

‘Mutations in *LAMB2* associate with albuminuria and Optic Nerve Hypoplasia with Hypopituitarism’

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Abstract/Summary

Context: Mutations in *LAMB2*, encoding the basement membrane protein, laminin β 2, are associated with an autosomal recessive disorder characterized by congenital nephrotic syndrome, ocular abnormalities and neurodevelopmental delay (Pierson Syndrome).

Case Description: This report describes a twelve year old boy with short stature, visual impairment and developmental delay who presented with macroscopic haematuria and albuminuria. He had isolated growth hormone deficiency, optic nerve hypoplasia and a small anterior pituitary with corpus callosum dysgenesis on his cranial MRI, thereby supporting a diagnosis of optic nerve hypoplasia syndrome. Renal histopathology revealed focal segmental glomerulosclerosis. Using next generation sequencing on a targeted gene panel for steroid resistant nephrotic syndrome, compound heterozygous missense mutations were identified in *LAMB2* [c.737G>A p.Arg246Gln, c.3982G>C p.Gly1328Arg]. Immunohistochemical analysis revealed reduced glomerular laminin β 2 expression compared to control kidney and a thin basement membrane on electron microscopy. Laminin β 2 is expressed during pituitary development and *Lamb2*^{-/-} mice exhibit stunted growth, abnormal neural retinae and here, we show, abnormal parenchyma of the anterior pituitary gland.

Conclusion: We propose that patients with genetically undefined optic nerve hypoplasia syndrome should be screened for albuminuria and if present, screened for mutations in *LAMB2*.

Key words: *LAMB2*, Pierson Syndrome, Optic Nerve Hypoplasia Syndrome

Introduction

Optic nerve hypoplasia syndrome (ONH), (also referred to as septo-optic dysplasia, SOD) represents a clinical spectrum associated with visual, pituitary and severe central nervous system structural abnormalities (1, 2). Traditionally, the cardinal features of the controversial term, SOD, involved two or more of the following: (1) pituitary hypoplasia with isolated or combined pituitary hormone deficiencies, (2) ONH and/or (3) midline brain defects. Risk factors include maternal exposure to recreational or prescription drugs, alcohol, diabetes and viral infection. Mutations in genes encoding transcription factors that regulate eye, forebrain and pituitary development have been identified in a small proportion of patients with ONH with hypopituitarism (1). *HESX1* homeobox 1 (*HESX1*), encodes a homeobox protein involved in forebrain and early pituitary development in mice. Mutations in *HESX1* account for less than 1% of hypopituitarism +/- ONH. An increasing number of genes implicated in ONH has been reported and include other transcription factors important for eye and forebrain development (*OTX2*, *SOX2*, *PAX6*, *NR2F1*, *VAX1*, *ATOH7*) whilst others identified regulate cellular processes such as RNA splicing, chromatin remodeling and the microtubular network (1).

Compound heterozygous mutations in *LAMB2*, encoding laminin β 2, an extracellular matrix glycoprotein were, herein identified, in a patient with three cardinal features of an ONH associated with growth hormone deficiency phenotype who also had proteinuric kidney disease. Screening for albuminuria may lead to further identification of *LAMB2* mutations in genetically undefined ONH diagnoses.

Methods

Ethics approval was obtained from Great Ormond Street Hospital NHS Foundation Trust. Following informed consent for genetic testing, targeted next generation sequencing of *ACTN4*, *ADCK4*, *CD2AP*, *COQ2*, *COQ6*, *INF2*, *ITGA3*, *LAMB2*, *LMX1B*, *MYO1E*, *NPHS1*, *NPHS2*, *PDSS2*, *PLCE1*, *PTPRO*, *SMARCAL1*, *TRPC6* and *WT1*) was undertaken for nephrotic syndrome and *HESX1*. Patient and control kidney sections were stained with anti-Laminin β 2 (1:500, AMAb91096, Atlas)

and anti-Podocin antibodies (1:100, P0372, Sigma) as previously described. (3) Pituitary tissue of *Lamb2^{-/-}* and *Lamb2^{+/-}* mice (4) were stained Haematoxylin & Eosin (H&E) and Diaminobenzidine (DAB) as previously described. (3)

Case History

A male infant presented at four months of age with roving eye movements and impaired visual function. Born at 35 weeks gestation to a healthy mother aged 33 years, he weighed 1.93kg (-1.47SDS) and measured 42cm (-2.13 SDS). No exposure to recreational drugs, alcohol or prescription medications were reported and his parents were non-consanguineous. By 31 months, he had global developmental delay with marked hypotonia. A cranial MRI revealed bilateral hypoplastic intraorbital optic nerves and anterior pituitary hypoplasia with a global reduction of white matter. The bulk of the posterior corpus callosum was reduced (Figure 1A). The septum pellucidum was present.

At 5 years of age, he presented with recurrent macroscopic haematuria associated with hypoalbuminaemia (25g/l) and significant albuminuria (urine albumin/creatinine ratio 1329mg/mmol) with normal renal function. A renal biopsy showed focal segmental glomerulosclerosis (FSGS) and a thin lamellated glomerular basement membrane (GBM) with podocyte foot process effacement (Figure 1B).

On follow up, he had short stature; height was 117.7 cm (-1.4SDS) with a weight of 18.5 kg (-2.54SDS) at the chronological age of 7.9 years (Figure 1C). Investigations revealed a low IGF1 of 29ng/ml and normal IGF-BP3 (2.48ng/l), normal T4 and TSH, with a low free T3 of 5.3pmol/l (6.2-9.5). A glucagon stimulation test revealed a peak growth hormone (GH) concentration of 4.5µg/l, suggesting GH insufficiency. At the start of the test, the basal glucose was 2.8 mmol/L with a nadir of 2.3 mmol/L, reflecting significant hypoglycaemia. The peak cortisol to glucagon stimulation testing was normal, measuring 819 nmol/l. Measured prolactin concentrations ranged between 80 - 244 mU/l (Normal: 44-479mU/l). At a chronological age of 10.5 years, the patient's bone age was delayed at 9 years and therefore, the low IGF-1 could not be solely accounted by undernutrition. Given the features

of GH insufficiency along with his poor height velocity at 3.7cm/year with a low IGF1 and neuroradiological findings, daily subcutaneous GH injections at 10IU/m²/week were commenced (Figure 1C). Following commencement of GH, his growth rate initially improved to 6.6cm per year from a growth rate of 3.7cm per year before commencement of GH. We suspect that adherence to GH treatment may have been an issue initially. Following transfer of the patient's care to grandparents, the excellent growth rate observed from the age of 12 years occurred while he was still prepubertal. At the age of 12.74 years, his height velocity was 10.6cms per year with pubertal ratings of G2 P1 A1 and testicular volumes of 02mls on the right and 03mls on the left. His bone age at that stage was 10.4 years.

Genetic testing for nephrotic syndrome revealed compound heterozygous missense mutations in *LAMB2*, the gene encoding laminin β 2, a structural component of the GBM, previously implicated in Pierson syndrome (OMIM 609049) (5) (Figure 1D). One mutation in exon 7 (c.737G>A, p.Arg246Gln) was that originally reported (5), and the other, identified in exon 25 (c.3982 G>C, p.Gly1328Arg), was novel. Both variants were predicted to be deleterious to protein function using PolyPhen and SIFT software analyses (Figure 1E) and affected highly conserved amino acids (Figure 1F). No mutations were detected in *HESX1* and the clinical phenotype did not match with that documented for *SOX2* nor *OTX2* mutations. Reduced glomerular expression of laminin β 2 was observed in the patient biopsy compared to the control (Figure 1G).

H&E staining of pituitary sections of *Lamb2*^{-/-} mice suggested abnormal morphology of the anterior pituitary parenchyma, exhibited by cellular clusters that were not evident in their wild-type littermates (Figure 2A). Somatotrophin signal was absent in *Lamb2*^{-/-} compared to *Lamb2*^{+/+} pituitaries (Figure 2B). As the patient had isolated GH deficiency, analysis of other pituitary cell types was not undertaken.

Discussion

Following presentation with recurrent macroscopic haematuria and albuminuria, compound heterozygous mutations in *LAMB2*, encoding laminin β 2, were identified in a boy with short stature, visual impairment and developmental delay. Investigations revealed ONH associated with anterior pituitary hypoplasia and GH deficiency. Whilst the septum pellucidum was present, dysgenesis of the posterior corpus callosum was evident thereby supporting the traditional diagnosis of ONH with GH deficiency and midline defects. Several publications have highlighted that ONH with hypopituitarism now represents a spectrum of developmental defects involving the eye, neural retina and forebrain (including pituitary) with a range of midline defects that don't always involve an absent septum pellucidum.

Albuminuria associated with FSGS has not yet been reported in the ONH spectrum. Interestingly, nonsense and truncating mutations in *LAMB2* are associated with Pierson syndrome, an autosomal recessive disorder characterized by congenital nephrotic syndrome, ocular abnormalities (commonly microcoria), muscular hypotonia, and neurological deficits (5, 6). Indeed, Pierson syndrome may be within the ONH spectrum. Hypomorphic missense mutations have been reported with milder phenotypes, manifesting later in childhood, whereby defective secretion of the mutant laminin β 2-trimer leads to compromised GBM integrity (7). The other variant (p.G1328R) is a novel missense mutation, in which glycine is replaced by an arginine at amino acid 1328 in the coiled coil (CC) domain of the peptide. Supporting evidence for a pathogenic mutant laminin β 2 protein, was the finding of a thin GBM on electron microscopy and reduced glomerular laminin β 2 expression on patient kidney sections.

To date, genes implicated in ONH play a role in the transcriptional regulation of pituitary and forebrain development (1). *In situ* hybridization studies have revealed expression of laminin isoforms throughout pituitary morphogenesis. (8, 9) In early murine gestation, laminin β 2 mRNA is expressed in the epithelium of Rathke's pouch and by mid gestation within the pars distalis and tuberalis. (9) Expression later extends to the parenchyma

and marginal cell layers of the anterior and intermediate pituitary lobes as well as the vasculature of the anterior lobe, both in late gestation and the early postnatal period (9) Laminin $\beta 2$ expression is observed within the parenchyma and vasculature of the anterior lobe in the adult pituitary. (9)

Our examination of *Lamb2*^{-/-} mice revealed abnormal parenchymal morphology of the anterior pituitary compared to controls and *Lamb2*^{-/-} mice exhibit stunted growth. (4,10) Further investigations involving assessment of pituitary function with measurement of GH levels will be of interest. In *Lamb2*^{-/-} mice, the outer segment of the rod photoreceptor layer is associated with disorganized synapses in the outer plexiform layer and a reduced physiological response. (4, 10) Both human and murine findings support the proposal that mutations in *LAMB2* may underlie genetically undefined ONH and urine dipstick testing may alert the clinician to screen to for mutations in *LAMB2*.

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List of Abbreviations

DAB	Diaminobenzidine
HESX1	Homeobox expressed in embryonic stem cells 1
GBM	Glomerular Basement Membrane
LAMB2	Laminin β 2
SOD	Septo-Optic Dysplasia
SOX2	SRY (Sex Determining Region Y)-related HMG box 2
OTX2	Orthodenticle homeobox 2

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Figure Legends

Figure 1 (A) Sagittal T1 weighted images (WI) of the pituitary region shows a small adenohypophysis (WI) (arrow in A), with a normal T1 posterior hyperintense focus corresponding to the neurohypophysis (dotted arrow in A). Note the reduction of the bulk of the posterior aspect of the corpus callosum. Axial T2 WI shows a dysplastic cortex in the right anterior temporal and insular regions (arrows in B). Axial T2 WI of the orbits shows small optic nerves (arrows in C) and bilateral buphtalmos. (B) H&E and PAS staining of patient renal biopsy sections: (a-b) H&E showed segmental sclerosis, narrowing of the Bowman's space and adhesions to its capsule (c) PAS staining showed mesangial proliferation with diffuse sclerosis and obliteration of the tip of the capillary tuft (d) Electron microscopy revealed a thin lamellated GBM and podocyte foot process effacement. Scale bar 50 micron (C) Growth chart showing height (cm) and weight (kg) of the proband from 1 to 14 years; initiation of growth hormone (arrow) (D) Sanger sequencing confirmed compound heterozygous *LAMB2* mutations; c.737G>A, Chr3:g.49168561C>T, p.Arg246Gln and c.3982G>C, Chr3:g.49160880C>G, p.Gly1328Arg. (E) Location of *LAMB2* detected variants at the level of exons and protein. The c.737G>A variant is located in exon 7 encoding part of the laminin N-terminal (LN') domain while the c.3982G>C variant is located in exon 25 encoding part of the laminin coiled-coil domain (LCC') of laminin β 2 protein. The LN' domain is responsible for laminin trimer polymerization to form the basement membrane. The LCC' domain is involved in assembly of individual laminin α , β and γ chains into trimers. (F) Conservation of the *LAMB2* amino acids that are altered in the patient; c.737G>A and c.3982G>C (upper and lower rows, respectively) alongside disparately related species. (G) Dual immunofluorescence staining of laminin β 2 in patient renal biopsy (*LAMB2* 737G>A/3983G>C; mutant) compared to time zero protocol renal transplant biopsy (*LAMB2*^{+/+};

control). The figure shows reduced expression of laminin β 2 (red) in the mutant, seen in a reduced thickness and integrity/continuity of the GBM compared to the control.

Figure 2: H&E and DAB immunostaining of pituitary gland sections from wild type and knockout *Lamb2* mice **(A)** The upper row shows H&E staining of anterior pituitary sections from *Lamb2*^{+/+} mice, which show uniform staining across the gland. The lower row shows anterior pituitary sections from the *Lamb2*^{-/-} mice, which show evidence of abnormal cell staining, seen as randomly distributed patches of unstained cells (indicated with arrow heads); imaged at magnifications of 5x (left) and 20x (right). Scale bar of 500 μ m and 200 μ m respectively **(B)** Growth hormone (GH) DAB immunostaining confirmed the abnormality of these cell clusters in *Lamb2*^{-/-} pituitary sections, which show negative staining for GH (lower row; indicated with arrow heads) compared to *Lamb2*^{+/+} mice (upper row); Scale bar of 500 μ m.

Figure 1

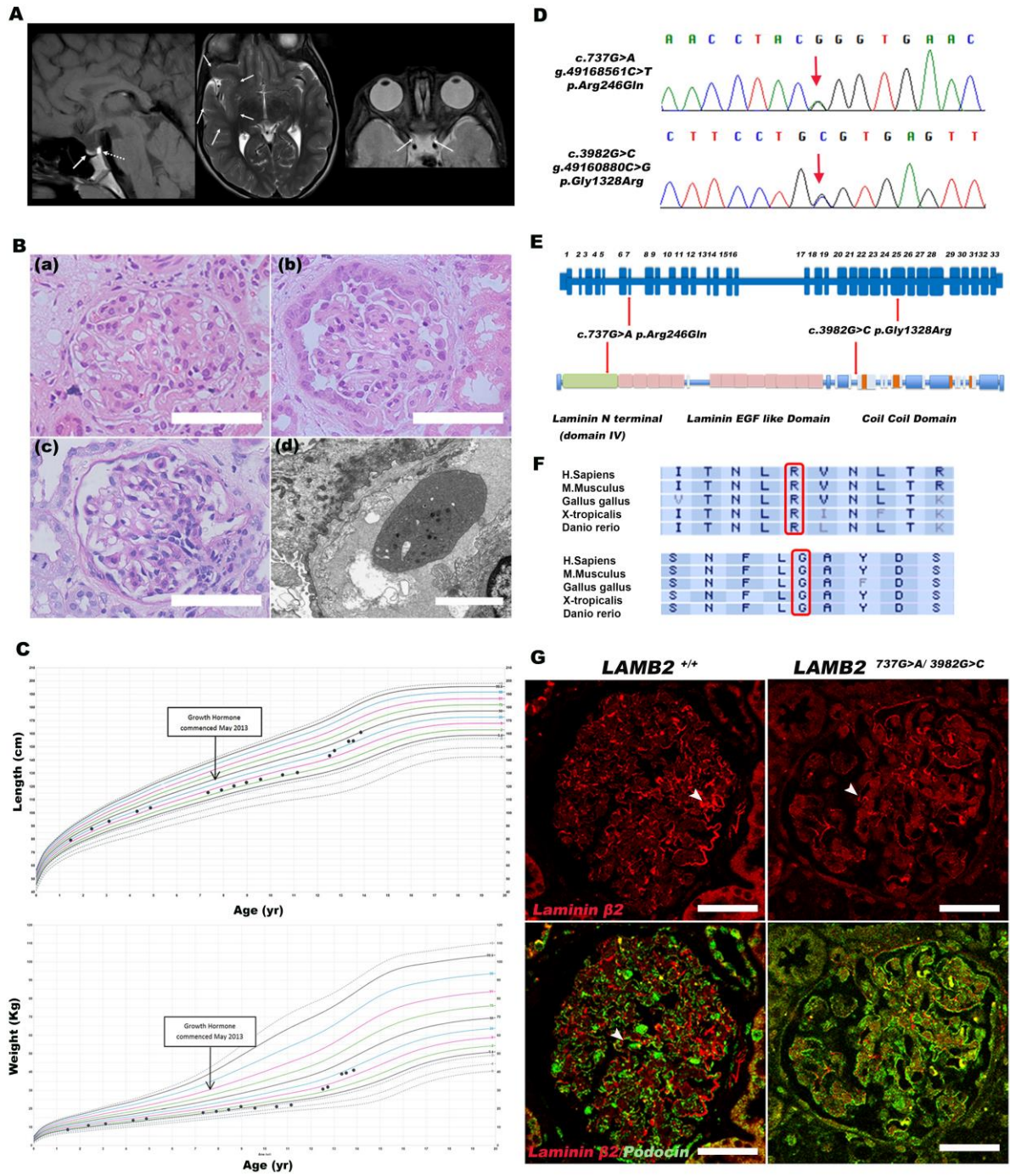
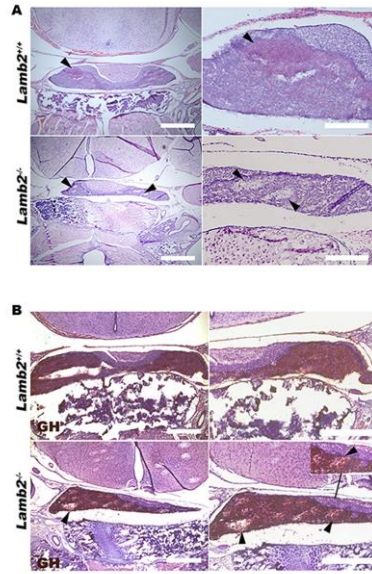


Figure 2



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