

SCIENTIFIC REPORT

Cone dystrophy phenotype associated with a frameshift mutation (M280fsX291) in the α -subunit of cone specific transducin (*GNAT2*)

M Michaelides, I A Aligianis, G E Holder, M P Simunovic, J D Mollon, E R Maher, D M Hunt, A T Moore

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Aim: To describe the phenotype of a three generation consanguineous Pakistani family containing six individuals with autosomal recessive cone dystrophy caused by mutation in *GNAT2*.

Methods: Five of the six affected individuals underwent an ophthalmological examination, electrodiagnostic testing, fundus photography, autofluorescence imaging, and detailed psychophysical testing.

Results: All five examined patients had a history of nystagmus from infancy, photophobia, defective colour vision, and poor visual acuity. The nystagmus in three of the individuals had lessened with time. Fundus examination revealed an abnormal foveal appearance, without frank atrophy or pigmentation. Electroretinography (ERG) revealed absent ISCEV cone flicker ERGs with some preservation of responses to short wavelength stimulation. Rod ERGs showed no definite abnormality, but maximal (mixed rod-cone) response α -wave amplitudes were mildly subnormal. Rudimentary residual colour vision was detected in three individuals. There is clinical evidence of progressive visual acuity reduction in two older individuals.

Conclusion: Mutation in the α -subunit of cone specific transducin (*GNAT2*) is characterised by an infantile onset cone dystrophy. Some affected individuals may show deterioration of visual acuity with time.

Fundus examination is usually normal, but electroretinography reveals absent photopic (cone) responses and normal scotopic (rod) responses. Individuals with incomplete achromatopsia retain some colour vision.

Achromatopsia is recessively inherited and genetically heterogeneous. To date, three achromatopsia genes have been characterised, the first two described being *CNGA3*^{8–10} and *CNGB3*,^{11–13} located at 2q11 and 8q21 respectively. *CNGA3* and *CNGB3* code for the α and β subunits of the cGMP gated cation channel in cone cells, respectively. The gene coding for the α -subunit of cone specific transducin (*GNAT2*) was proposed as a candidate gene for achromatopsia by Mollon in 1997,¹⁴ and mutations in this gene have recently been described in patients with achromatopsia.^{15–16} However, a detailed description of the phenotype associated with *GNAT2* inactivation has not been presented. In this report we have reviewed the phenotype of the large consanguineous Pakistani family in whom we identified a novel frameshift mutation in *GNAT2* (c842_843insTCAG; M280fsX291).¹⁶

PATIENTS AND METHODS

Five affected members of a three generation, consanguineous Pakistani family with cone dystrophy were assessed after informed consent was obtained (Fig 1).

A full medical history was taken and an ophthalmological examination performed. Examined subjects also underwent colour fundus photography and fundus autofluorescence imaging using the confocal scanning laser ophthalmoscope (cSLO) (Zeiss Prototype; Carl Zeiss Inc, Oberkochen, Germany). Electrodiagnostic assessment included electro-oculography (EOG), full field electroretinography (ERG) and pattern ERG (PERG), incorporating the protocols recommended by the International Society for Clinical Electrophysiology of Vision.^{17–19} S-cone ERGs were also recorded using a previously described protocol.²⁰

Colour vision testing included the use of the Hardy, Rand, Rittler (HRR) plates (American Optical Company, New York, NY, USA), Sloan achromatopsia plates, enlarged Farnsworth D-15 (PV-16), the enlarged Mollon-Reffin (M-R) minimal test,²¹ and the Nagel anomaloscope. The PV-16, Sloan achromatopsia plates, and the M-R test were all performed under CIE Standard Illuminant C from a MacBeth Easel lamp.

The PV-16 and the enlarged M-R test were used in order to detect any residual colour discrimination that might be present in patients with low vision: the coloured discs of the PV-16 were 33 mm in diameter and those of the enlarged M-R test were 26 mm in diameter (corresponding to visual angles of 3.8 and 3.3 deg at a viewing distance of 500 mm). With the same purpose in mind, we also used a modification of the Cambridge colour test, a computerised test that allows the measurement of colour discrimination along different

The cone and cone-rod dystrophies form part of a clinically heterogeneous group of retinal dystrophies that are a major cause of childhood blindness. The major clinical features of cone dystrophies are reduced visual acuity, abnormal colour vision, photophobia, central scotomata, and often nystagmus. Cone dystrophies have been described with autosomal dominant, autosomal recessive, or X linked patterns of inheritance.^{1–2}

Cone and cone-rod dystrophies are also phenotypically heterogeneous.² Various subtypes have been identified on the basis of natural history and psychophysical and electrophysiological testing.^{3–5} These disorders may be stationary or progressive. The two well characterised stationary cone dystrophies are blue cone monochromatism, an X linked disorder in which there are only two functional classes of photoreceptor (rods and S-cones), and rod monochromatism.

Rod monochromatism or complete achromatopsia is a stationary cone dystrophy, with an incidence of approximately 1 in 30 000, in which there is an absence of functioning cone photoreceptors.^{6–7} Affected individuals usually present in infancy with nystagmus, poor visual acuity, photophobia, and complete colour blindness.

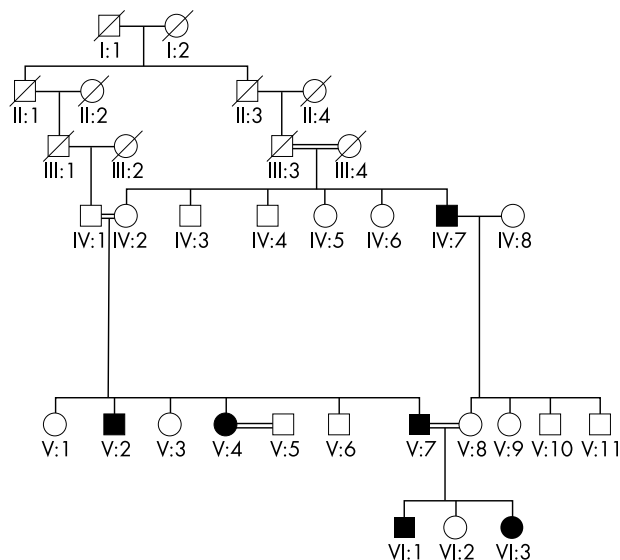


Figure 1 Family pedigree. Solid symbols indicate clinically affected individuals and open symbols represent unaffected individuals.

directions in colour space.^{22, 23} In the modified test, the stimulus array consisted of only four large discs, organised in a diamond pattern. Each disc subtended 4 degrees of visual angle at the viewing distance of 1 metre. On any presentation, one of the discs differed in chromaticity from the remaining three, and the patient's task was to identify this disc by pressing one of four buttons within 4 seconds. To ensure that the discrimination was on the basis of chromaticity, the luminance of each disc was given a random value chosen from six levels between 4 cd/m² and 24 cd/m². To establish the patient's threshold for a given direction in colour space, the chromatic difference between the target and distractor discs was adjusted by a double staircase procedure.²⁴

RESULTS

All five patients had a history of nystagmus from infancy, mild photophobia, defective colour vision, and poor visual acuity (6/60 to CF). They all described improved vision in mesopic conditions. Examination of the anterior segment was unremarkable, except for one individual (VI:3) who had a unilateral congenital cataract. Fundus examination revealed a mildly abnormal foveal appearance but without frank atrophy or pigmentation (Fig 2). Peripheral retinal examination was normal in all subjects. ERG showed absent cone responses to 30 Hz flicker, small responses to short wavelength stimulation, and normal rod specific ERGs, but mildly subnormal maximal response a-wave amplitudes (Fig 3). Autofluorescence imaging was normal in all individuals. Clinical findings are summarised in Table 1.

Two older individuals (V:2 and V:7) described a gradual deterioration of vision. Visual acuity in V:7 was 6/36 in both eyes when he first presented 30 years ago, while his current best corrected acuity is counting fingers. His brother V:2, also showed evidence of deterioration of visual acuity from 6/60 in both eyes documented 10 years ago, to counting fingers at present. Both subjects have more prominent horizontal pendular nystagmus than other affected family members.

All five patients had abnormal colour vision. At the Nagel anomaloscope all five patients exhibited a scotopic spectral sensitivity. Four patients displayed a scotopic pattern of arrangement on PV-16. One patient, V:7, unlike a typical patient with complete achromatopsia, produced only one major transposition on the PV-16. Rudimentary colour

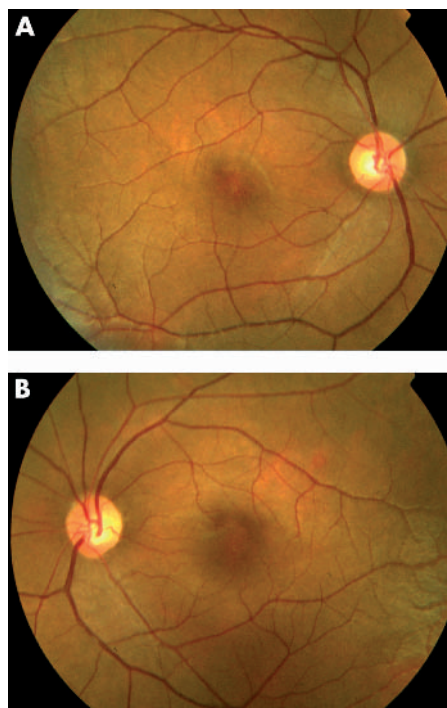


Figure 2 Fundus photographs. Mildly abnormal foveal appearance, but without frank atrophy or pigmentation.

discrimination was also detected in three individuals on testing with the M-R minimal test, with variable ability to identify the most saturated chip along the deutan and/or tritan axes. Further evidence of residual colour vision was provided by the colour discrimination ellipses produced on computerised colour vision testing. The other two individuals, V:2 and V:4, displayed no residual colour vision and showed typical achromatopic matches on Sloan plates.

DISCUSSION

The phenotype in this family with a novel homozygous frameshift mutation in the cone α -transducin gene, *GNAT2*, is characterised by mild photophobia, nystagmus, abnormal colour vision, and poor visual acuity (6/36 to CF). Electroretinography using the ISCEV protocol revealed absent cone responses, with normal rod specific ERGs, but mild reduction in maximal response a-wave amplitudes. Small photopic responses to short wavelength stimulation were detectable. On detailed colour vision testing, residual colour discrimination was detected in three individuals.

This phenotype is similar therefore to the incomplete form of achromatopsia arising from certain mutations in *CNGA3*, the gene encoding the α -subunit of the cGMP gated cation channel in cones.¹⁰ Unlike complete achromatopsia, we have been able to record S-cone ERG responses in our patients and, in two older subjects, a worsening of visual acuity with age has been documented, although we have no definite evidence of progressive deterioration in retinal function. In achromatopsia we have found only one case report of progressive retinal degeneration in the form of mid-peripheral retinal pigmentation and concentric constriction of the peripheral visual fields.²⁵ It was also reported that a few of the younger subjects in that achromatopsia series had small residual cone responses on ERG. Taken together, therefore, these findings may represent evidence that progression in retinal dysfunction may be present in at least some individuals with achromatopsia, but no natural history studies are available to corroborate this.²⁵

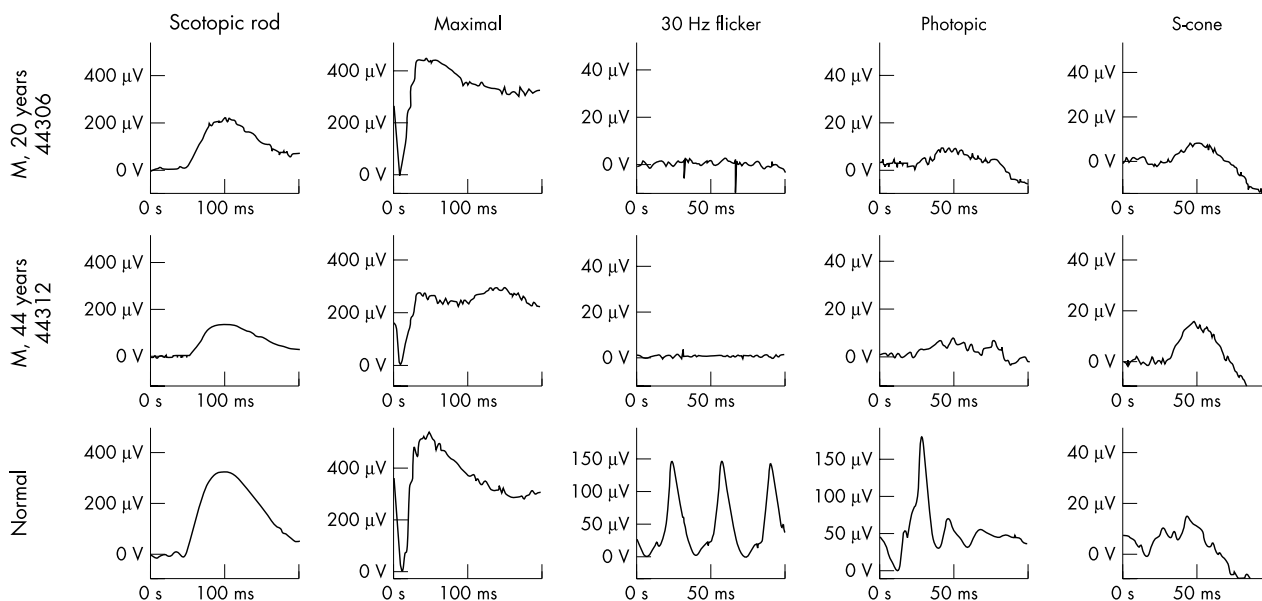


Figure 3 Electrophysiological data from two patients, a father (V:7) and son (VI:1). A normal control is shown for comparison. Note the differences in calibration between the normal and the two patients for the cone derived ERGs. Both patients have normal rod specific ERGs, with borderline subnormal a-wave in the maximal response of the son, mildly subnormal in the father. Flicker ERG is undetectable in both patients, but there is some very low amplitude activity with photopic single flash stimulation. S-cone specific stimulation, using a blue light superimposed on an orange background,²⁰ suggests some preservation of mechanisms sensitive to short wavelengths. Note the presence of an earlier peak at ~30 ms in the normal, absent in the two patients, which reflects activity from L/M cone systems.

Retinal dysfunction in our family is predominantly confined to cone photoreceptors. In cone cells, light activated photopigment interacts with transducin, a three subunit guanine nucleotide binding protein, stimulating the exchange of bound GDP for GTP. The cone α -transducin subunit (encoded by *GNAT2*), which is bound to GTP, is then released from its β and γ subunits and activates cGMP phosphodiesterase by removing the inhibitory γ subunits from the active site of this enzyme. cGMP phosphodiesterase lowers the concentration of cGMP in the photoreceptor which results in closure of cGMP gated cation channels and consequent hyperpolarisation of the photoreceptor.²⁶ Thus, the finding of a germline *GNAT2* mutation in a family with cone dystrophy is consistent with the known function of the

GNAT2 product. Furthermore, mutations in human rod specific α -transducin, which is 83% homologous to cone α -transducin,^{27, 28} have been shown to be associated with the Nougaret form of congenital stationary night blindness.²⁹

The frameshift mutation identified in our family results in a truncated protein that lacks 63 amino acids from the carboxy terminal.¹⁶ All the *GNAT2* mutations identified by Kohl *et al* would also result in premature translation termination and in protein truncation at the carboxy terminal.¹⁵ This region contains important functional domains of α -transducin which have been shown to interact with the rhodopsin³⁰ and phosphodiesterase γ -subunits.³¹ However, if this mutation were to lead to a complete lack of α -transducin function, it is difficult to explain the residual

Table 1 Summary of clinical findings

Patient	Age	VA	Refraction	Horizontal pendular nystagmus	ERG	Fundus	M-R colour vision test
V:2	35	R CF	-3.5/+3.0×30	Prominent	Absent 30 Hz cone responses; Normal rod specific responses	Abnormal foveal appearance	P(no)D(no)T(no)
		L CF	-4.0/+3.0×160				P(no)D(no)T(no)
V:4	41	R 6/60	-2.0/+2.0×110	Absent	Absent 30 Hz cone responses; Normal rod specific responses	Abnormal foveal appearance	P(no)D(no)T(no)
		L 6/60	-2.5/+1.5×70				P(no)D(no)T(no)
V:7	44	R CF	-2.0/+2.0×135	Prominent	Absent 30 Hz cone responses Normal rod specific responses	Abnormal foveal appearance	P(no)D(7)T(5)
		L CF	-1.5/+1.5×45				P(no)D(6)T(5)
VI:1	20	R 6/60	+1.5/+1.5×110	Absent	Absent 30 Hz cone responses Normal rod specific responses	Abnormal foveal appearance	P(no)D(7)T(4)
		L 6/60	+1.0/+2.0×80				P(no)D(no)T(5)
VI:3	19	R CF	Balance	Minimal	Absent 30 Hz cone responses Normal rod specific responses	Abnormal foveal appearance	NA
		L 6/60	-2.0/+3.0×75				P(no)D(6)T(5)

ERG = counting fingers; M-R = Mollon-Reffin test. The letters give the axis P = protan, D = deutan, and T = tritan. The number in parentheses gives the least saturated chip that could be discriminated from the greys; VI:3 has a right sided congenital cataract.

colour vision along deutan and tritan axes in these individuals, if these are entirely cone mediated mechanisms. It is possible that the mutation results in a protein that, although severely reduced in efficacy, may still show some residual α -transducin function. Alternatively, there may be some redundancy within the cone phototransduction pathway that allows a level of continued function despite suboptimal or absent function of one of the components of the cascade.

The human cone transducin α -subunit (*GNAT2*) gene was completely characterised by Morris and Fong in 1993²⁷ and the evidence that this gene is expressed in all three cone types comes from the immunohistochemical demonstration that an antibody raised against cone α -transducin peptides cross-reacts with all three classes of cone photoreceptor in the human retina.³² This does not however definitively rule out the possibility that S-cones may express an alternative form of α -transducin since identical epitopes may be present on both forms. It may also be significant that Southern blot analysis of human genomic DNA indicated that there may be more than one cone α -transducin gene.³² Therefore, although there are no subsequent studies that provide any direct evidence for an S-cone specific cone α -transducin, it remains a possibility that *GNAT2* is not expressed in S-cones, and that the residual S-cone function detected in our family arises from the use of this other distinct form of α -transducin. In this case therefore, the residual tritan colour discrimination may be accounted for by a comparison of quantum catches in the remaining functional S-cones and rod photoreceptors, in the manner proposed to underlie colour discrimination detected in blue cone monochromatism.³³

A detailed description of the phenotype associated with mutation in the α -subunit of cone specific transducin has not been previously reported. It is characterised by a cone dystrophy with an infantile onset, a deterioration of visual acuity with time in older individuals, and residual S-cone function.

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Authors' affiliations

M Michaelides, D M Hunt, A T Moore, Institute of Ophthalmology, University College London, 11–43 Bath Street, London EC1V 9EV, UK
I A Aligianis, E R Maher, Section of Medical and Molecular Genetics, Department of Paediatrics and Child Health, University of Birmingham, Edgbaston, Birmingham B15 2TT, and West Midlands Regional Genetics Service, Birmingham Women's Hospital, Birmingham B15 2TG, UK
G E Holder, Moorfields Eye Hospital, City Road, London EC1V 2PD, UK
M Simunovic, J D Mollon, Department of Experimental Psychology, University of Cambridge, Downing Street, Cambridge CB2 3EB, UK

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

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