LETTERS TO THE EDITOR

Mutations in the Norrie disease gene: a new mutation in a Japanese family

EDITOR,—Norrie disease (ND) is a genetic disorder causing bilateral blindness in early infancy. The major ocular disease is retrolental masses caused by undifferentiated, proliferated vitreous and retina, accompanied by maldeveloped anterior segments. In later years, corneal opacities and secondary cataract develop, eventually leading to atrophy of the eyelid. Histological features include prominences, vitreoretinal dysplasia consisting of undifferentiated vitreous and retina. A certain number of patients show psychomotor retardation or hearing impairment as part of a multisystem disorder. 1

ND is believed to be a rare disease transmitted in an X linked manner. The disease has received attention as a severe disease and in the context of maldevelopment of the neuroectodermal involving the retina and brain. In recent years, a candidate gene for ND was isolated and characterised. 2 3 The disease association of the ND gene has been substantiated by identification of microdeletions or mutations in white patients and also in Japanese families. There is marked inter-familial variability in the nature of the genetic defect causing ND, but the molecular-clinical correlation remains to be elucidated. 4 9 A mutation in the ND gene may also be responsible for a separate clinical entity — X linked familial exudative vitreoretinopathy. 10 We report a Japanese ND family with a previously unreported mutation in the ND gene, together with a review of the literature regarding molecular genetic defect of ND.

CASE REPORT
The proband was a 16-year-old Japanese male who had been blind in both eyes since childhood. On examinations, he had normal psychomotor development and normal hearing ability. Pupils were not responsive to light. The right eye had a retrolental fibrous mass accompanied by posterior synechiae, and the left eye had posterior synechiae and dense secondary cataract. Both retinas were invisible. Eyeballs showed a gradual shrinkage in subsequent follow up studies. The parents were normal. The other family members of the proband had lived in the Tokyo area, located in central Japan. Being compatible with an X linked trait, two maternal uncles of the proband were blind in both eyes and were otherwise normal.

We obtained peripheral blood with informed consent and analysed DNA samples of the proband, his mother, two possible carriers of his family, and 40 unrelated controls, for mutations in the ND gene by the method described in detail elsewhere. 8 PCR amplification of coding exons of the ND gene 4 was performed using wild type oligo-nucleotide primer pairs. 5 Amplified DNA fragments of exon 2 and exon 3 were purified, and the nucleotide sequence was determined by dye terminator cycle sequencing with an automated nucleotide sequencer (ABI, model 373A). For identification of the mutation, PCR products of exon 3 of the ND gene were digested with Aci I and separated on 6% polyacrylamide gel electrophoresis. The T to C mutation at codon 95 of exon 3 created an Aci I restriction site, allowing the detection of individuals with hemizygous or heterozygous for the mutation.

Nucleotide sequencing of PCR products of exon 3 of the ND gene from the proband showed a single nucleotide alteration at codon 95 (TGC to TGC), which converts cysteine to arginine. 3 As illustrated in Figure 1, PCR restriction revealed that the proband was hemizygous for the mutation, and that his mother and two other possible carriers showed both the wild type and mutant type DNA at codon 95, as expected for heterozygotes. On the other hand, 40 unrelated normal controls and 15 unrelated patients with other ocular diseases showed only the wild type DNA at codon 95 of exon 3. Other mutations in ND reported in the literature 4-9 and in X linked familial vitreoretinopathy 10 were not detected by nucleotide sequencing in any samples.

Table 1 summarises mutations of the ND gene that have been reported to date. 2-10 A variety of mutations has been identified in the ND gene in ND families, including missense mutation, nonsense mutation, deletion or insertion of bases, initiation codon mutation, and splicing defect resulting in conversion of amino acids, production of truncated protein, etc. Clinical manifestations in these molecular diagnosed cases are also variable, with all of the cases showing severe ocular disease with variable mental retardation and hearing impairment. It is thus worthwhile establishing if a correlation exists between the genotype and the phenotype, although from the results obtained to date there is no definite genotype-phenotype correlation. Indeed, there is inter- and intrafamilial variation, as patients with identical mutations show a diversity of clinical manifestations, either restricted to the eye or syndromic in the eye and brain. This is also the case for microdeletions of the gene (Table 1). A definite conclusion cannot yet be

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<th>Reference family</th>
<th>Mutation codon</th>
<th>Type</th>
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<th>Mental retardation</th>
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Figure 1 Identification of a mutation at codon 95 by PCR-restriction digest in family 1. M = molecular weight markers (bp). The mutation at codon 95 creates an Aci I restriction site. The proband (III-1) shows a single digested fragment (163 bp, closed arrowhead), compatible with hemizygosity for mutant DNA; residual smaller digested fragment (36 bp) is not visible in the figure. His mother (II-1) and two female relatives (I-1, II-2) show both the mutant DNA and undigested wild type DNA (192 bp, open arrowhead), as expected for heterozygotes. C = an unrelated normal male who shows only wild type DNA.

*Family or patient identification from the literature.

COMMENT
The present ND family shows a distinct genetic defect with a missense mutation at codon 95 of exon 3 of the ND gene, in a manner expected for an X linked genetic disease. This is the third Japanese family with ND in whom mutations of the ND gene were identified. 9 Table 1 summarises mutations of the ND gene that have been reported to date. 2-10 A variety of mutations has been identified in the ND gene in ND families, including missense mutation, nonsense mutation, deletion or insertion of bases, initiation codon mutation, and splicing defect resulting in conversion of amino acids, production of truncated protein, etc. Clinical manifestations in these molecular diagnosed cases are also variable, with all of the cases showing severe ocular disease with variable mental retardation and hearing impairment. It is thus worthwhile establishing if a correlation exists between the genotype and the phenotype, although from the results obtained to date there is no definite genotype-phenotype correlation. Indeed, there is inter- and intrafamilial variation, as patients with identical mutations show a diversity of clinical manifestations, either restricted to the eye or syndromic in the eye and brain. This is also the case for microdeletions of the gene (Table 1). A definite conclusion cannot yet be
reached as most of the mutations reported to date have only been found in one family. Although ND is believed to be rare, it is likely that many cases remain undiagnosed. The predominant ocular feature of ND – retrolental mass – is not necessarily pathognomonic, and it is clinically difficult to exclude other diseases of undefined aetiology, particularly in sporadic and non-syndromic cases. The ongoing molecular assessment of the ND gene will enable us to make definitive diagnoses in cases in which mutations are detected and will also provide useful information for genetic counselling. From the molecular studies to date, we are aware of the marked heterogeneity of pathogenic mutations in the ND gene and, hence, direct sequencing of the gene has to be performed to test for unknown mutations.

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Choroidal ischaemic plaques in sarcoidosis

EDITOR—Sarcoidosis is a multisystem granulomatous disorder of unknown aetiology. It may present with a variety of ocular inflammatory signs, including anterior or posterior uveitis, vitritis, periphlebitis, and choriotremitis; choroidal lesions may manifest as hyper- or hypopigmentation.1

We present a patient manifesting multiple placoid choroidal lesions with a previous history of sarcoidosis.

CASE REPORT

A 45-year-old white man presented in July 1994 with a severe bilateral acute anterior uveitis with raised intraocular pressure in the left eye. He also had bilateral punctate epithelial erosions and band keratopathy of his left cornea. Fundal examination revealed multiple placoid areas of choroidal pallor widespread throughout both fundsi, but concentrated around the posterior poles (Fig 1).

Past medical history included pulmonary consolidation in 1989 at which point bronchial biopsy revealed non-caseating granulomata. He had had an episode of left herpes zoster opthalmicus with corneal involvement in 1991. In April 1994 he presented with a recurring, itchy skin rash of oedematous papules on the shoulders, back, chest, and proximal limbs; biopsy again revealed non-caseating granulomata.

He was admitted for investigation and his uveitis treated with topical steroids, central visual acuity returning to normal as this subsided. Intravenous fundal angiography was performed with fluorescein (IVF) and indocyanine green (ICG); IVF showed no early marking (Fig 2A) but areas of late staining hyperfluorescence corresponding to the placoid lesions (Fig 2B) and ICG revealed a marked, sustained hypofluorescence in the same areas (Fig 3).

Routine blood testing revealed a marked hypercalcaemia (corrected calcium 3.60 mmol/l) and renal impairment (urea 19.2 mmol/l, creatinine 334 umol/l). He was also noted to have a benign paraproteinemia (IgG kappa 7 g/l). He was treated with intravenous hydration, diuretics, and corticosteroids and improved rapidly.

Four weeks after presentation his anterior uveitis had cleared and the fundal placoid lesions had begun to show signs of hypopigmentation.

COMMENT

Ocular sarcoidosis may present with choroidal lesions in 29–50% of cases.1

Appearances may vary from patches of creamy yellow depigmentation to a serpiginous appearance extending out from the peripapillary region; and have also mimicked birdshot chorioidopathy.3 The single reported case of serpiginous choriditis associated with sarcoidosis in the literature was marked by early masking and late staining on IVF.4

Lesions mimicking birdshot chorioidopathy have been described as hypopigmented, flat lesions which manifested late staining on IVF.3 However, fundal distribution was mainly confined to the peripapillary and nasal regions and active investigation of patients with birdshot chorioidopathy has revealed no link with sarcoidosis.

The appearance demonstrated in our patient is more reminiscent of acute posterior multifocal placoid pigment epipletiopath, and indeed this has been linked with areas of

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