Idiopathic hypoparathyroidism in two patients with 22q11 microdeletion

Recently, Scire *et al*¹ reported the presence of haploinsufficiency 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 (Kurahashi *et al*, submitted). cHKAD26 and cHKAD9² were provided by the

In a preliminary observation on a small sample, among the 19 pedigrees of inherited disorders, 14 could be maternal in origin and three could be paternal in origin.⁵ More recently, Seaver *et al*⁶ reported four cases of pulmonary atresia associated with maternal 22q11 deletion using the D22S624 dinucleotide repeat polymorphism.

A case of 22q11 deficiency with abnormal face and hypernasal speech, not accompanied by cardiac anomaly, thymic hypoplasia, cleft palate, or hypocalcaemia, has been reported.⁷ Nickel *et al*⁸ reported three patients with meningomyelocele, congenital heart defect, and 22q11 deletion. These recent reports and our observations suggest that the CATCH 22 syndrome has a wider variability of clinical phenotype than previously reported. However, the range of the mild phenotype of this syndrome has not been delineated. Further investigations are needed to identify the clinical spectrum of the CATCH 22 syndrome

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Clinical and laboratory findings in present cases

	Case 1	Case 2
Sex	F	M
Age	18 y	11 y
Hypocalcaemia in 1st year	+	+
PTH level during hypocalcaemia	Not detected	Not detected
Ellsworth-Howard test	Normal*	Normal
Learning disabilities	+†	Attention deficit disorder
Cleft palate	—	—
Congenital heart disease	—	—
T cell impairment	+‡	—
Nasal speech	+	+
Abnormal face	+	+
Psychiatric problem	Symptomatic psychosis	—
Intracranial calcification	+	—

* 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-cHKAD26-D0832-cen (Kurahashi *et al*, submitted). A distal marker, cHKAD9 assigned to 22q13.3, was used as a control probe to identify the chromosome 22 homologues. D0832, cos71, and cHKAD26 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 face, Thymic hypoplasia, Cleft palate, and Hypocalcaemia, resulting from 22q11 deletion.³ 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 haploinsufficiency of 22q11. All probes we 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 DGS can occur because of teratogenic exposure (alcohol, maternal diabetes, retinoids).⁴ 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.

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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.¹ The complementation group C coding sequence (FAC) has been described² and 15% of FA patients harbour mutations in FAC.^{3,4} 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 sequence⁵ near the 3' end of the gene that contains only a single mismatch (table A). Experimental evidence shows that a 3' to 5' orientation of the consensus sequence, such as observed in the FAC gene, is less effective, but does not prevent p53 binding.⁶ The location of a p53 binding site other than within the 5' promoter region is somewhat unusual, but has a precedent in the mdm2 gene.⁷ FA cells show chromosomal instability and a defective cell cycle, both of which could derive from impairment of some p53 mediated function.⁸

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

Relative positions of the proposed motifs within the human FAC protein sequence (drawing not to scale).

A p53 binding sites

Consensus	RRRCW	WGYYY	(N) ⁰⁻¹³	RRRCW	WGYYY
Human FAC sequence	¹⁸⁴⁸ AGACA	TGTTC	C	AGACC	AGTTC ¹⁸²⁸
Mouse FAC sequence	²⁰¹⁰ AGACA	TGTTC	C	AGACC	AGTTC ¹⁹⁹⁰

B Leucine zipper motifs

Consensus	LXXXXXXXXXXXXXXXXXXXXXXXXL-----LXXXXXXXXXXXXXXXXXXXXXXXXL
Human FAC sequence	³⁸ VAQFQEFRLRKMVEALKEMDSNT ⁵⁹ ----- ²³⁴ LPMSAVVCLWLRHLPLEKAMLHL ²⁵⁷
Mouse FAC sequence	³⁸ LSGFQEFRLRQMYEILKEMDSAIL ⁶¹ ----- ²³⁵ VPTSSVSVSLWFRHLPLEKATLHL ²⁵⁸

repeats. The second of the zipper motifs is preceded both by a helix-turn-helix region (with the crucial glycine 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.⁹ The strong conservation between the murine¹⁰ and human motifs (table) argues for their functional significance: the conservation amounts to 100% for the p53 binding site motif (*v* 78% in the remainder of the sequence), and to 90% *v* 79% for the amino acid residues within the leucine zipper domains.

The apparent contradiction between nuclear¹¹ and cytoplasmic location^{12,13} of the FAC protein can be reconciled if one assumes that the inactive bulk of the protein is located in the cytoplasm, and that transfer into the nucleus and subsequent activation occurs only in response to DNA damage.

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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 myofibrillar disarray.^{1,2} FHC is genetically heterogeneous. Four loci have been identified: CMH1 on chromosome 14, CMH2 on chromosome 1, CMH3 on chromosome 15, and CMH4 on chromosome 11, and a fifth locus exists.³ On these loci, three genes have been identified, respectively encoding the cardiac sarcomeric proteins β myosin heavy chain, troponin T, and α tropomyosin.^{4,5} For these three genes, there is also allelic heterogeneity.³ The gene implicated in the CMH4 locus⁶ is not yet known. We attempted to ascertain whether the gene COX8, which is localised at 11q12-q13 and encodes the subunit VIII of the cytochrome c oxidase complex (COX),⁷ 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.⁸ 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.^{9,10} All these characteristics make the COX8 gene a good candidate for the CMH4 locus, and analyses were performed on family 714 in which this locus was described.⁶

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 (*Bam*HI, *Bcl*II, *Bgl*II, *Eco*RI, *Eco*RV, *Hind*III, *Hinf*I, *Msp*I, *Pst*I, *Pvu*II, *Rsa*I, and *Taq*I). 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,⁷ northern blot analyses were performed, using 10 μ g of total RNA purified from lymphoblastoid cell lines of four affected and four healthy people, and COX8 cDNA was used as specific probe.¹² 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 μ g of total RNA purified from lymphoblastoid cell lines of four affected and four healthy people. The strategies of am-

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COX8 cDNA amplification and sequencing strategies. COX8 cDNA is depicted as described in Rizzuto *et al.*⁷ (A) Reverse transcription and amplification using poly-T primer and F 1-22 specific primer. (B) Amplification of cDNA 5' end using the 5'-RACE method: reverse transcription of COX8 cDNA with R 231-250 specific primer, followed by addition of a poly-C tail at the 5' end of this cDNA, on which what is termed a 5'-RACE primer is anchored, to amplify the DNA fragment with the R230-250 specific primer. Arrows indicate the different sequencing steps of DNA fragments A and B. The hitherto unidentified sequence of 42 nucleotides at the 5' end of the cDNA is also presented.