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Clinical characterisation of the *CABP4*-related retinal phenotype

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ABSTRACT

Background Calcium binding protein 4 (CABP4), specifically located in photoreceptor synaptic terminals, has been associated with congenital stationary night blindness based on this clinical diagnosis being made for three individuals from two Swiss families with *CABP4* mutations; however, the few reported cases limit phenotype–genotype correlation. We expand the number of reported patients with *CABP4* mutations and clinically characterise the *CABP4*-related phenotype.

Methods A retrospective case series of 11 individuals (age 2–26 years; four consanguineous families) with early-onset retinal dysfunction found to harbour *CABP4* mutations after a strategy of homozygosity analysis and/or candidate gene testing.

Results The 11 patients from four families harboured the same homozygous *CABP4* mutation (c.81_82insA; p.Pro28Thrfs*4) and shared a common haplotype. All patients had congenital nystagmus, stable low vision, photophobia and a normal or near-normal fundus appearance. None complained of night blindness when specifically questioned. Eight had hyperopic cycloplegic refractions (≥+ 1.00 dioptre). Electroretinography showed an electronegative waveform response to scotopic bright flash, near-normal to subnormal rod function, and delayed and/or decreased cone responses or was non-recordable. Although these and previously reported families with homozygous mutations were labelled with different clinical diagnoses, all had similar clinical features.

Conclusion These typical clinical features, which do not include a symptom of night blindness, suggest *CABP4* mutations. The phenotype is best uniformly termed congenital cone-rod synaptic disorder. In Saudi Arabia a founder homozygous c.81_82insA *CABP4* mutation is a recurrent cause.

INTRODUCTION

Calcium binding protein 4 (CABP4), a member of the CABP family of neuronal Ca^{2+}-binding proteins, is specifically located in photoreceptor synaptic terminals, where it probably modulates photoreceptor Ca^{2+} channels and transmitter release.[1 2] Because there are only a few reported patients harbouring *CABP4* mutations, phenotype–genotype correlation is limited.[3–5] Affected individuals have been labelled as incomplete congenital stationary night blindness (CSNB; two families),[3] congenital cone-rod synaptic disorder (one family)[4] and Leber congenital amaurosis-like (one family).[5] In order to characterise the associated clinical phenotype better, we report the ophthalmic findings of additional identified patients harbouring *CABP4* mutations in the context of clinical features that have been described in previous reports.[3–5]

METHODS

Institutional review board approval was granted for this study. Patients with early-onset retinal dysfunction whose phenotypes segregated with recessive *CABP4* mutations were identified and reviewed. These *CABP4* mutations were identified in consanguineous Saudi families with early-onset retinal dysfunction who were referred for genetic testing. A previously described strategy[6] of homozygosity analysis to identify candidate genes for sequencing was used for all but the final family; for that final family the affected individual directly underwent *CABP4* gene testing because by that time we had become familiar with recurrent clinical features of the *CABP4*-related phenotype. All affected family members underwent complete ophthalmic examination including cycloplegic refraction (cyclopentolate 1%) and most had full-field electroretinography (ERG) as per the standards of the International Society for Clinical Electrophysiology of Vision.[7]

Primers and PCR conditions used for *CABP4* sequencing are available in supplementary tables 1 and 2 (available online only). Affected individuals from all four families underwent haplotype analysis of a recurrent mutation. For haplotype analysis, the GeneChip Human Mapping 2 × 250K (500K) Array Set (Affymetrix, Santa Clara, CA) was used in conjunction with GeneChip Genotyping Analysis Software (Affymetrix, Santa Clara, CA). Relevant SNPs were selected which span a 3 Mb region (65,526,356-68,767,227) that includes the *CABP4* gene.

RESULTS

A homozygous c.81_82insA *CABP4* mutation (p.Pro28Thrfs*4; accession number NM_145200.3) segregated with congenital retinal dysfunction in 11 affected individuals (aged 2–26 years) from four consanguineous families (family A: four affected siblings; family B: three affected siblings; family C: two affected siblings and their affected mother; family D: an affected boy) (table 1). Family A has previously been reported.[5] Haplotype analysis confirmed a shared haplotype surrounding the mutation for all four families (supplementary table 3, available online only).

Clinical features are summarised in table 1. All 11 patients had congenital nystagmus, low vision that was considered stable and photophobia. No patient complained of night blindness, and all patients had been specifically questioned for this potential symptom. For all patients fundus appearance was normal or near normal. Eight were

Table 1 Summary of *CABP4*-related cases to date

ID	Family	Ethnicity	Clinical diagnosis	Age (years)	Sex	*CABP4* mutation	BCVA	Refraction	Flash scotopic/photopic	Isolated rod	References	Comments
1	1	Swiss	CSNB	45	M	c.800_801del2/ c.800_801del2	20/200 20/400	NA NA	Electronegative/delayed and depressed	NA	Zeitz *et al*[3]	
2				39	M		20/200 20/200	NA NA	Electronegative/delayed and depressed	NA		Absent foveal reflexes
3	2	Swiss	CSNB	15	M	c.800_801del2/ c.370C>T	20/30 20/30	NA NA	Electronegative/delayed and depressed	NA	Zeitz *et al*[3]	Only patient with night blindness, worsening vision, and no nystagmus
4	3	Dutch	CRSD	12	M	c.646C>T/ c.646C>T	20/200 20/200	+5.00 +5.50	Electronegative/depressed	Near normal	Littink *et al*[4]	
5				10	F		20/200 20/400	+4.50 +4.50	Electronegative/depressed	Depressed		
6	4 (A)	Saudi	LCA-like	16	F	c.81_82insA/ c.81_82insA	20/400 20/400	+1.00 +2.50	Unrecordable/unrecordable	Unrecordable	Aldahmesh *et al*[5] and current	
7				15	F		20/400 20/400	+7.50 +7.50	Unrecordable/unrecordable	Unrecordable		
8				12	F		20/400 CF	+7.50 +7.50	Electronegative/delayed and depressed	Borderline		
9				6	M		20/400 20/400	+4.50 +5.00	Unrecordable/unrecordable	Unrecordable		
10	5 (B)	Saudi	CRSD	18	F	c.81_82insA/ c.81_82insA	20/200 20/200	−4.00–3.00×010 −4.50–3.00×010	Electronegative/delayed and depressed	Depressed	Current	
11				14	M		20/300 20/300	+4.75 +4.75	Mild delay and electronegative/delayed and depressed	Delayed and depressed		
12				2	M		CSM CSM	−2.50–2.00×180 −2.50–2.00×180	NA	NA		
13	6 (C)	Saudi	CRSD	26	F	c.81_82insA/ c.81_82insA	20/80 20/80	+2.00–3.75×010 +3.00–3.00×170	Mild delay and electronegative/delayed and depressed	Delayed and depressed	Current	Affected mother and carrier father (pseudodominant)
14				7	M		20/400 20/400	+8.25–2.00×180 +8.25–2.00×180	Electronegative /delayed and depressed	Depressed		Had head shaking, less apparent with time
15				5	M		20/200 20/200	+8.00–2.00×180 +7.50–2.00×180	NA	NA		
16	7 (D)	Saudi	CRSD	11	M	c.81_82insA/ c.81_82insA	20/200 20/200	+2.00 +2.00	Mild delay and electronegative/delayed and depressed	Delayed and depressed	Current	

All patients had normal or near-normal fundus examination.
All patients had nystagmus, photophobia and no night blindness except for patient 3 (family 2), the only patient with a missense mutation.
In a given family, affected individuals are siblings except for family 6 (patient 13 is the mother in a pseudodominant pedigree).
BCVA, best-corrected visual acuity; CF, count fingers; CRSD, cone-rod synaptic disorder; CSM, central steady maintained; CSNB, congenital stationary night blindness; ID, patient identification number; LCA, Leber congenital amaurosis; NA, not available.

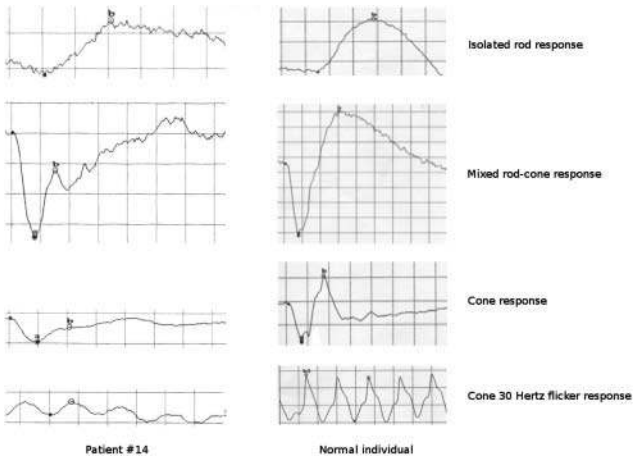

Figure 1 Electroretinography of the right eye in patient 14 and tracings from the right eye of a normal individual: for patient 14 under scotopic conditions, the isolated rod response is slightly delayed and depressed. The mixed rod-cone response to flash is an electronegative tracing with normal a-wave implicit time, a-wave amplitude, and b-wave implicit time. Under photopic conditions, both the cone response to flash and to 30 Hertz flicker are delayed and depressed. For patient 14, box represents 100 mV vertically and 20 ms horizontally but for the normal individual scales are different. For patient 14, normative scotopic means and ranges are as follows: a-wave implicit time 14 ms (11–17), a-wave amplitude 271 mV (164–378), b-wave implicit time 51 ms (42–60), b-wave amplitude 495 mV (284–705). For patient 14 under photopic conditions, normative means and ranges are as follows: a-wave implicit time 13 ms (11–15), a-wave amplitude 86 mV (54–117), b-wave implicit time 35 ms (33–37), b-wave amplitude 168 mV (96–239), flicker implicit time 28 ms (25–32), flicker amplitude 158 mV (96–259).

hyperopic (≥+1.00 dioptre spherical equivalent). In family A (the previously reported family),[5] three of the four affected family members had ERGs that were non-recordable. The fourth affected family member from family A and all affected individuals from families B and C had similar ERGs: an electronegative waveform to scotopic flash, near-normal or subnormal rod function and decreased and delayed cone responses to photopic flash (figure 1).

A review of the phenotypes of the previously reported Swiss and Dutch patients with *CABP4* mutations revealed all but one patient (the only reported compound heterozygote harbouring the only reported missense mutation)[3] to have similar clinical features and symptoms (table 1). ERGs in the current study and previous reports[3–5] were similar with the exception of the three affected individuals from family A with non-recordable ERG tracings (table 1).

DISCUSSION

Despite different clinical diagnoses, previously reported Swiss and Dutch patients with homozygous *CABP4* mutations and these Saudi patients harbouring a homozygous c.81_82ins1 mutation had similar clinical features, and none complained of night blindness. When recordable, the ERG showed cone-rod dysfunction with an electronegative waveform response to scoptopic flash. Rather than CSNB, the *CABP4*-related phenotype is better uniformly considered congenital cone-rod synaptic disorder, the term that was used to describe the previously-reported Dutch family.

The first three reported patients (from two Swiss families) were clinically labelled as incomplete CSNB and were found to harbour mutations in *CABP4* after it was sequenced as a candidate gene because of its known function in the photoreceptor synapse (table 1).[3] In the two patients who were siblings a homozygous c.800_801del2 was found, predicted to cause a frameshift and elongation of the protein by 96 amino acids (p.Glu267fs) that probably disrupted its tertiary structure and interactions.[3] The third patient was compound heterozygous for the same mutation with a missense variant (c.370C>T) that was predicted to be functionally damaging to protein function.[3] There seem to have been two factors that led Zeitz *et al*[3] to label these three patients as CSNB. One is that they had an electronegative ERG, a classic (although not specific) feature of CSNB. The other is the fact that mutations in another CABP protein gene at the photoreceptor synapse—*CACNA1F*—are a recognised cause of incomplete CSNB (in which both rods and cones are affected on the ERG, as was the case in the these three patients, as opposed to rods only in complete CSNB).[8] However, in these three Swiss patients cones were more affected than rods (rather than rods being more affected than cones), and only one of the three complained of night blindness when specifically questioned. That one patient is the only reported patient with *CABP4* mutations with this symptom. He is also atypical from other reported patients with *CABP4* mutations in other ways—in addition to having the symptom of night blindness, he is the only one with compound heterozygosity, a missense *CABP4* variant, visual acuity as good as 20/30, symptoms of progressive visual loss and no nystagmus (table 1).[3] In the context of all other described patients[3–5] and the new patients reported in this study, his phenotype is not characteristic of *CABP4* mutations (table 1).

The third and fourth reported patients (siblings from a Dutch family) were found to harbour the homozygous *CABP4* mutation (c.646C>T;p.Arg216X) after homozygosity mapping and subsequent candidate gene analysis (table 1).[4] Although these Dutch siblings had electronegative ERGs that resembled what is seen in incomplete CSNB, Littink *et al*[4] recognised that cones were more severely affected than rods, that the isolated rod response was near normal or subnormal, and that neither patient had the symptom of night blindness. In light of these observations and the localisation of CABP4, the authors felt the best term for the phenotype was cone-rod synaptic disorder.

In the current study, 11 Saudi patients (four consanguineous families) harboured the same underlying homozygous c.81_82insA *CABP4* mutation. Family A was previously described as Leber congenital amaurosis-like[5] based on non-recordable ERG tracings in three of the four affected family members. Clinically, however, these patients had features and symptoms similar to those of all other patients with homozygous *CABP4* mutations—congenital nystagmus, low vision that was considered stable, photophobia, no night blindness symptoms, a normal or near-normal fundus appearance and a typically hyperopic refraction (table 1), which suggests that the low ERG readings in family A were related to the severity of the condition rather than a disease entity different from that of other affected patients. Supporting this concept is the fact that the one affected family member from family A who had a recordable ERG (patient 8 in table 1) and all affected individuals from families B, C and D (patients 10–16 in table 1) had ERG tracings similar to each other and to those of all other previously reported *CABP4*-related patients: cone-rod dysfunction and an electronegative waveform response to scotopic flash (table 1, figure 1).

Our findings in these new cases and reappraisal of previously described cases define the phenotype that should raise suspicion for *CABP4* mutations. Homozygous loss-of-function *CAPB4* mutations cause a relatively stable congenital cone-rod dysfunction with congenital nystagmus and a normal or near-normal fundus appearance. Affected individuals do not complain of night blindness and are usually hyperopic. When recordable the ERG shows an electronegative response to scotopic flash in the setting of cone-rod dysfunction. As has been previously suggested,[4] this phenotype is best uniformly termed congenital cone-rod synaptic disorder. In Saudi Arabia, a founder homozygous c.81_82insA *CABP4* mutation is a recurrent cause.

Contributors AOK: conception and design, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, and final approval. MA: analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, and final approval. FSA: analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, and final approval. AOK and MA are co-first authors.

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Competing interests None.

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Correction notice This article has been corrected since it was first published Online First. The sentence, "A retrospective case series of 11 individuals (age 2–26 years; four consanguineous families) with early-onset retinal dysfunction (age 2–26 years) found to…" has been updated to read "A retrospective case series of 11 individuals (age 2–26 years; four consanguineous families) with early-onset retinal dysfunction found to…".

REFERENCES

1. **Haeseleer F,** Imanishi Y, Maeda T, *et al*. Essential role of Ca2+-binding protein 4, a Cav1.4 channel regulator, in photoreceptor synaptic function. *Nat Neurosci* 2004;**7**:1079–87.
2. **Maeda T,** Lem J, Palczewski K, *et al*. A critical role of CaBP4 in the cone synapse. *Invest Ophthalmol Vis Sci* 2005;**46**:4320–7.
3. **Zeitz C,** Kloeckener-Gruissem B, Forster U, *et al*. Mutations in CABP4, the gene encoding the Ca2+-binding protein 4, cause autosomal recessive night blindness. *Am J Hum Genet* 2006;**79**:657–67.
4. **Littink KW,** van Genderen MM, Collin RW, *et al*. A novel homozygous nonsense mutation in CABP4 causes congenital cone-rod synaptic disorder. *Invest Ophthalmol Vis Sci* 2009;**50**:2344–50.
5. **Aldahmesh MA,** Al-Owain M, Alqahtani F, *et al*. A null mutation in CABP4 causes Leber's congenital amaurosis-like phenotype. *Mol Vis* 2010;**16**:207–12.
6. **Aldahmesh MA,** Safieh LA, Alkuraya H, *et al*. Molecular characterization of retinitis pigmentosa in Saudi Arabia. *Mol Vis* 2009;**15**:2464–9.
7. **Marmor MF,** Fulton AB, Holder GE, *et al*. ISCEV standard for full-field clinical electroretinography (2008 update). *Doc Ophthalmol* 2009;**118**:69–77.
8. **Bech-Hansen NT,** Naylor MJ, Maybaum TA, *et al*. Loss-of-function mutations in a calcium-channel alpha1-subunit gene in Xp11.23 cause incomplete X-linked congenital stationary night blindness. *Nat Genet* 1998;**19**:264–7.

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Notes