Compound heterozygous variants in LAMC3 in association with posterior periventricular nodular heterotopia

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Abstract
Background: Periventricular nodular heterotopia (PNH) is a malformation of cortical development characterized by nodules of abnormally migrated neurons. The cause of posteriorly placed PNH is not well characterised and we present a case that provides insights into the cause of posterior PNH.

Case presentation: We report a fetus with extensive posterior PNH in association with biallelic variants in LAMC3. LAMC3 mutations have previously been shown to cause polymicrogyria and pachygyria in the occipital cortex, but not PNH. The occipital location of PNH in our case and the proposed function of LAMC3 in cortical development suggest that the identified LAMC3 variants may be causal of PNH in this fetus.

Conclusion: We hypothesise that this finding extends the cortical phenotype associated with LAMC3 and provides valuable insight into genetic cause of posterior PNH.

Keywords: LAMC3, Periventricular nodular heterotopia, Occipital, Lobe, Case report

Background
Malformations of cortical development (MCD) are a group of disorders encompassing macroscopic and microscopic abnormalities of the cerebral cortex that have arisen during prenatal development [1]. The spectrum of MCD includes periventricular nodular heterotopia (PNH), where nodules of mis-localised neurons are abnormally arrested in their migration to the developing cerebral cortex. These ectopic neurons instead collect in the periventricular white matter, the location of the embryonic ventricular zone (VZ) [1, 2]. Peak neuronal migration occurs predominantly between the 12th to 24th weeks of gestation, meaning PNH can potentially be identified on ultrasound and magnetic resonance imaging (MRI) in pregnancy, but is difficult to diagnose before the third trimester [3].

Classical bilateral, symmetric PNH is associated with mutations affecting FLNA at Xq28 [4]. In the majority of individuals with PNH secondary to FLNA mutations, the nodules are located in the anterior bodies and frontal horns of the lateral ventricles (fronto-parietal) [4]. Individuals with classical PNH are typically female, and usually present with seizures and normal to borderline intelligence [4]. In addition to classical PNH, multiple chromosomal abnormalities as well as variants affecting 9 genes (ARFI, ARFGEF2, DCHS1, ERMARD, FAT4, INT58, MAP1B, MCPH1, and NEDD4L) have been

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linked to PNH, indicating the genetic heterogeneity of the disorder [5–13].

Considerable heterogeneity is also observed in clinical presentation, with multiple subtypes of PNH having been described [2]. Bilateral posterior PNH, involving the occipital cortex, accounts for ~25% of all PNH cases, and differs from fronto-parietal, FLNA-associated PNH not only in the location of the nodules, but also in the increased likelihood of associated cortical malformations [14]. The genetic cause of many posterior PNH cases remains unknown, with mutations in only 3 genes (ARFGEF2, ERMARD and NEDD4L) being implicated as causal [6, 8, 12].

Here we report a male fetus with a severe presentation of isolated posterior PNH in which we identified compound heterozygous missense variants affecting the LAMC3 gene (MIM 604349), which encodes the laminin subunit γ3. To our knowledge, this is the earliest identification of PNH by morphology ultrasound and the first report of LAMC3 variants in a case of posterior PNH. Since mutations in LAMC3 have previously been associated with other occipital MCD, it was an ideal candidate for the abnormalities identified in this fetus.

Case presentation
Study subjects
Written informed consent was provided by both parents for inclusion in the the Genomic Autopsy Study, an National Health and Medical Research Council (NHMRC) funded trio exome research study, approved by the Women’s and Children’s Health Network Human Research Ethics Committee (HREC/15/WCHN/35). All procedures performed were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments.

Case description
The parents of the male proband were non-consanguineous and healthy, with no known medical conditions. They have had 5 pregnancies; two healthy males, the proband, a healthy pregnancy currently in progress, and a history of one previous early miscarriage (Fig. 1a).

The proband pregnancy was conceived naturally and there were no early exposures to teratogens. Maternal health was good, although there was one episode of minimal vaginal bleeding at 6 weeks. The 12-week nuchal translucency scan was normal (NT 2.3 mm) and first trimester biochemical screening was low risk for trisomies 13, 18 and 21. The 19-week morphology ultrasound demonstrated unilateral ventricular dilatation and irregularity of the cerebral ventricular wall, suggestive of a possible diagnosis of PNH (Fig. 1b). The femur and humerus measured at the 10th centile, whilst the remainder of the long bones measured normal. No other abnormalities were seen. A fetal MRI performed at 20+0 weeks gestation showed left sided colpocephaly and irregularity of the fetal ependymal lining in the lateral ventricles, representative of subependymal nodular heterotopia (Fig. 1c). No other brain abnormalities were identified. Following the abnormal ultrasound results, a microarray (Illumina Infinium Global Screening Array-24v1.0. Illumina Inc, San Diego, CA, USA.) was performed on amniotic fluid cells. This confirmed a male fetus but no copy number variants (CNVs) were identified.

Based on the ultrasound findings, the couple elected for a termination of pregnancy at 21+5 weeks gestation and a perinatal autopsy was undertaken. The fetus weighed 440 g and growth parameters were consistent with a 21-week gestational age. Subtle dysmorphic features were apparent with a triangular face, widened anterior fontanelle and prominent heels. The remainder of the external examination was normal and there was no histological abnormality of the placenta. The internal thoracic and abdominal organs were normally formed and sited, and a radiological skeletal survey was normal. A post mortem MRI confirmed extensive PNH, predominantly involving the occipital horns of the lateral ventricles (Fig. 1d). The PNH was visible macroscopically at autopsy (Fig. 1e) and histology of affected tissue showed nodules of circumscribed glioneuronal tissue with disorganised, moderately dense aggregates of NeuN positive, primitive neurons (Fig. 1f, Additional file 1: Figure S1a). Some heterotopic nodules were also noted adjacent to the germinal matrix (Additional file 1: Figure S1b). No abnormalities of the corpus callosum were identified. The remainder of the cortex, brainstem and cerebellum were also macroscopically and histologically normal, with no evidence of polymicrogyria or pachygyria (Fig. 1e, Additional file 1: Figure S1c).

Genetic analysis
Genomic DNA was isolated from whole blood (parents) or lung tissue (proband) and sequenced at the Broad Institute of MIT and Harvard’s Genomics Platform (Boston, MA, USA). Exonic regions were enriched using an Illumina exome capture (38 Mb target) and sequenced (150 bp paired reads) on an Illumina HiSeq (Illumina Inc, San Diego, CA, USA). Sequencing reads were aligned to the hg19 reference genome using BWA version 0.7.12, duplicate reads were removed and variants called using GATK HaplotypeCaller version 3.8.0. CNV’s were detected using an in-house (unpublished) algorithm, which partitions read depth signals into bin sizes optimal for the exome capture. CNV calling for the trio was performed in conjunction with 98 unrelated samples.

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sequenced in the same batch, used to normalise read depth signals and remove sequencing-induced errors.

Single nucleotide variants (SNVs) were retained for being rare, with rare defined as ≤1% population frequency and ≤3 homozygous individual in gnomAD for autosomal or X-linked recessive inheritance, and ≤0.001% population frequency for autosomal dominant de novo inheritance [15]. Subsequent filtering selected for missense, truncating or canonical splice site protein-altering variants which were either called ‘de novo’ or inherited in an X-linked or autosomal recessive manner from the unaffected parents. Using the same

Fig. 1 Neurological phenotype of a family with biallelic missense variants in LAMC3. a Pedigree of the non-consanguineous family. b Morphology ultrasound at 19 + 4 weeks gestation showing unilateral ventricular dilatation and irregularity of the cerebral ventricular wall (indicated by arrows). c Fetal MRI at 20 + 0 weeks gestation showing multiple foci of periventricular nodular heterotopia in the occipital lobes (indicated by arrows) [Axial T2 weighted image, 4 mm thick slices]. d Post mortem MRI at 21 + 5 weeks gestation shows extensive bilateral posterior periventricular heterotopia (indicated by arrows) [Coronal T2 weighted image, 2 mm thick slices]. e Coronal sections of the cerebrum (frontal top left to occipital bottom right). Macroscopically the brain shows normal gyration for gestation, with no evidence of polymicrogyria or loss of sulci usually visible at 21 weeks gestation to suggest early pachgyria. Subependymal nodularity is present within the occipital horns of the lateral ventricles (indicated by arrows). No further structural malformations of the brain are evident. f Microscopic image of a subependymal periventricular nodular heterotopia within the occipital horn of the lateral ventricle. The well circumscribed nodule is composed of disorganised primitive neurons within glioneuronal tissue. (Scale = 500 µm)
in the clinical setting but no mutations were identified.

**heterozygous LAMC3 clones, associated with colpocephaly, in which compound
PNH located in the occipital horns of the lateral ventricle, consistent with a normal 16-week pregnancy, currently at 28-weeks gestation, carries only frequent pregnancies. The first male pregnancy was found
villus sampling at 10-weeks gestation, in their two subsequent
ents elected for prenatal diagnostic testing, via chorionic
variants, the par -
LAMC3
variants were identified. Further prioritisation of vari -
variants in 4 genes considering autosomal recessive
inception and 3 potential de novo variants (Additional
file 4: Table S1). No significant copy number
variants were identified. Further prioritisation of vari
ants affecting genes associated with brain development
indicated compound heterozygous variants in LAMC3:
 NM_006059.3:c.2726A>G,). Both
p.Arg356Cys (paternal; Chr9(GRCh37):g.133914340C>T,
and p.Gln909Arg (maternal; Chr9(GRCh37):g.133943597A>G,
NM_006059.3:c.1066C>T,) and p.Gln909Arg
(paternal variant (Fig. 1a, Additional file 2: Figure S2).

**Prenatal diagnosis**
Following identification of the LAMC3 variants, the parents
elected for prenatal diagnostic testing, via chorionic
tissue sampling at 10-weeks gestation, in their two subsequent
pregnancies. The first male pregnancy was found
to not carry either variant, consistent with the normal
16-week ultrasound and normal morphology scan at
20-weeks. At birth there was no evidence of PNH and
at 1 year the child is developing normally. The second
pregnancy, currently at 28-weeks gestation, carries only
the maternal variant, consistent with a normal 16-week
ultrasound and normal morphology scans (Fig. 1a, Additional
file 2: Figure S2).

**Discussion and conclusions**
Here we present a 21-week-old male fetus with posterior
PNH located in the occipital horns of the lateral ventricles,
associated with colpocephaly, in which compound
heterozygous LAMC3 variants were identified.

LAMC3 is located on chromosome 9 and encodes the
laminin subunit γ3. Laminins are extracellular matrix
glycoproteins required for cell adhesion, differentiation
and migration. They are made of an alpha, beta and
gamma chain, with different combinations of the subunit
variants combining to form the various laminin isoforms
[16]. The laminin γ3 subunit is utilised in 3 laminin
isoforms, two of which have only been observed to be expressed in the central nervous system [16].

In the developing human fetal brain, LAMC3 is
expressed in both the VZ and throughout the cortical
plate, with greatest expression in the region of the
temporal and occipital lobes. At the subcellular level,
LAMC3 expression is localized to the cell bodies and
dendrites of pyramidal neurons [17]. This expression is
similar to that observed in known PNH genes, FLNA
and ARFGEF2, although LAMC3 does not seem to show
the same localisation within the VZ to neuroependymal
progenitors [18]. The spatial and temporal expression
pattern of LAMC3 is therefore consistent with mutations in the gene potentially causing a PNH phenotype.
The expression pattern of human LAMC3 is however in
contrast to that of mice, where expression is localized to
the pial basement membrane and cerebral vasculature, as
well as to the intermediate zone, and the marginal zone
of the cortical plate [17, 19].

The functional importance of Lamc3 is suggested by a
homozygous knockout mouse model which shows cortical
lamination defects and reduced brain size compared to
wild-type [19]. Lamb2-Lamc3 double null mice show
profundely abnormal lamination, likely resulting from a
combination of disruptions to the integrity of the pial
basement membrane, altered radial glial cell morphology
and aberrant distribution of Cajal-Retzius cells [19].
While the double null mouse has a phenotype that is
more severe than either single null alone, it is currently
unclear what the individual contribution of Lamb2 and
Lamc3 is to these functions. Additional insight into the
function of LAMC3, comes from Lamc3 knockout mice
which demonstrate significantly delayed retinal astrocyte
migration before later resolution to wild-type-like
distribution [20]. Lamb2-Lamc3 double null mice show
severely halted migration which does not resolve, and
is again more severe than observed in either null individu -
ally [20]. Further support for a functional role of
LAMC3 in migration is given by a knockdown zebrafish
model which demonstrates defects in rostral primary
motor neuron migration [21]. This proposed function of
LAMC3 in migration and cortical development is consis-
tent with mutations in the gene resulting in a PNH
phenotype through failed migration and subsequent ter-
minal differentiation of a subset of progenitor cells within
the VZ.
In humans, biallelic mutations in LAMC3 are responsible for a rare autosomal recessive condition characterized by pachygyria and polymicrogyria restricted to the occipital lobes (MIM 614,115) [17]. To date, 11 individuals from 5 consanguineous families have been described, with both truncating mutations and missense mutations reported [17, 22, 23]. In addition to the occipital gyration abnormalities, affected individuals typically present with seizures (11/11 reported cases, all childhood onset where noted), and mild to moderate developmental delay (8/10 reported cases, not specified in 1 case) [17, 22, 23]. In one individual, while the pachygyria remained confined to the occipital lobe, the polymicrogyria extended to the frontal, parietal and temporal lobes of both hemispheres [23]. Although PNH has not been observed in any of the reported LAMC3-related cases, the predominantly occipital location of their MCD is consistent with the variants identified in our proband being the cause of the occipital restricted PNH.

Both of the LAMC3 variants identified in our proband, p.Arg356Cys (94 alleles, 0.03%) and p.Gln909Arg (1 allele, 0.0004%), are rare in the population database gnomAD and have never been observed in homozygosity [15]. An alternate variant at amino acid 356, p.Arg356His, has been observed in homozygosity once, but the physicochemical properties of histidine are more similar to arginine than cysteine. Both variants are highly conserved (Additional file 3: Figure S3a and b) and computational pathogenicity prediction tools suggest the variants to have a deleterious effect (CADD: p.Arg356Cys: 26; p.Gln909Arg: 23). One pathogenic LAMC3 missense variant has been previously reported, p.Gly350Arg (VCV000030419.1), which is located only 6 amino acids upstream from the p.Arg356Cys variant in our individual and within the same laminin-type EGF-like (LE) domain [20]. The introduction of a new cysteine at amino acid 356 may alter the disulfide binding pattern, interfering with the highly conserved structure of the region and potentially affecting laminin-laminin interactions (Additional file 2: Figure S2) [24]. The p.Gln909Arg variant is also located in a LE domain in a region shown to be involved in nitrogen binding, an interaction important for basement membrane assembly [25].

This is the first report of biallelic LAMC3 variants in a non-consanguineous family, and the first clinical association with posterior PNH, potentially broadening the phenotype of LAMC3-related disease. To our knowledge, this case is also the first to identify PNH on morphology ultrasound and MRI at such an early gestation. Our observation of this early phenotype should help in further elucidating the function of LAMC3 and its role in cortical development. For this family, exploratory exome analysis and the identification of likely causative variants facilitated prenatal diagnostic testing which provided reassurance in their two subsequent pregnancies.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12920-021-00911-4.

**Additional file 1: Figure S1.** Post mortem neurological findings. a NeuN positive nuclear labelling of primitive neurons within the heterotopic nodules. (Scale = 100 μm) b Thickening of the richly vascular germinal matrix with an adjacent heterotopic nodule. (Scale = 500 μm) c Cortical mantle showing normal layering and normal overlying meninges. (Scale = 500 μm).

**Additional file 2: Figure S2.** Family pedigree and segregation of the LAMC3 variants by Sanger sequencing. Both LAMC3 variants were confirmed to be present in compound heterozygosity in the proband. The paternal variant, p.Arg356Cys, and the maternal variant, p.Gln909Arg, were confirmed as heterozygous. Only the paternal variant was present in unaffected sibling 1, and only the maternal variant was present in the compound heterozygous unaffected pregnancy. The third unaffected sibling carries neither variant.

**Additional file 3: Figure S3.** Conservation of the LAMC3 variants. a The arginine residue at the location of the p.Arg356Cys variant (red box) is highly conserved across 100 species. The introduction of a cysteine at amino acid 356 may disrupt the completely conserved disulfide binding pattern of cysteines (blue boxes) in the region. b The glutamine residue at the location of the p.Gln909Arg variant (red box) is highly conserved across 100 species.

**Additional file 4: Table 1.** Exome sequencing results and variant filtering outcomes.

**Abbreviations**

CNV: Copy number variant; HREC: Human Research Ethics Committee; LE: Laminin-type EGF-like; MRI: Magnetic resonance imaging; MCD: Malformations of cortical development; MIT: Massachusetts Institute of Technology; NHMRC: National Health and Medical Research Council; NT: Nuchal translucency; OMIM: Online Mendelian inheritance in man; PNH: Periventricular nodular heterotopia; SNVs: Single nucleotide variants; VZ: Ventricular zone; WCHN: Women's and Children's Health Network; NT: Nuchal translucency.

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**Authors’ contributions**

CDA, ABB, PA, RM and CB drafted the manuscript, with ABB and CDA contributing equally and considered as co-first authors. ABB, MB and SLK-S coordinated the study and substantially contributed to the acquisition and analysis of data. JF, TH, PW and AWS processed WES data, and ABB and PA performed data analysis. ABB, IM, CDA, PA, TH, QS, HSS and CB contributed to interpretation and discussion of results. NW performed surgical pathology investigations and contributed substantial contributions to the interpretation of data. CB provided clinical care. AT interpreted the neuroimaging. HSS and CB conceived and supervised the study, contributing equally, and should be considered co-senior authors. All authors have agreed to be personally accountable for their own contributions. All authors read and approved the final manuscript.

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Availability of data and materials
Sequence data has been deposited at the European Genome-phenome Archive (EGA), which is hosted by the European Bioinformatics Institute and the Centre for Genomic Regulation, under accession number EGAS00000104479. Sequence variations and clinical assertions have been submitted to ClinVar (VCV000691960.2, VCV000691961.1). All unique materials and datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The study was conducted in full compliance with NHMRC ethical standards, with written informed consent from all parents involved in the study. As the subjects of the study are deceased infants/fetuses, written consent on their behalf was obtained from the parents or legal guardians. If living siblings under the age of 16 were involved in the testing process (for example, as the result of cascade testing) written informed consent was obtained from their parents/or legal guardians.

Consent for publication
As the subjects of the study are deceased infants/fetuses, written informed consent on their behalf for publication of identifying images or other personal or clinical details was obtained from their parents or legal guardians. Written informed consent for publication of identifying images or other personal or clinical details of siblings under the age of 18 was obtained from their parents or legal guardians.

Competing interests
The authors have no conflicts of interest to declare.

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