Variability of retinopathy consequent upon novel mutations in LAMA1.

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Abstract

Purpose Bi-allelic mutations in *LAMA1* (laminin1) (OMIM #150320) cause Poretti-Boltshauser Syndrome (PTBHS), a rare non-progressive cerebellar dysplasia disorder with ophthalmic manifestations including oculomotor apraxia, high myopia and retinal dystrophy. Only 38 variants, nearly all loss of function have been reported. Here we describe novel *LAMA1* variants and detailed retinal manifestations in two unrelated families.

Methods Whole genome sequencing was conducted on three siblings of a consanguineous family with myopia and retinal dystrophy and on a child from an unrelated non-consanguineous couple. Clinical evaluation included full ophthalmic examination, detailed colour, autofluorescence retinal imaging, retinal optical coherence tomography (OCT), fluorescein angiography under anesthesia and pattern and full-field electroretinography.

Results Genetic analysis revealed a novel homozygous *LAMA1* frameshift variant, c.1492del p.(Arg498Glyfs*25), in the affected siblings in family 1 and a novel frameshift c.3065del p.(Gly1022Valfs*2) and a deletion spanning exons 17-23 in an unrelated individual in family 2. Two of the three siblings and the unrelated child had oculomotor apraxia in childhood; none of the siblings had symptoms of other neurological dysfunction as adults. All four had myopia. The affected siblings had a qualitatively similar retinopathy of wide-ranging severity. The unrelated patient had a severe abnormality of retinal vascular development which resulted in vitreous haemorrhage and neovascular glaucoma in the left eye and a rhegmatogenous retinal detachment in the right eye.

Conclusions This report describes the detailed retinal structural and functional consequences of *LAMA1* deficiency in 4 patients from two families and these exhibit significant variability with evidence of both retinal dystrophy and abnormal and incomplete retinal vascularisation.

Keywords: LAMA1; Poretti-Bolthauser syndrome; cerebellar dysplasia; oculomotor apraxia; myopia; retinal dystrophy

Introduction

Poretti-Boltshauser Syndrome, PTBHS, OMIM #615960 is a rare autosomal recessive disorder characterised by cerebellar dysplasia, vermis hypoplasia, cysts, non-progressive cerebellar ataxia, and sometimes delayed cognitive development and language and motor delay[1]. The disorder is associated with bi-allelic mutations in the *LAMA1* gene[2]. Ophthalmic manifestations including oculomotor apraxia, high myopia and retinal dystrophy are present in most patients.

LAMA1 (OMIM #150320) on 18p.11.31 encodes Laminin alpha-1 which is the alpha 1 (α1) subunit of the laminin proteins, a large family that form a major component of the basement membrane, a thin layer of the specialized extracellular matrix (ECM). The laminins are heterotrimeric glycoproteins consisting of an alpha, beta and gamma chain. 16 isoform members have been identified from combinations of five α, four ß, and three γ chains (encoded by 12 genes), with the combination of chains conferring tissue specificity and unique physiological functions. The laminin α1 subunit combines with ß, and γ chains in two of the 16 identified laminin molecules, laminin- α1ß1γ1 or laminin-111 (previously laminin-1) and laminin- α1ß2γ1 (or laminin-121).

The laminins have a dual role in scaffolding through their N-terminal domain interacting with basement membrane biomacromolecules, and signalling, through their C-terminal domains binding to cell surface receptors[3]. Laminin α1 plays a role in cell adhesion, migration and organization of cells into tissues during embryonic development[4]. The laminin-111 heterotrimer is ubiquitous in the embryo, while it is restricted to a small subset of basement membranes in the adult. A critical role for *lama1* in both retinal and cerebellum development has been suggested through study of conditional knockouts in mice[5,6] and in zebrafish[7].

In this study, we describe the retinal manifestations and intrafamilial variability in three affected siblings homozygous for a novel frameshift mutation and an additional unrelated individual found to harbour novel biallelic loss of function variants.

Methods

This study was endorsed by the Local Research Ethics Committee at Moorfields Eye Hospital and adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all participating subjects.

Participants and Molecular genetic analysis

Three affected siblings from a distantly consanguineous family of Asian origin presented to the inherited retinal disorders service at Moorfields Eye Hospital (family GC23208) (figure 1A). The proband, her mother, one affected sibling and one unaffected sibling underwent whole genome sequencing (WGS) through the 100,000 Genomes Project[8] using the Illumina platform (150bp paired-end reads, average x30 read-depth)[9]. Filtering for rare variants (those with an allele frequency in the gnomAD dataset (http://gnomad.broadinstitute.org/) of less than 0.001) in genes known to be associated with retinal disease (https://panelapp.genomicsengland.co.uk/panels/307/) and for genome-wide variants in autozygous regions in the affected individuals was employed. A third affected sibling underwent familial variant confirmation by bidirectional Sanger sequencing. An 11-year-old girl (family GC20722) (figure 1B) underwent WGS through participation in the National Institute of Health Research BioResource Rare Diseases (NIHRBR-RD)[10] study.

Clinical assessment

Systems enquiry for dysmorphology was followed by ophthalmic examination consisting of Snellen visual acuity (VA) and dilated fundus examination. Clinical imaging including slit lamp photography, spectral-domain optical coherence tomography (SD-OCT), fundus autofluorescence imaging (FAF) (Spectralis HRA+OCT (Heidelberg Engineering GmbH, Heidelberg, Germany), and widefield fundus photography including fluorescein angiography (Optos plc, Dunfermline, UK or Retcam, Natus, Pleasanton, USA). Full field and pattern electroretinogram (ERG; PERG) testing in the adult patients were performed using gold foil corneal electrodes, incorporating the Standards of the International Society for Clinical Electrophysiology of Vision (ISCEV)[11]. Additionally, photopic On-Off ERGs and short wavelength flash (S-cone) ERGs were performed[12–14]. An abbreviated paediatric flash ERG protocol was used in the youngest patient at the age of 37 months, using lower eyelid skin electrodes[15].

Results

Genetic analysis

A novel homozygous frameshift mutation GRCh37 (hg19): Chr18:7038880del NM_005559.4 (*LAMA1*):c.1492del p.(Arg498Glyfs*25) was found in all three affected siblings and heterozygous in both parents of family GC23208. This frameshift is predicted to be pathogenic according to ACMG/AMG guidelines[16]. The frameshift affects exon 11 of 63 and thus would be expected to result in nonsense mediated decay of the truncated transcript and abolish protein expression representing a biallelic null. No other pathogenic variants have been reported in exon 11. No other candidate variants were identified in the gene panel.

The affected individual of family GC20722 was unsolved following whole genome sequencing and rare variant analysis in an in-house curated virtual retinal disease gene panel as previously described[10]. Fifteen rare (MAF<0.01 in the ExAC dataset) variants were identified in the virtual gene panel. No biallelic variants in recessive IRD genes and no variants in dominant IRD genes survived filtering. Subsequent reanalysis for rare candidate variants in an updated gene panel including structural and copy number variant (SV/CNV) analysis using the CANVAS and MANTA algorithms for copy gain/loss and split read data[10] revealed a heterozygous deletion of exons 17-23, Chr18:7013230_7031988del (see supplementary figure S4), unique in the dataset, and predicted to lead to an inframe deletion, *LAMA1* c.2275_3363del, p.(Ala759_Lys1121del). ~~,~~ Also identified was~~and~~ a hemizygous single nucleotide deletion, Chr18:7015784del, *LAMA1* c.3065del in exon 22 leading to an out of frame codon followed by a termination codon, p.(Gly1022Valfs*2). These variants were demonstrated to be biallelic by direct interrogation of the individual read data using the Integrative Genomics Viewer[17]. These and all previously reported mutations in *LAMA1* are shown in figure 2.

Clinical features

Clinical, imaging and electrophysiological findings are summarized in Table 1. Parents and asymptomatic family members were not examined.

GC23208

The proband (III-2), a 42-year-old woman of Indian ancestry, noticed scotomata and mild reduction of her vision. She had long standing night blindness, nystagmus, pathological high myopia and was known to be a beta-thalassemia carrier. She was otherwise fit and well. Clinical examination revealed visual acuities of 6/24 in both eyes. Her VA had been reported

as 6/36 in the right eye and 6/24 in the left eye 7 years earlier. The ocular media were clear through posterior chamber implants and the intraocular pressures were normal. Both fundi revealed anomalous tilted discs and symmetric outer retinal atrophy in the posterior pole and mid-periphery (figure 3A). There was an area of diffuse reduction of the FAF signal in the temporal periphery and patchy atrophic lesions in the macular region of both eyes, and the atrophic area extended nasal to the optic disc in the left eye. The rest of the periphery was relatively preserved (figure 3A). The SD-OCT showed bilateral central atrophy of the retina and choroid (figure 3A). The retinal changes were atypical for high myopia.

Dark-adapted (DA) dim flash (DA 0.01) ERGs were subnormal and strong flash (DA 10) ERG a- and b-waves were reduced with a normal b:a amplitude ratio. The light-adapted (LA) 30Hz flicker ERGs were delayed and markedly subnormal and single flash (LA 3) cone ERG a- and b- waves were subnormal with a reduced b:a ratio bilaterally (figure 4). Photopic On-Off ERGs showed evidence of a-, b- and d-wave reduction with a low b:a ratio. S-cone ERGs were relatively preserved. The PERG P50 components were undetectable, and although likely partly attenuated by the effects of nystagmus, suggested severe macular dysfunction bilaterally. The ERG findings were consistent with generalised cone more than the rod system dysfunction and were in keeping with a cone-rod dystrophy, with S-cone ERGs suggesting preservation of short-wavelength retinal sensitivity.

Patient III-3 is a 40-year-old male with a 2-year history of reduced visual acuity in the right eye. His ocular history revealed high myopia, previous squint surgery and oculomotor apraxia. He suffered from night vision problems and bi-nasal field loss, which was causing him difficulty with navigation. As a child he had delayed speech. His VA was 6/12 in the right eye and 6/6 in the left eye. Retinal examination showed significant cataract in the right eye, symmetrical bilateral patches of atrophy in the macula and temporal retina (figure 3B). FAF imaging showed reduced autofluorescence in the central macula and was surrounded by alternating rings of increased and reduced FAF signal extending to the temporal retina (figure 3B). OCT revealed central atrophy of the retina and choroid which remained stable over time, suggesting a stationary developmental retinopathy (supplementary figure S1). Full-field ERGs showed a generalized cone-rod pattern of dysfunction with findings similar to those described for case III-2 (figure 4).

Patient III-4 is a 37-year-old male who had no visual complaints and no night vision problems. He reported difficulties moving his eyes as a youngster and had to move his head instead. He was diagnosed as having oculomotor apraxia. However, this was no longer a problem for him in adulthood. He had speech therapy as a child to help pronounce consonants. His VA was 6/9 in both eyes. He correctly identified all the colour plates of the Ishihara test when each eye was tested. There was no nystagmus, and he had a full field of vision to confrontation. Retinal examination showed largely normal retina with bilateral small areas of degeneration slightly temporal to the macula (figure 3C). FAF imaging showed evident reduction in autofluorescence corresponding to the same areas of degeneration (figure 3C). SD-OCT showed a well-preserved foveal architecture with multiple pigment epithelial detachments in both eyes. (figure 3C).

The DA 0.01 and DA 10 ERGs were of normal timing and amplitude; LA 30Hz ERGs were of normal timing and mildly subnormal amplitude; LA3 and On-Off ERGs showed a mildly reduced b:a ratio and possible subtle distortion of the d-wave bilaterally. S-cone ERGs were unremarkable (figure 4). The PERG P50 components were subnormal. The findings were consistent with a mild cone dystrophy with moderate macular involvement.

The parents, who are in their fifties and sixties, have normal vision with glasses, with no other family history of ocular disease and 2 unaffected children.

Patient II-1 is an 11-year-old girl born at term whose parents were concerned about poor visual responses in early infancy. Examination at 3 months of age revealed high myopia and ocular motor apraxia. She was prescribed spectacles for high myopia. Fundus examination raised concern about bilateral retinal abnormalities and an examination under anaesthetic (EUA) and RETCAM fluorescein angiography (FFA) was performed (supplementary figure S2). This examination revealed bilateral iris hypoplasia (figure 5 OD-A and OS-A, B), bilateral dysplastic optic discs and hypopigmented fundi with situs inversus of the retinal vessels in the right and a persistent fetal vasculature (PFV) in the left eye. There was a focal area of chrorioretinal atrophy in each eye (figure 5 OD-B, OS-C, D). In each eye there was a lack of retinal vessels in the temporal periphery. FFA of the right eye demonstrated incomplete retinal vascularisation of the temporal retina (imaging of the left eye was difficult due to the PFV. Electrodiagnostic evaluation revealed a mild cone-rod dystrophy. Over time it became apparent that she had delayed speech and motor development and hypoplasia of dental enamel (figure 5 T). MRI Scan revealed bilateral cerebellar hemispheric dysplasia with cysts, and hypoplasia of the cerebellar vermis with a 'molar-tooth' appearance (supplementary figure S3).

At the age of three, the patient started developing recurrent mild vitreous haemorrhages in the right eye and denser ones in the left eye, which initially resolved completely without the need of an operation bilaterally. Ultrasound B-scans in that period did not show evidence of retinal tears and confirmed flat retinae. Best corrected vision at age 4 years was 6/24 right eye and 6/30 left eye. During follow-up visits, she was noted to have microscopic hyphema and rubeosis iridis of the left eye and there was a dense vitreous haemorrhage reducing vision to light perception. Examination under anaesthesia with FFA again showed situs inversus with very incomplete retinal vascularisation and virtually no choroidal circulation in the non-perfused retina of the right eye. An antero-posterior fold running from the disc to the nasal border of the lens with a completely detached temporal retina was seen in the left eye. Subsequently evisceration of the left eye was required for neovascular glaucoma unresponsive to treatment.

The right eye later developed a retinal detachment. This was presumed initially to be exudative and therefore treated with intravitreal Lucentis 0.05ml, posterior sub-tenons triamcinolone 40mg, topical steroids and mydriatics. Subsequently she underwent vitreo-lensectomy, silicone oil tamponade and endolaser.

Discussion

This study describes the clinical and retinal features in three siblings and an unrelated fourth patient with biallelic *LAMA1* variants. Three novel variants are reported including two frameshift variants: p.(Arg498Glyfs*25) and p.(Gly1022Valfs*2) and a multi exon deletion (Chr18:7013230_7031988del) predicted to lead to loss of 363 aminoacid residues. Almost all *LAMA1* PTBHS-causing mutations reported to date are loss of function. Including our study, 41 PTBHS disease-causing unique *LAMA1* variants have been identified[2,4,18–25] (figure 2, supplementary Table S1); 12 (29.3%) are nonsense, 14 (34.1%) are frameshift, 6 (14.6%) are canonical splice site variants, 5 (12.2%) are multi-exon deletions or duplications and 4 (9.8%) are missense, with the predicted consequence of complete loss of function of the protein in the majority of cases. Consistent with the loss of function disease model, the variants are scattered throughout the gene with no apparent hotspots. Two of the four missense mutations were reported in trans in one of only two patients with normal cognitive development described by Micalizzi et al.[18]. The other two missense mutations were also in trans and reported to co-occur with a novel missense in *PMCA3*, an X-linked gene in a patient with

cerebellar ataxia[19]. The authors suggest that these mutations in two genes may work synergistically in a digenic mechanism, resulting in the cerebellar ataxia. However one of the two missense variants p.(Arg2381Cys) has an allele frequency of 0.008 in South Asian alleles in the gnomAD dataset and is observed in the homozygous state suggesting that this is unlikely to represent a fully penetrant allele. No other similar cases have been reported and therefore digenic *LAMA1*-disease is as yet unproven. Biallelic null mutations can be associated with mild systemic features, as evidenced in two siblings described separately by Aldinger et al. (pedigree UW160) and Vilboux et al (family1) with null mutations and in the three siblings reported here, who all had normal cognitive development. Phenotypic variability may be caused by other or modifying factors. In conclusion, there is no demonstrable genotype/phenotype correlation in *LAMA1*.

A summary[25] of the eye findings of 33 biallllelic *LAMA1*-PTBHS patients shows myopia in 12 of 33 (36%) patients, retinal dystrophy in 15 out of 33 (45%) and strabismus in 17 out of 33 (52%). In an earlier study, 13 of 17 (76%) patients had ocular motor apraxia[18]. Neurodevelopmental delay (97%), cerebellar dysplasia (97%), cerebellar cysts (94%) and ataxia (67%) were more common features. In our study, one patient presented with nystagmus and three with oculomotor apraxia. Three patients had speech or language delay but no other developmental nor intellectual disability was present. All four patients were myopic with varying degrees of retinal dystrophy. The intra-familial phenotypic variation observed in the sibs with respect to the ocular manifestation of PTBHS, suggest the presence of unknown trans-acting genetic and or environmental modifying factors.

To the best of our knowledge this is the first report of ISCEV-standard ERG and PERG findings in *LAMA1*-related retinopathy, and is the first to further characterise retinal dysfunction using photopic On-Off and S-cone ERGs. The ERGs revealed a cone-rod dystrophy in 3 cases and a mild cone dystrophy in one other individual, with preservation or relative sparing of short-wavelength retinal sensitivity in 3 of 3 adult cases. The same 3 siblings showed a low b:a ratio in the LA 3 ERG but with mild a-wave reductions in two, suggesting greatest cone system dysfunction post-phototransduction and consistent with On-Off ERG evidence of both on- and off- bipolar cell system involvement. This is broadly consistent with the low photopic ERG b:a ratio described in a 3-year-old child with PTBHS, but tested using a non-standard ERG protocol[26]. It is emphasized that the DA ERG a-wave reductions in 3 of 4 of the current series were consistent with significant loss of rod photoreceptor function, and that the normal DA 10 ERG b:a ratios suggest that additional dysfunction post-phototransduction is confined to the cone system. PERG P50 components were undetectable in 2 cases and subnormal in one other, as is common in other forms of cone and cone-rod dystrophy and in keeping with relatively early macular dysfunction.

Retinal vascular abnormalities in PTBHS have been described in two previous studies in 3 patients reported to have incomplete retinal vascularisation[21,26]. This was seen in our subject individual II-1 (family GC20722) who had a retinal detachment in one eye at presentation in infancy and evidence of situs inversus and persistent fetal vasculature in addition to incomplete vascularisation in the other eye. Although fluorescein angiograms have not been performed in the siblings of family GC23208, the pseudocolour and autofluorescence images of the two severely affected patients (III-2 and III-3) are consistent with incomplete vascularisation. A mouse model of mutations in *LAMA1* predicts the human phenotype of aberrant retinal and choroidal vascularisation which can also include failure of the hyaloid vasculature to regress[5]. Disruption of the inner limiting membrane seems to be the key feature and is shown in the OCT scans of patient II-3 and II-4 (GC23208) resulting in the formation of glial cell membranes on the surface of the retina. Severe retinal thinning in the avascular retina can result in small atrophic round holes that can be difficult to detect clinically. Individual II-1 in our series is the first report of such holes resulting in

rhegmatogenous retinal detachment which is challenging to treat when the holes are posterior and the vitreous is attached. The child is also the first case in whom dental and iris hypoplasia are reported as part of the phenotype. LAMA1 is expressed in the primary dentition in the mouse model and could explain the phenotype in human milk teeth[27]. MRI verified cerebellar and frontal lobe dysplasia and it is unknown if the sibs have such changes as MRI has not been performed in them.

The two families described here were negative following clinical WGS analysis through the NIHR-RD or 100KGP pipeline (panelapp[10]) because *LAMA1* was not included in the retinal gene panel at the time of analysis. Only subsequent gene-agnostic analysis was able to identify the pathogenic variants. Other similar cases may remain undiagnosed and we suggest that biallelic *LAMA1* should be considered in cases of high myopia/retinal dystrophy even in the absence of additional clinical signs of *LAMA1*-disease.

This report describes the detailed retinal structural and functional consequences of *LAMA1* deficiency in 4 patients from two families, exhibiting intra- and inter familial variability and showing ERG evidence of cone-rod or cone dystrophy, with preservation of short-wavelength sensitivity. Hypoplasia of the enamel and iris are new findings. Fluorescein angiography is recommended to map the extent of retinal non perfusion which can be masked by fundus hypopigmentation. Rhegmatogeneous retinal detachment is reported for the first time, secondary to atrophic round microholes in non-perfused retina. Biallelic *LAMA1* mutations, even when the genotype is likely to be null, can cause a non-syndromic ocular condition. This, and the variability in the reported family, suggests the action of trans-acting modifying factors. Biallelic mutations should be considered in myopia and retinopathy, even in the absence of systemic signs.

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Disclosure statement

The authors report there are no competing interests to declare.

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Supplementary material

Supplementary data for this article can be accessed on the publisher's website.

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Figure Legends

Figure 1. Pedigrees with phenotypic features in families with molecularly confirmed *LAMA1* biallelic mutations. (1A) Family GC23208, distantly consanguineous, affected individuals homozygous for c.1492delC p.(Arg498Glyfs*25). (1B) Family GC20722, proband compound heterozygous for c.3065delG p.(Gly1022Valfs*2) and c.2275_3363del p.(Ala759_Lys1121del). Square symbols indicate males; circles, females; diamond, either gender; n, number of children not specified; filled symbols, affected; quadrants indicating respective phenotypes, see key in figure.

Figure 2. Schematic representations of *LAMA1* gene (NM_005559.4) and LAMA1 protein domain structure (UniProtKB_P25391), with variants identified in the current study above, and previously reported variants below.

Figure 3. Retinal imaging—colour and autofluorescence (Optos) fundus images and foveal optical coherence tomography (OCT) (Heidelberg Spectralis) and optic discs of siblings III-2 (A) proband) showing outer retinal atrophy in the posterior pole and mid-periphery, and pigmentary changes in the temporal retina in the two lesser affected siblings III-3 (B), and III-4 (C) respectively, family GC23208.

Figure 4. Full-field ERG and pattern ERG (PERG) recordings from patients III-2, III-3 and III-4 (family GC23208), compared with those from a representative control subject (N). Recordings are shown for the right (OD) eye only. The dark-adapted (DA) ERGs (flash strengths 0.01 and 10.0 cd.s/m^2; DA 0.01 and DA 10) and light-adapted (LA) ERGs for a flash strength of 3.0 cd.s/m^2 (LA 3; 30Hz and 2Hz) are shown together with photopic On-Off ERGs (stimulus duration 200ms) and S-cone ERGs. The PERG is recorded to an alternating chequerboard. Broken lines replace blink artefacts occurring after ERG b-waves. Patient responses are superimposed to demonstrate reproducibility. See text for details.

Figure 5. Multimodal imaging of right (OD) and left (OS) eyes of patient 4 over the course of 3 examinations under anesthesia around 5 years of age. Iris atrophy was present(A). Prior to the development of retinal detachment abnormalities in the fundus seen with Retcam included chorioretinal atrophy (red star (OD-B, OS-C, D) and an abnormal exit of retinal vessels from the optic nerve termed situs inversus. The fundus was hypopigmented especially temporally (white star in OD-B). An infrared reflectance image (OD-C) and arteriovenous phase angiogram (OD-D) performed following the development of retinal detachment showed retinal non perfusion in this area. Two microholes were seen (Yellow arrows: OD-C) and confirmed with OCT (OD-G) which also showed large vitreous opacities (red arrow), disruption of the ILM (blue arrow) and retinal microcysts (grey arrows). Multimodal imaging of the left eye also shows Iris atrophy (OS-A, B) and macular coloboma (OS-C,D: red star). The fundus appears to suggest the presence of a funnel retinal detachment. However, the presence of intact atrophic retina is confirmed in horizontal and vertical peripapillary OCT scans (OS-E). The source of the recurrent vitreous was initially thought to be a persistent hyaloid artery remnant (blue dot to arrowed blue line in the Retcam montage (OS-C) and the late phase fluorescein angiogram (OS-D). The enamel of the primary dentition (T) was hypoplastic as evidenced by pitting (Black arrows) and discoloration (White arrow). The incisional edge of teeth had a curved margin.