



BRIEF COMMUNICATION

Deletion of the last two exons of *FGF10* in a family with LADD syndrome and pulmonary acinar hypoplasia

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Abstract

Pulmonary acinar hypoplasia (PAH) and lacrimo-auriculo-dento-digital (LADD) syndrome have both been associated with loss-of-function variants in, or deletions of *FGF10*. Here we report a multi-generational family with seven members manifesting varying features of LADD syndrome, with one individual dying in early infancy of PAH. Whole genome sequencing in one family member identified a 12,158 bp deletion on chromosome 5p12 that removes two of the three exons of *FGF10*. Allele-specific PCR demonstrated that all affected family members, including the individual with PAH, carried the 12 kb deletion. We conclude the deletion is pathogenic and expands the mutational spectrum of *FGF10* variants in LADD syndrome. The common mechanism underlying the variable clinical features of LADD syndrome is defective terminal branching of salivary and lacrimal glands and pulmonary acini, regulated by the *TBX4-FGF10-FGFR2* pathway. The variable phenotypic expressivity of *FGF10* haploinsufficiency from relatively benign to lethal is likely due to variation at other genetic loci.

Introduction

Pulmonary acinar hypoplasia (PAH) or acinar dysplasia of the lung, is a rare neonatal disorder with a near complete fatality rate. It is characterised by absent or immature acinar structures, with no alveoli. The recurrence of PAH within families is well-recognised [1–3], but until recently the genetic basis of the disorder was unknown. In 2016, PAH was linked to variants in the genes *TBX4* and *FGFR2* [4, 5]. The protein products of these genes are components of an epithelial-mesenchymal signalling pathway that regulates

the developmental branching of lungs and limbs, among other organs [4–7].

Fibroblast growth factor 10 (*FGF10*) is also a key component of this pathway [8]. Pathogenic variants in *FGF10*, along with *FGFR2* and *FGFR3*, cause the dominantly inherited allelic disorders lacrimo-auriculo-dento-digital syndrome (LADD – OMIM 149730) and aplasia of the lacrimal and salivary glands (ALSG – OMIM 180920) [9–12]. In 2019, Karolak et al. reported five cases of PAH from four families who were heterozygous for copy-number deletions, or SNVs, in *FGF10* [9].

Here, we describe an additional family with LADD syndrome caused by a novel 12 kb heterozygous deletion that encompasses the last two exons of *FGF10*. DNA extracted from a Guthrie card sample from a neonate who died of PAH in the early postnatal period, confirmed that she too carried the deletion.

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Subjects and Methods

Clinical history

The index case (III.6, represented by an arrow on Fig. 2B) was born with hypoplasia of the left thumb (Fig. 1A) and an

Fig. 1 Clinical findings in the affected family. **A** Hypoplastic thumb in the index case, an accessory thumb was removed surgically in infancy. **B** Bifid uvula in the index case. **C** Lung histology from the infant (individual IV.4) who died from pulmonary acinar hypoplasia (PAH), showing reduced density of bronchioles and absence of alveoli. **D** For comparison, normal lung histology from a 2-week old who died of sudden infant death syndrome (SIDS) showing normal alveolar structure. H & E stain, magnification $\times 250$.

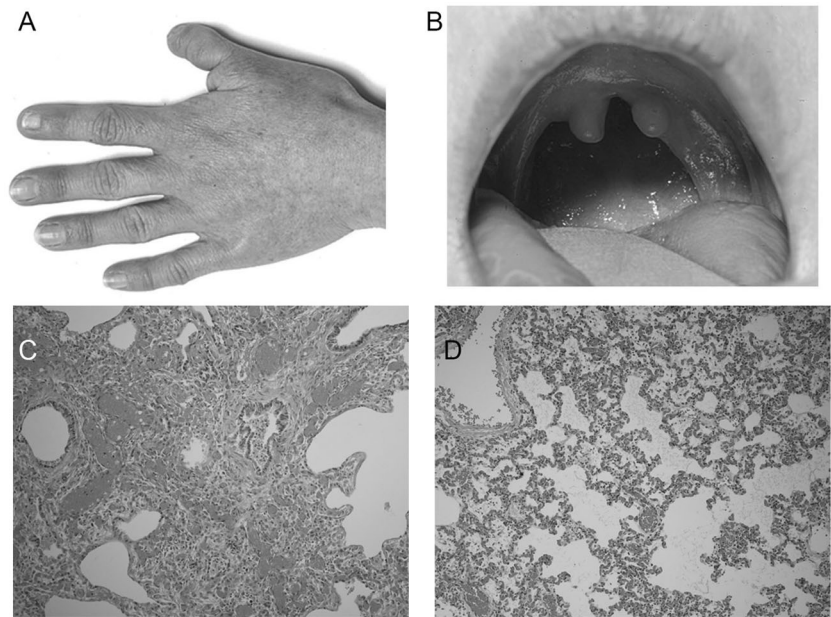
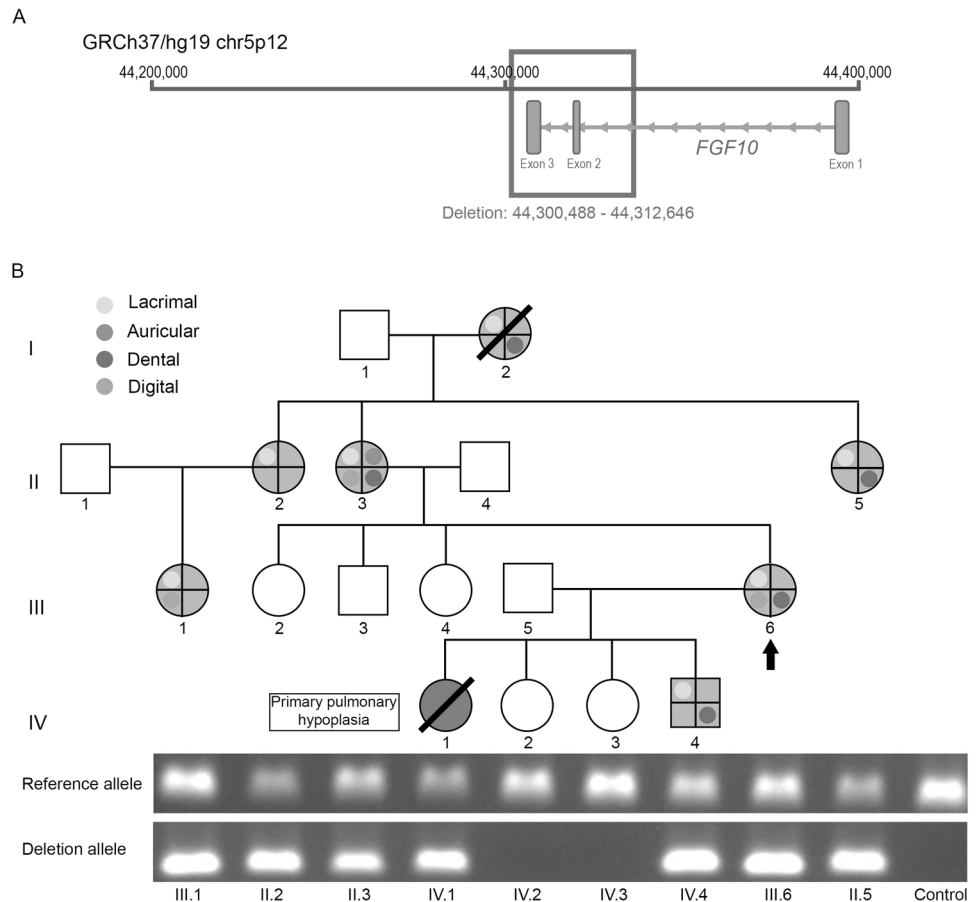


Fig. 2 Genetic findings in the affected family. **A** Schematic representation of the NC_000005.9: g.44300489_44312646del deletion in context with the *FGF10* gene. **B** Detailed pedigree to show degrees of affectedness in the four-generation family, the index case is marked with an arrow. Results from the allele specific PCR show that all affected individuals are heterozygous for the deletion, whereas unaffected family members, and a healthy control individual, only amplify the reference allele. No DNA was available for individual I.2.



extra left thumb, which was removed surgically in infancy. She lost her primary teeth through severe caries at the age of 5 years and was subsequently found to have absent parotid and submandibular glands with poor salivary flow, and a

bifid uvula (Fig. 1B). She has epiphora because of absent lacrimal ducts. A diagnosis of LADD syndrome was made at the age of 32 years. There was a striking three-generation history of the disorder (Fig. 2B).

The index case had her first child (IV.1) at the age of 27. The infant was born after a normal full-term pregnancy but, within a few minutes of birth, she developed respiratory distress and central cyanosis. In Neonatal Intensive Care she required ventilation, requiring 100% oxygen to maintain saturations above 90%. An echocardiogram on day 2 indicated persistent pulmonary hypertension of the newborn. Her respiratory status continued to deteriorate. After discussion with her parents, ventilator support was withdrawn on day 12.

The post-mortem examination showed a reduction in the number of large and small bronchi, bronchioles and an absence of alveoli, indicating a severe degree of PAH (Fig. 1C). No abnormalities were identified in any other organ. Clinical details of this case have been published previously [13]. The index case went on to have three other children, the youngest of whom had lacrimal duct hypoplasia and absent salivary glands (IV.4).

Human subjects and ethical approval

The study was conducted in accordance with the Declaration of Helsinki and the family consented to participate in a research study under approved protocols MEC/08/08/094 and 13/STH/56 (Health and Disability Ethics Committee, New Zealand). Clinical images are reproduced with consent from the family.

Genome sequencing

Genomic DNA was extracted from whole blood and genome sequencing was carried out on individual III.6, by the Kinghorn Centre for Clinical Genomics (Sydney, Australia) using the HiSeq2500 (Illumina, CA, USA) as described previously [14]. Briefly, DNA was prepared with the Illumina 30× GS (TruSeq Nano) v2.5 kit. Alignment of paired reads and variant calling was in accordance with the best practice guidelines from the Genome Analysis Tool Kit (GATK). SnpEff (v.4.3 S) and BCFtools annotate were used to annotate GVCFs. Manta (v1.6.0) was used to call large deletions and duplications using the genome sequences.

Variant confirmation

Genomic DNA was extracted as above for all but one family member. For individual IV.1, DNA was retrieved by the laboratory service at Auckland City Hospital from a heel-prick blood spot taken at birth. Allele-specific PCR was performed as follows: a common forward primer, 5' to the deletion (on the forward strand, *FGF10* is located on the reverse strand), was used together with either a reverse primer designed within the deleted region, therefore only amplifying from the non-deleted allele (product size

130 bp), or with a reverse primer located 3' to the deleted region (product size 12,271 bp reference allele, or 112 bp deletion allele). PCR conditions were such that the reverse primer, 3' of the deleted region, would only amplify with the common forward primer from the deletion allele (112 bp). The AmpliTaq Gold polymerase kit (Applied Biosystems, CA, USA) was used for all PCR reactions. Primer sequences are available on request.

Results

Because of the previous implication of 5p12 deletions and *FGF10* variants in LADD/ASG and PAH [9, 12], we examined this region in the genome sequence of the index case. This approach identified a heterozygous 12,158 bp deletion: NC_000005.9:g.44300489_44312646del; NM_004465.1:c.326–2014_*4608del. The deletion encompasses the last two (of three) exons of *FGF10* (Fig. 2A). The variant was classified pathogenic (1a) according to the ACMG/AMP guidelines [15] and has been submitted to ClinVar (SCV001548555).

The maternal grandmother (I.2), mother (II.3), two maternal aunts (II.2 and 5), and a first cousin (III.1) of the index case were also affected with LADD syndrome, as well as her son (IV.4). Her first daughter (IV.1) died in early infancy from PAH (Fig. 2B). To confirm that the deletion segregated with the phenotype, a PCR-based test was designed to specifically amplify from the reference or deleted allele as described in the methods. DNA was not available from individual I.2. DNA from individual IV.1, extracted from a heel-prick bloodspot, was highly fragmented. We were able to map the breakpoints of the deleted region, in the index case, to design PCR products to be as small as possible to increase the chances of amplification from individual IV.1. The six affected family members, from whom DNA was available, amplified both a reference allele product and a deletion allele product demonstrating that they are all heterozygous for the deletion. Two unaffected family members (IV.2 and IV.3), as well as an unrelated, healthy control individual only amplified a product from the reference allele (Fig. 2C).

The lung-enhancer region (17q23.1q23.2), previously associated with PAH in families with deletions or truncations of *FGF10* or *TBX4* [9], was analysed for variants in the genome sequence of index case III.6. We found eight of the non-coding variants in *BCAS3* identified by Karolak et al. in individuals with PAH and copy-number variation or point mutations in *TBX4* [9]. One insertion variant (rs71148400) is present on both alleles, but the expansion is two nucleotides longer on one allele. The minor allele frequency of the single nucleotide variants found varies from 0.18 to 0.01. The two common variants found in

individuals, with a *TBX4* mutation and PAH, by Karolak et al. (rs35827636 and rs192153557) were not found in individual III.6. The complete details of the variants found are listed in Table S1.

Discussion

Loss-of-function variants, and whole gene deletions of *FGF10* have previously been implicated in LADD syndrome and PAH [9]. In the family presented here, genome sequencing identified a novel 12,158 bp deletion encompassing the last two exons of *FGF10*. This variant is classed as pathogenic according to the ACMG/AMP guidelines. Using a targeted, allele-specific approach, we were able to show that a further six affected individuals in the family had inherited the same deletion, including the infant affected with PAH.

This deletion removes the last two (of three) exons of *FGF10*. The 3' breakpoint lies in the intron between exons one and two (Fig. 2A). The transcription initiation site of *FGF10* is therefore left intact, but if a transcript were to be made from the deletion allele, it would likely be subject to nonsense-mediated decay, rendering individuals with the deletion deficient of the *FGF10* gene product. Previous studies have shown that heterozygous loss-of-function of *FGF10* is sufficient to cause LADD/ALSG but other inherited factors could be required for these *FGF10* variants to result in PAH [9]. Entesarian et al. describe a 53 kb deletion segregating with aplasia of lacrimal and salivary glands in an extended pedigree that also removes exons 2 and 3 of *FGF10*. However there is no reported PAH in the family [12].

Amongst their many functions, FGF10 and its receptor are key regulators of terminal differentiation of acinar structures including the salivary and lacrimal glands [16, 17]. In the lungs, this results in the arrest of pulmonary development at 10–20 weeks' gestational age with no alveolar formation [8, 9]. FGF10 is also involved in the development of palatal structures [18]. Two members of the family we report had a bifid uvula, a finding that has been reported previously in one patient with LADD syndrome [19]. We suggest that bifid uvula may be a feature of LADD.

How both a relatively benign condition (LADD) and a fatal one (PAH) can be caused by the same deletion or single nucleotide variant requires explanation. Karolak et al. found that individuals who develop PAH with loss-of-function *TBX4* or *FGF10* variants also harbour non-coding single nucleotide variants in a putative lung-enhancer region at 17q23.1q23.2, whereas individuals with the same variants and normal lungs do not [9], implicating epistatic genetic interactions. We examined the genome of the index

case (III.6) and discovered eight of the non-coding variants found by Karolak et al. It is uncertain from the Karolak paper if individuals with *FGF10* mutations, without PAH, were also found to have risk alleles at 17q23.1q23.2. From our data, it seems as though the eight variants found in our index case, do not predispose to PAH when inherited with a *FGF10* null allele. Unfortunately, because of the fragmented state of the DNA from individual IV.1, we were unable to test the burden of variation at 17q23.1q23.2. The contribution of trans-acting factors to the manifestation of *FGF10*-related LADD syndrome will be important to ascertain in future presenting families.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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