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ONLINE MUTATION REPORT

Broader geographical spectrum of Cohen syndrome due to *COH1* mutations

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Cohen syndrome (COH1: MIM 216550) is an autosomal recessive disorder, first described in 1973.¹ Cardinal clinical features of Cohen syndrome include microcephaly, non-progressive mental retardation, characteristic facial features, neutropenia, and ophthalmologic findings.² It is overrepresented in Finland, though cases have been reported worldwide.

The genetic locus for Cohen syndrome was mapped to chromosome 8q in several Finnish pedigrees.^{3,4} Recently, a novel gene, *COH1*, in this locus was shown to carry mutations in many patients with Cohen syndrome.⁵ *COH1* is a large gene, consisting of 62 exons and encoding a protein of 4022 amino acids, whose biological function is not known. So far all the patients with reported mutation in the *COH1* gene are of Finnish or other northern European origin. Therefore it has not been known if the *COH1* gene is responsible for those cases of Cohen syndrome outside northern Europe.

Here we report novel mutations in the *COH1* gene in four non-Finnish (Omani, Saudi Arabian, Japanese, and French) pedigrees, demonstrating that *COH1* mutations are responsible for Cohen syndrome in non-Finnish populations. Variable phenotypes among these patients supports the idea that non-Finnish Cohen syndrome due to *COH1* mutations has a broader clinical spectrum than the Finnish subtype. We also perform the first expression analysis of the mouse *COH1* homologue, in order to investigate the pathogenesis of microcephaly in Cohen syndrome.

METHODS

Patients

Four pedigrees with a total of 11 affected children from a wide geographical distribution (Omani, Saudi Arabian, Japanese, and French) were enrolled into the study of *COH1* mutations. The pedigree structures are shown in figure 1. The patients were identified as having features of Cohen syndrome according to the proposed diagnostic criteria.^{2,6} Clinical information of the four pedigrees is presented in table 1. We studied these pedigrees according to a study protocol approved by the institutional review board of Beth Israel Deaconess Medical Center and by the participating institutions. Formal ophthalmologic examinations were performed by ophthalmologists, though electroretinography was not performed. MRI images of the brain were obtained as a part of diagnostic work-up for mental retardation in some patients. Blood samples from affected children, as well as unaffected siblings and parents, when available, were collected after informed consent was obtained. DNA was extracted using standard protocols.

Mutation analysis

Primers were designed to amplify each individual exon of the full length *COH1* transcript (GenBank accession no. AY223814). Some larger exons required more than two

Key points

- Our work aimed at identifying new mutations in the *COH1* gene in non-Finnish Cohen syndrome patients, and to perform the first expression study of the *COH1* gene.
- We found four novel mutations of the *COH1* gene in four non-Finnish pedigrees, providing evidence that *COH1* is responsible for Cohen syndrome over a wide geographical distribution. Some patients deviate from the typical Finnish Cohen syndrome phenotype, for instance in lacking microcephaly, showing that the clinical spectrum of *COH1* mutations is wider in non-Finnish populations. This may be consistent with the absence of the Finnish founder mutation in our study population.
- We performed the first expression analysis of the *COH1* homologue (*Coh1*) in the mouse brain. *Coh1* is expressed widely in neurons of the postnatal brain, but has a low level of expression embryonically, suggesting that the cardinal role of the *COH1* gene may be in neuronal differentiation, but not in proliferation.

primer pairs, and a total of 88 primer pairs were used. Primer sequences used for mutation analysis are available upon request. PCR products from genomic DNA were purified using PSI Clone kit (Princeton Separations), and direct sequencing was performed using BigDye Terminators kit (Applied Biosystems) and ABI 3700 capillary sequencers (Applied Biosystems).

Identification of the mouse homologue of the *COH1* gene and in situ hybridisation

A BLAST analysis of the human *COH1* gene was performed against the public database (<http://www.ncbi.nih.gov/BLAST/>) and the Celera mouse genome sequence database (<http://www.celera.com/>). Predicted mouse transcripts for the RIKEN cDNA c330002D13 gene (GenBank accession no. XM_283276 and Celera ID no. mCT5410) were identified and appeared to represent a partial sequence of the mouse *COH1* homologue, and a PCR primer pair was designed to amplify a 907 base pair segment of this transcript, which corresponds to the amino acid residues 82 through 385 of the human COH1 protein. The primers were synthesised as chimeras, with T3 or T7 promoter sequence added to the 5'-end. PCR reaction was performed, using first-strand cDNA made from mouse 15 day embryo total RNA (BD Biosciences) as a template. Non-radioactive in situ hybridisation was performed as described,⁷ using a digoxigenin

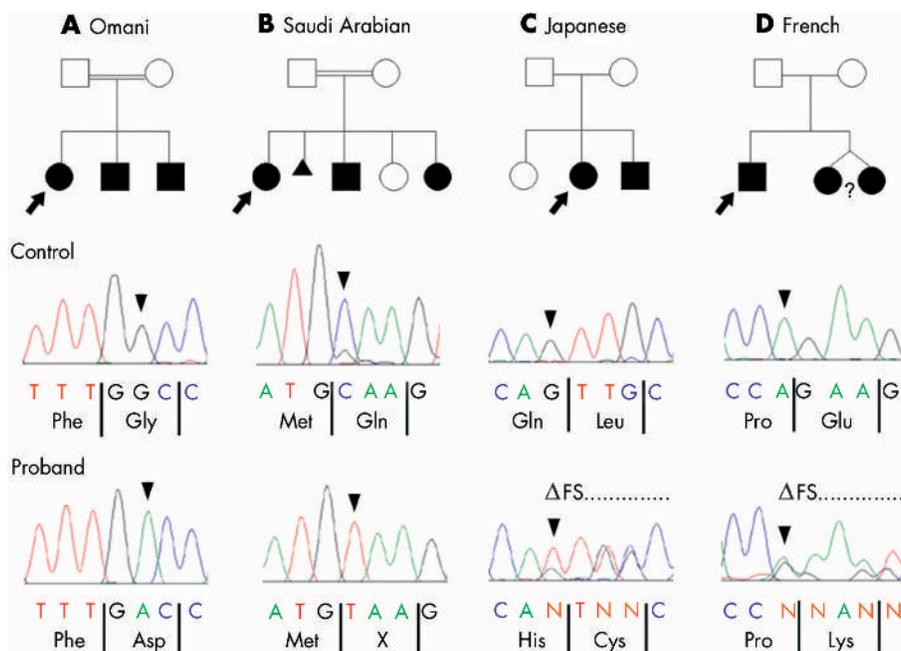


Figure 1 Pedigree structure and novel mutations in the four families with Cohen syndrome. (A) The Omani family carried a homozygous 7934 G>A transition in exon 43, which results in a G2645D missense mutation. (B) The Saudi Arabian family carried a homozygous 1219 C>T transition in exon 9, resulting in Q407X nonsense mutation. (C) The Japanese family was a putative compound heterozygote with a heterozygous one base pair deletion (7221delG) in exon 40, which results in frameshift. No other mutant allele was found in this family in the coding region of the *COH1* gene. (D) The French family was a compound heterozygote with a one base pair deletion (11598delA) in exon 61, which results in frameshift, and a previously reported 7051 C>T transition in exon 39, resulting in R2351X nonsense mutation (not shown). Nucleotides mutated in the patients are indicated by arrowheads. Deletion followed by frameshift is indicated by "ΔFS.....". In the heterozygous patients (probands of C and D), protein translation of the mutant allele is shown.

(DIG)-labelled cRNA probe generated from the PCR product and frozen mouse brain sections.

RESULTS AND DISCUSSION

Mutation detection

We have identified four novel *COH1* mutations and one previously reported mutation in the four pedigrees studied (fig 1). The identified mutations are summarised in table 2. Patients in the Omani pedigree were homozygous for a novel mutation, 7934 G>A in exon 43. This mutation is predicted to substitute aspartic acid for glycine at residue 2645 (G2645D), which changes

charge and so is very likely harmful. Both parents were confirmed to be heterozygous for this mutation. The clinical features of the patients in the Omani family are not milder than others with nonsense or frameshift mutations, suggesting that this missense mutation is also probably a null mutation.

Affected individuals in the Saudi Arabian pedigree were homozygous for a second novel mutation, 1219 C>T in exon 9. This mutation changes a glutamine residue at position 407 of 4022 to a termination codon (Q407X), creating a severely truncated protein. Parents were heterozygous for the mutation, and the unaffected sibling tested was homozygous for the wild-type allele.

Table 1 Summary of clinical findings in four non-Finnish Cohen syndrome pedigrees

Pedigree	Patient number	Age (years)	Sex	Head circumference	Typical facial features	Ophthalmologic findings*	Neutropenia†	Brain MRI
Omani	1	10	Female	-5.6 SD	Yes	Myopia, retinal dystrophy	No	Relatively large corpus callosum
	2	8	Male	-5.8 SD	Yes	Myopia	No	N/A
	3	5	Male	-5.3 SD	Yes	Myopia	No	N/A
Saudi Arabian	4	9	Female	-5.0 SD	Yes	Retinal dystrophy	N/A	Relatively large corpus callosum, mild inferior vermian hypoplasia
	5	7	Male	-4.6 SD	Yes	N/A	N/A	Mild vermian hypoplasia
Japanese	6	2	Female	-3.8 SD	Yes	N/A	N/A	Normal
	7	13	Female	+0.6 SD	Yes	N/A	No	N/A
French	8	11	Male	-2.7 SD	Yes	N/A	N/A	N/A
	9	27	Male	-2.1 SD	Yes	Retinitis pigmentosa, myopia	Yes	N/A
	10	24	Female	-1.2 SD	Yes	Maculopathy with amblyopia (left), peripheral pigmentation abnormalities (right), bilateral polar cataracts	Yes	N/A
	11	24	Female	-2.7 SD	Yes	Myopia, astigmatism, bilateral polar cataracts	Yes	N/A

*Ophthalmologic findings are according to formal examination by ophthalmologists. Electroretinography was not performed; †presence or absence of neutropenia was determined according to reference range set by each laboratory where the blood count measurement was performed. Patients with no neutropenia did not undergo repeated measurements. N/A, not available.

Table 2 Summary of the mutations in four non-Finnish Cohen syndrome pedigrees

Pedigree	Mutation	Protein change	Exon	Homozygous/heterozygous	Novel/reported
Omani	7934 G>A	G2645D	43	Homozygous	Novel
Saudi Arabian	1219 C>T	Q407X	9	Homozygous	Novel
Japanese	7221 del G	Q2407H...V2414X	40	Heterozygous	Novel
French	7051 C>T	R2351X	39	Heterozygous	Reported
	11598 del A	E3867K...V3877X	61		Novel

Patients in the Japanese pedigree were heterozygous for a novel *COH1* mutation, 7221delG in exon 40. This mutation creates a translational frameshift after residue 2407, with predicted premature termination at residue 2413 (Q2407H...V2414X). Their mother also carried this base pair change in the heterozygous state, while their father was not available for testing. These siblings are presumably compound heterozygotes for two different *COH1* gene mutations, but a second mutation was not found despite complete sequencing of all known coding exons of the full-length *COH1* transcript.

The patients in the French pedigree were all compound heterozygotes for *COH1* mutations. One mutation, 7051 C>T in exon 39, has already been reported in a Belgian patient,⁵ and is predicted to change an arginine residue at position 2351 to a termination codon (R2351X). The other mutation, 11598delA in exon 61, has not been reported before, and causes a translational frameshift, leading to premature termination after the addition of 10 abnormal amino acids (E3867K...V3877X; fig 1). None of the four novel mutations we found were observed in at least 56 Caucasian (112 chromosomes) and 26 Middle Eastern (Omani) unrelated normal control individuals (52 chromosomes).

It is interesting to note that we did not find in any of the pedigrees we studied the mutation that seems to be most prevalent in Finland, 3348_3349delCT. Out of 27 Finnish patients who were studied previously, 26 had at least one allele of this mutation.⁵ Furthermore, it appears likely that different mutations prevail among different ethnic groups, although further mutation screening is necessary to confirm this.

Genotype–phenotype correlation

No clear genotype–phenotypic correlation was apparent in the patients we studied. However, the patients reported here seem to show more phenotypic variability compared to the Finnish Cohen syndrome patients, who have a highly homogeneous clinical phenotype. For example, patients 7 and 10 in our study lack microcephaly. In addition, patients 1, 2, 3, and 7 were not found to have neutropenia, though neutropenia may be intermittent, and this cannot be ruled out as these patients had blood counts tested on one occasion. Both microcephaly and neutropenia appear to be invariant features in reported Finnish patients,² and reported patients from other populations may lack these features.⁶ The variability in clinical features that we found may be consistent with the absence of a Finnish founder mutation in our study population and, possibly, in other reported non-Finnish Cohen syndrome patients.

One of the mutations found in the French family was a novel frameshift mutation that truncates only the C-terminal region of the predicted protein. Perhaps this mutation results in partial loss of function of the COH1 protein, which may account for the relatively preserved head circumference in this pedigree, though otherwise it shows a classic phenotype similar to the Finnish Cohen syndrome. Though the biological function of the COH1 protein is not known, there

is a potential endoplasmic reticulum retention signal detected by the PSORT II program at the C-terminal region^{5, 8}; therefore, the French mutation may partially disrupt the function of the protein by interfering with subcellular localisation.

Identification of the mouse homologue of the *COH1* gene and in situ hybridisation

A partial sequence for the mouse homologue of the *COH1* gene was identified through BLAST searches. Predicted mouse transcripts for the RIKEN cDNA c330002D13 gene (GenBank accession no. XM_283276 and Celera ID no. mCTS410), which appear to represent a partial sequence of the mouse *COH1* homologue (*Coh1*), were identified. The predicted amino acids translated from XM_283276, for example, are 90% identical to the first 415 amino acids of the human COH1 protein. In situ hybridisation was performed using a probe designed from this sequence. In the mouse brain at postnatal day 21, roughly equivalent to early childhood to juvenile age in humans, the *Coh1* gene was expressed widely in the central nervous system. In the cerebellum, a high level of expression was seen in the internal granule layer and Purkinje cells, but not in the white matter (fig 2A). In the cerebral cortex, a high level of expression was seen in all cortical layers except for layer I, but expression was absent in the subcortical white matter (fig 2B). A high level of expression was also seen in the dentate gyrus of hippocampus and throughout Ammon's horn. The adult mouse brain showed a similar pattern of expression (data not shown). These data suggested that all or most neurons in the adult brain express *Coh1*, with no obvious regional differences.

In contrast to the adult brain, the embryonic mouse brain showed low or undetectable levels of *Coh1* expression using

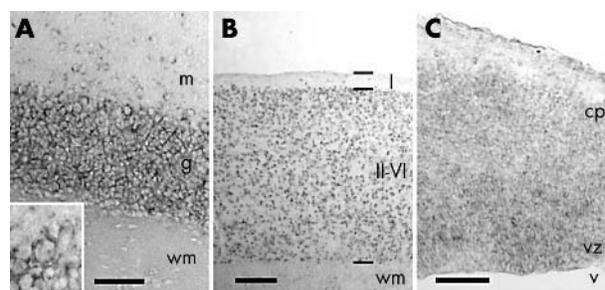


Figure 2 In situ hybridisation of the mouse homologue of the *COH1* gene. (A) Cerebellum of the mouse at postnatal day 21. A high level of expression is seen in the internal granule layer (g), and little expression is seen in the molecular layer (m) and white matter (wm). Purkinje cells (inset) also show expression. (B) Cerebral cortex of the mouse at postnatal day 21. Many cells in layers II through VI of the cerebral cortex (II–VI) show expression, but only a few cells are positive in layer I of the cortex (I) and white matter (wm). (C) Cerebral wall of the mouse at embryonic day 14. A low level of expression is seen in both the cortical plate (cp) and ventricular zone (vz). Scale bars represent 100 μ m in A and C, 250 μ m in B.

the same labelled probe (fig 2C). Repeated attempts gave a low signal not greatly different from background and less intense than hybridisation seen with a sense control probe. Thus, *Coh1* appears to be widely expressed in neurons of the postnatal and adult brain, but is not highly expressed in the embryonic brain.

Our results indicate that the gene is expressed widely in the neurons of the central nervous system. However, there was little expression in the white matter tracts of cerebrum and cerebellum, suggesting that the gene is not expressed in glial cells at a high level. In addition, the level of expression appeared to be higher in the postnatal brain than in the embryonic brain. This may suggest that the *COH1* gene primarily functions in postmitotic neurons, and not in neuronal progenitor cells. These findings are consistent with the fact that patients with Cohen syndrome are generally born with normal head circumference and develop microcephaly postnatally.² *COH1* mutations may cause microcephaly by disrupting, for example, dendritic or axonal outgrowth, which continues postnatally and is important for normal postnatal brain growth.

Further molecular genetic studies, in combination with biochemical studies of the COH1 protein, will likely help us understand the pathogenetic mechanism of Cohen syndrome. In addition, studying additional patients with atypical phenotypes (for example, less severe mental retardation or without neutropenia) may provide additional insights into the function of the *COH1* gene. Furthermore, defining the clinical-genetic spectrum of Cohen syndrome will potentially benefit patients and their families by allowing accurate diagnosis of the syndrome.

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REFERENCES

- 1 **Cohen MM Jr**, Hall BD, Smith DW, Graham CB, Lampert KJ. A new syndrome with hypotonia, obesity, mental deficiency, and facial, oral, ocular, and limb anomalies. *J Pediatr* 1973;**83**(2):280-4.
- 2 **Kivitie-Kallio S**, Norio R. Cohen syndrome: essential features, natural history, and heterogeneity. *Am J Med Genet* 2001;**102**(2):125-35.
- 3 **Tahvanainen E**, Norio R, Karila E, Ranta S, Weissenbach J, Sistonen P, de la Chapelle A. Cohen syndrome gene assigned to the long arm of chromosome 8 by linkage analysis. *Nat Genet* 1994;**7**(2):201-4.
- 4 **Kolehmainen J**, Norio R, Kivitie-Kallio S, Tahvanainen E, de la Chapelle A, Lehesjoki AE. Refined mapping of the Cohen syndrome gene by linkage disequilibrium. *Eur J Hum Genet* 1997;**5**(4):206-13.
- 5 **Kolehmainen J**, Black GC, Saarinen A, Chandler K, Clayton-Smith J, Traskelin AL, Perveen R, Kivitie-Kallio S, Norio R, Warburg M, Fryns JP, de la Chapelle A, Lehesjoki AE. Cohen syndrome is caused by mutations in a novel gene, COH1, encoding a transmembrane protein with a presumed role in vesicle-mediated sorting and intracellular protein transport. *Am J Hum Genet* 2003;**72**(6):1359-69.
- 6 **Chandler KE**, Kidd A, Al-Gazali L, Kolehmainen J, Lehesjoki AE, Black GC, Clayton-Smith J. Diagnostic criteria, clinical characteristics, and natural history of Cohen syndrome. *J Med Genet* 2003;**40**(4):233-41.
- 7 **Berger UV**, Hediger MA. Differential distribution of the glutamate transporters GLT-1 and GLAST in tanycytes of the third ventricle. *J Comp Neurol* 2001;**433**(1):101-14.
- 8 **Nakai K**, Kanehisa M. A knowledge base for predicting protein localization sites in eukaryotic cells. *Genomics* 1992;**14**(4):897-911.