude the possible presence of mutations in intronic splicing sites, which would have led to transcript deletion or insertion or both. Lastly, COX8 transcripts were sequenced. Reverse transcription and amplification of COX8 mRNA (RT-PCR) were carried out with 1 μg of total RNA purified from lymphoblastoid cell lines of four affected and four healthy people. The strategies of am-
Prototype sequence clues within the Fanconi anaemia group C gene.

W Liebetrau, M Bühner and H Hoehn

doi: 10.1136/jmg.32.8.669-a

Updated information and services can be found at:
http://jmg.bmj.com/content/32/8/669.2.citation

**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.

**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/