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# A Novel Mutation Involving the Initiation Codon of *FGF3* in a Family Described with Complete Inner Ear Agenesis, Microtia and Major Microdontia (LAMM Syndrome)

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## Abstract

LAMM syndrome (OMIM #610706) is a rare autosomal recessive syndrome characterized by the association of Michel aplasia, microdontia and malformation of the external ear. Different mutations in *FGF3* gene were reported in several families presenting with this syndrome.

Clinical features and genetic results observed in a family with LAMM syndrome are reported. The diagnosis of isolated Michel aplasia was initially made in this family composed of two affected children. Microtia and microdontia was recently evidenced in both patients suggesting the diagnosis of LAMM syndrome. New auditory and orodental iconography was performed permitting to describe the patients' phenotype in depth and to report rare findings of LAMM syndrome.

The sequencing of *FGF3* gene identified a novel missense mutation (c.2T>G), substituting the first initiator methionine in arginine, in the fibroblast growth factor 3 (*FGF3*) at the homozygous state in both patients. LAMM syndrome was confirmed and appropriate genetic counseling performed.

**Keywords:** LAMM syndrome; FGF3 gene; Autosomal recessive inheritance; Michel aplasia; Microtia; Genetic counseling

## Introduction

Whereas developmental defects of ears and/or teeth are very common, the combination of both remains a rare syndromic event. Michel aplasia, first described in 1863 [1] is a very rare malformation with complete bony and membranous aplasia of the inner ear. Michel aplasia in association with microdontia and malformation of the external ear (microtia type I) defines the LAMM syndrome (Labyrinthine Aplasia, Microtia, and Microdontia; OMIM 610706), which is a rare recessive syndrome recently related to mutations in the *FGF3* gene.

Only a dozen of FGF3 mutations have been reported to date in various studies. Initially, three different mutations were described in 3 unrelated Turkish families: p.Arg104*, p.Ser156Pro and p.Val206Serfs*13 [2]. Since then, 9 other mutations were reported in 6 different studies [3-8].

We report herein the identification of a novel mutation affecting the initiation codon of *FGF3* gene at the homozygous state (p.[Met1Arg];[Met1Arg] / c.[2T>G];[2T>G]) in two adults' siblings with LAMM syndrome for whom the inner ear aplasia was reported in 1997 [9]. In addition to the identification of the mutation, we provide here the clinical and radiological phenotype of these 2 patients with novel RMI imaging features as well as the in depth description of the orodental phenotype.

## Methods

### Patients and related phenotype

The two patients described are the two children of a non-consanguineous couple, native of France. Informed consent was obtained from all the family, in accordance with the tenets of the Declaration of Helsinski.

### Case 1

The first child, a boy, was referred to the genetic clinic when he was 1 year old due to a profound sensorineural hearing loss diagnosed a few months after birth. The pregnancy had been uneventful with no history of antenatal exposure to medical treatment, toxics or infections. The tympanogram was flat and the brainstem auditory evoked potential were absent on both sides. Moreover, X-rays of temporal bones revealed asymmetric diameters of external auditory canals. The clinical examination evidenced no dysmorphia, small anteverted auricles (Figures 1A-1D) and microdontia of the primary dentition. A dextrorotation of the heart and ocular dryness were also diagnosed during the first year of life. The external ear was operated on for esthetic purposes (Figures 1 E and 1F). The boy, although profoundly deaf,

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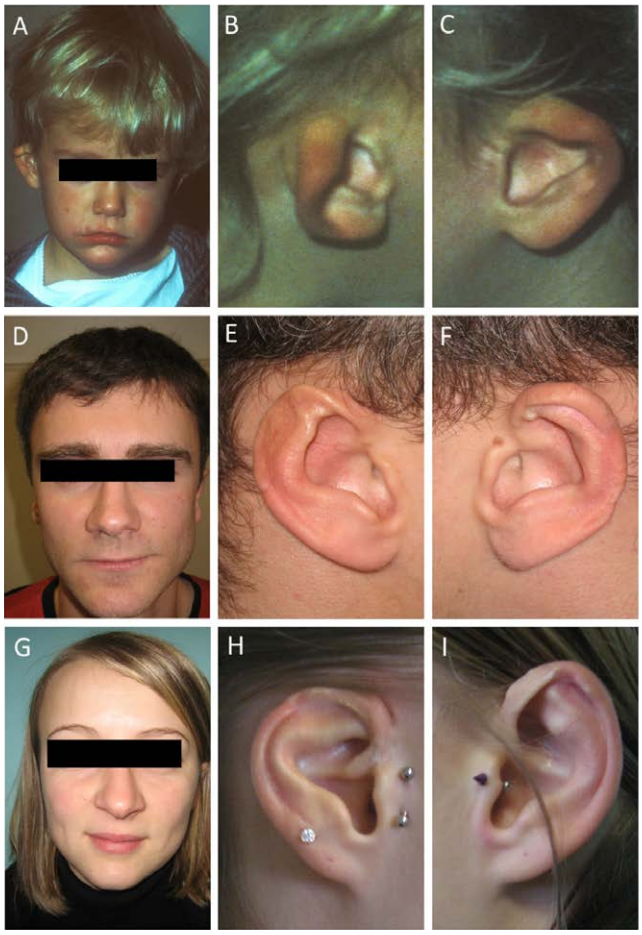


**Figure 1:** Photographs of the two affected patients. Frontal view of the patient I.1 at the ages of 1 year (A, B, C) and at adulthood (D, E, F) and of the patient I.2 at adulthood (G, H, I) showing the absence of facial dysmorphia (A, D, G) and the microtia before (B, C) and after surgery (E, F, H, I).

learnt to use phonic vibrations and labial lecture enabling a perfectly integrated school and young adult life. At the age of 17, an MRI showed an internal ear aplasia [9], also called "Michel abnormality" in reference to the initial description reported in 1863 [1].

As a young adult (31 years old), the patient sought for genetic counseling in order to evaluate the risk of recurrence for his offspring. At this occasion an in-depth dental and otic evaluation was performed. The dental examination revealed a generalized microdontia with widely spaced teeth, associated with tooth agenesis (Figures 2A-2D). More precisely, at the maxillary level, the right first premolar was absent with persistence of the deciduous molar (white arrow, Figure 2C). On the left side, the first deciduous molar also persisted despite the fact that no permanent premolars were absent there (star, Figure 2C). At the mandibular level, permanent central incisors were absent, with persistence of the deciduous incisors (Figures 2C and 2D). The right second premolar and first molar were also missing due to previous avulsions (Figure 2C). In addition to microdontia and agenesis, the clinical examination also showed a dyschromia of the teeth (Figures 2A and 2B), as well as enamel defects corresponding to a thinning of enamel mantle and the presence of pits on the molar and premolar occlusal tables (Figure 2E). The radiographs and the CT scan, carried out for dental implant planning surgery, confirmed these clinical signs (Figures 2C, 2F and 2G) and highlighted a taurodontism (elongation of the pulp chamber) on the first permanent molars (Figures 2F and 2G). The radiographs also revealed root abnormalities, consisting in root elongation (Figures 2C, 2D and 2F) and C-shaped morphology (Figures 2H and 2I).

To better characterize the otic abnormalities found in this patient, a novel generation CT scan was performed. Compared to Marsot-Dupuch et al. [9] this analysis confirmed the Michel aplasia (characterized by the bilateral aplasia of the inner ear structures associated with bilateral aplasia of the petrous apex and absence of development of the internal auditory canal) as well as the hypoplasia of the middle ear ossicles associated with a normal external auditory canal (Figures 3A-3C). However, abnormalities of the middle ear ossicles were better described with this new imagery. Indeed, the stapes, initially reported as absent, was in fact very hypoplastic. Moreover, the incus and the malleus, initially considered as normal, were deformed with a shortening of the long process of the incus. A new MRI was also realized, confirming the initial findings (described but not shown), notably the bilateral absence of the cochlea vestibular nerve and absence of development of the internal auditory canal in high resolution T2 weighted sequences focusing on the cerebellopontine angles (Figure 3D). The resolution of the new radiographic images permitted to define the abnormal course of the facial nerve. Indeed, after a normal origin, the course of the facial nerve passed in the lower part of the cerebellopontine angle and then followed a usual posterior path in the petrous apex. The CT scan confirmed its usual emergence in the stylo-mastoid foramen behind the styloid processes and sideward of jugular foramen.

On the basis of these clinical and radiological data, the diagnosis of isolated Michel aplasia initially established [9] moved to LAMM syndrome.

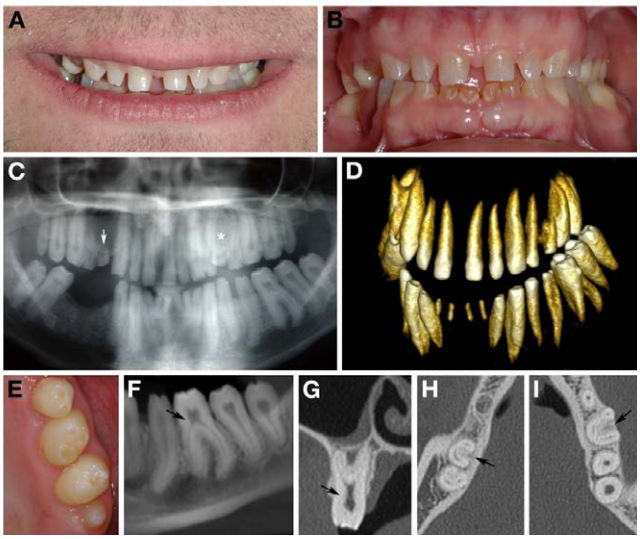


**Figure 2:** Clinical views and radiographs illustrating tooth abnormalities of affected patient I.1. (A, B) Frontal clinical views showing the microdontia and the dyschromia of the teeth. (C, D) Panoramic radiograph (C) and 3D CT-scan reconstruction of the teeth (D) showing oligodontia and root abnormalities. (E) Occlusal view of the left maxillary arcade showing the pits in the enamel of the premolars and molars occlusal tables. (F, G) Panoramic focus on the left mandibular teeth (F) and cross-sectional CT view (G) exhibiting respectively the taurodontism (black arrows) on the mandibular left (F) and the maxillary right (G) first molars. (H, I) Axial mandibular slice displaying the C-shaped roots (black arrows) of the right (H) and left (I) molars.

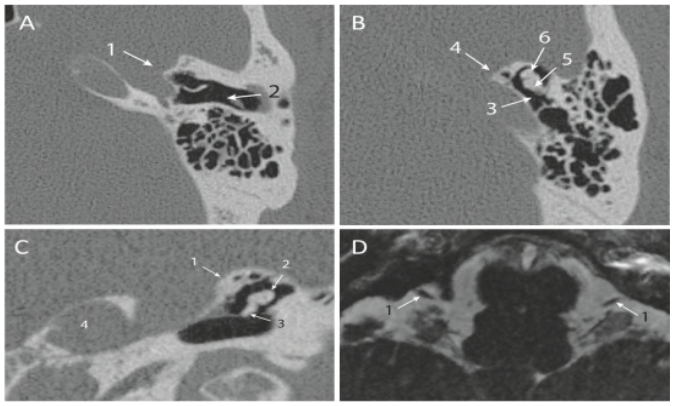


**Figure 3:** Radiological images illustrating ear abnormalities of affected patient I.1. (A, B) Axial CT scan showing the bilateral aplasia of the inner ear structures (1), normal external auditory canal (2), hypoplasia of the middle ear (3) and bilateral aplasia of the petrous apex and non-development of the internal auditory canal (4). Incus and Malleus are shown by arrows (5) and (6) respectively. (C) Coronal CT scan of the skull base and petrous bones showing the bilateral absence of the inner ear structures associated with bilateral aplasia of the petrous apex and non-development of the internal auditory canal (1). Incus and Malleus are shown by arrows (2) and (3) respectively. (4) Left jugular foramen. (D) Axial MRI of the cerebellopontine angles in high resolution T2 weighted sequence showing the bilateral absence of the cochleovestibular nerve and the non-development of internal auditory canal. (1) Facial nerves.

### Case 2

His sister, born 3 years later, presented with similar symptoms, namely profound deafness, teeth agenesis, microdontia associated with widely spaced teeth and malformation of the external ears with small anteverted auricles (Figures 1G-1I). Her deafness was more profound than her brother with only some residual auditory perception on low frequencies. CT scan and MRI disclosed the same anomalies of the inner ear than her brother. The teeth agenesis and the microdontia were however less severe. The diagnosis of LAMM was also established for her.

### Parents reports

The parents of the two patients are non-consanguineous and native of France. Both of them are in good health and their examination was normal especially for hearing and for the appearance of the external ears. The teeth examination was normal.

MRI and CT scan were performed for the two parents and revealed no malformation of the inner, middle or external ear.

### Genotyping

The *FGF3* gene was screened for mutations by direct Sanger sequencing. Informed consent was obtained from the 4 members of the family for DNA analysis. Genomic DNA was extracted from blood samples according to the manufacturer's protocol (Flexigene DNA kit, Qiagen). The three exons and exon-intron boundaries of *FGF3* gene were PCR amplified with 50 ng of genomic DNA template. The primers were designed with Primer 3 (http://frodo.wi.mit.edu/primer3); detailed protocols and primers are available on request. Bidirectional sequencing of purified PCR products was performed by the GATC Sequencing Facilities (http://www.gatcbiotech.com/en/about-us/gatc.html). All exons, intron-exon boundaries were sequenced.

### Mutation analysis

Sequences were aligned and compared with consensus data obtained from the human genome databases (www.ensembl.org) and examined for variation using Seq Scape® Software (Applied Biosystems®) program.

### Homozygosity mapping

A genowide scan of the affected boy was undertaken using the Affymetrix Gene Chip Human Mapping 10K 2.0 Array (average resolution of about 0.3cM) and Assay kit according to the manufacturers' instructions. Genotypes were called with GTYPE version 4.1 (Affymetrix) as previously described [10]. Detailed information on specific SNPs was accessible on supplementary table.

## Results

Sequencing of *FGF3* in both patients identified a novel homozygous missense mutation (p.[Met1Arg]; c.[2T>G]) in the first exon. The parents were heterozygous for the mutation (Figure 4A). This mutation substitutes the first amino acid, methionine, into an arginine. Because of the homozygous status of the mutations, we suspected a distant consanguinity, and performed a genome scan for case II.1 using an Affymetrix setting. Haplotype sharing of SNPs identified a homozygote region of 10 Mb on chromosome 11 encompassing *FGF3* gene, between rs 1944130 and rs 568421 corresponding to physical position of 69,291,704 - 78,714,313 bp (Figure 4B). This homozygous block, associated with several others disturbed along the patient's genome, suggested a distant consanguinity.

## Discussion

The first case of LAMM syndrome was reported in 1991 by Hersch et al., who described a patient presenting with Michel/labyrinthine aplasia, microtia and microdontia [11]. The acronym "LAMM syndrome" was used first in 2007 by Tekin et al. with the identification of a homozygous mutation in *FGF3* in three unrelated Turkish families including nine affected individuals [2]. The authors described three major phenotypic manifestations common to all individuals, which were then confirmed by further studies [3-8]: i) profound congenital neurosensorial deafness associated with the complete absence of inner ear structures bilaterally, including cochlea, vestibule and semicircular canals (Michel aplasia), ii) type I microtia with shortening of auricles, and iii) microdontia with widely spaced teeth. The individuals appeared to have normal middle ears structures and normal cognitive abilities, even if a delay in gross motor skills was noted for all of them, probably in relation with absence of inner ear and impaired balance. Type I microtia was associated with anteverted auricles in seven of nine individuals. The dental anomalies reported were variable with supernumerary teeth, absence of teeth, peg-shaped lateral incisors and loss of tooth heights due to abrasion. A mild micrognathia was also reported in some patients [2].

Herein, we report the identification of a novel *FGF3* mutation in two siblings presenting with LAMM syndrome with the characteristic association of Michel/labyrinthine aplasia, microtia and microdontia. Compared to the literature, our patients presented the classical clinical and radiological symptoms of LAMM syndrome associated to mutations in *FGF3*, i.e., inner ear agenesis, microdontia and microtia associated with the anteverted auricles. Radiological investigations demonstrated anomalies classically described of the inner ear, but also defects at the level of the middle ear. This anomaly was reported only once before by Sensi et al., in two sibs presenting a bilateral involvement of middle ear structures in addition to the inner ear aplasia [8] (Figure 3). Our observation therefore confirms that middle ear defects can be found in LAMM syndrome. We also described bilateral absence of the cochlea vestibular nerve with otherwise normal brain and cerebellum

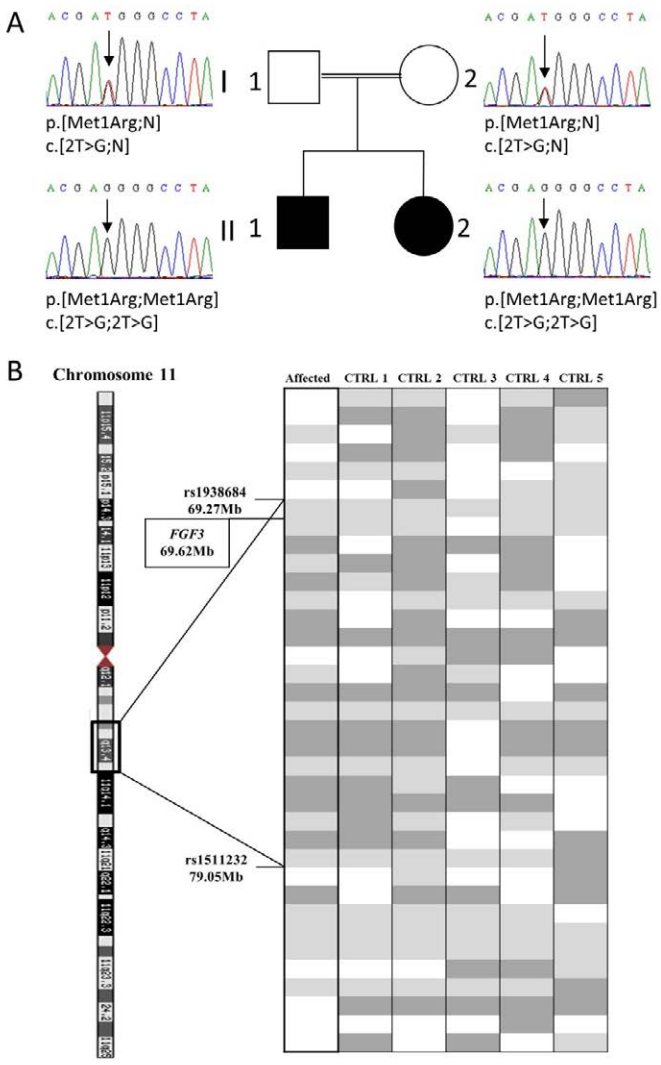


**Figure 4:** Mutation detection in the family and homozygosity mapping performed for patient I.1. (A) Pedigree of the family and electropherograms of a part of the *FGF3* exon 1 showing a homozygous p.Met1Arg mutation in the two affected patients (II.1 and II.2) and a heterozygous p.Met1Arg mutation in the two unaffected parents (I.1 and I.2). (B) Schematic representation of the results of homozygosity mapping showing a homozygous region on chromosome 11 including *FGF3* for one affected patient compared to 5 controls. Grey box: homozygous SNPs (AA or BB). White box : heterozygous SNPs (AB).

for the two patients. This finding was previously reported once in a patient affected by LAMM syndrome [2].

The orodental examination showed abnormalities very similar to those reported in the literature; the CT-scan carried out for dental implant planning surgery, however also highlighted new features such as the presence of a taurodontism, as well as root abnormalities consisting in root elongation and C-shaped morphology. To our knowledge, this is the first time that such defects are reported with *FGF3* mutations.

Interestingly, the abnormal external ears and orodental phenotype were initially overlooked as the diagnosis of isolated Michel aplasia was suggested at first [9]. Years later a reappraisal by the medical genetic department and the dentistry department completed the syndromic description to LAMM. Although autosomal dominant transmission was initially suggested [9], the identification of the homozygous mutation in the *FGF3* gene confirmed an autosomal recessive inheritance reassuring the patient for his offspring, as he was not related to his wife.

*FGF3* gene is composed of 3 exons and since 2007, 12 exonic mutations have been reported in 15 families: 6 missense, 2 nonsense and 4 frame shift mutations [2-8]. Two mutations were common to in several families, namely: p.Arg95Trp and p.Arg104* (Figure 5). Here, we describe the identification of a novel mutation, which substitutes the first amino acid, methionine, into an arginine. The methionine is the first amino acid common to all genes, permitting the initiation of protein translation; a function that could explain why methionine is highly conserved during evolution. We made the hypothesis that this mutation prevents translation of FGF3 protein, then resulting in no functional FGF3 protein, as described in other syndromes such as thalassemia, with mutation in the alpha 1-globin gene [12], oligondontia with mutation in *PAX9* [13] or albinism with mutation in the tyrosinase gene [14]. However, we could not exclude that this mutation generates an aberrant protein product due to the presence of in-frame methionines in the FGF3 transcript. Unfortunately, we could not have access to cells from the patient and could not perform further analysis at the RNA or protein level, then preventing to exclude this possibility.

Interestingly, one heterozygous deletion of the entire *FGF3* gene was reported in 3 families suffering from otodental syndrome with an autosomal dominant transmission [15]. Otodental syndrome (OMIM 166750) is a differential diagnosis of LAMM syndrome characterized by grossly enlarged canine and molar teeth (globodontia) associated with sensorineural hearing loss [16]. In LAMM syndrome, hearing loss is congenital and profound whereas in otodental syndrome, patients present high-frequency and progressive hearing-loss. The dental phenotype is also different between the two syndromes; indeed, the microdontia in LAMM syndrome contrasts with the globodontia in otodental syndrome. The stunning opposite clinical phenotypes between otodental syndrome due to deletion of one allele of the entire *FGF3* gene and LAMM syndrome with homozygous or compound heterozygote nonsense mutations in FGF3 remains unsolved [2,15].

Lacrimo-Auriculo-Dento-Digital (LADD) Syndrome (OMIM #149730) is another differential diagnosis of LAMM syndrome associating hearing loss, malformations of the auricles and dental anomalies, including small and peg-shaped lateral maxillary incisors as well as mild enamel dysplasia. Contrasting with LAMM syndrome, the auricles features are cup-shared pinnae and the hearing loss has a mixed origin. Moreover, LADD syndrome includes lacrimal and digital features not present in LAMM syndrome. Lacrimal anomalies consist in aplasia or hypoplasia of the puncta with obstruction of the nasal lacrimal ducts. The digital features are variable but include fifth finger clinodactyly, duplication of the distal phalanx of the thumb, triphalangeal thumb, and syndactyly. Interestingly, LADD syndrome, inherited as an autosomal dominant condition (like the otodental syndrome), has been attributed to heterozygous mutations in *FGFR2*, *FGFR3* and *FGF10* genes, pointing to a common Fibroblast Growth Factors pathway implied in common steps of the development of the ear and the teeth [17, 18].

In conclusion, we describe a novel homozygous mutation implying the first methionine of FGF3 in a family with LAMM syndrome with unexpected consanguinity. The identification of the mutation permitted to confirm the diagnosis of LAMM syndrome, clinically suspected at the adulthood and was mandatory for proper genetic counseling.

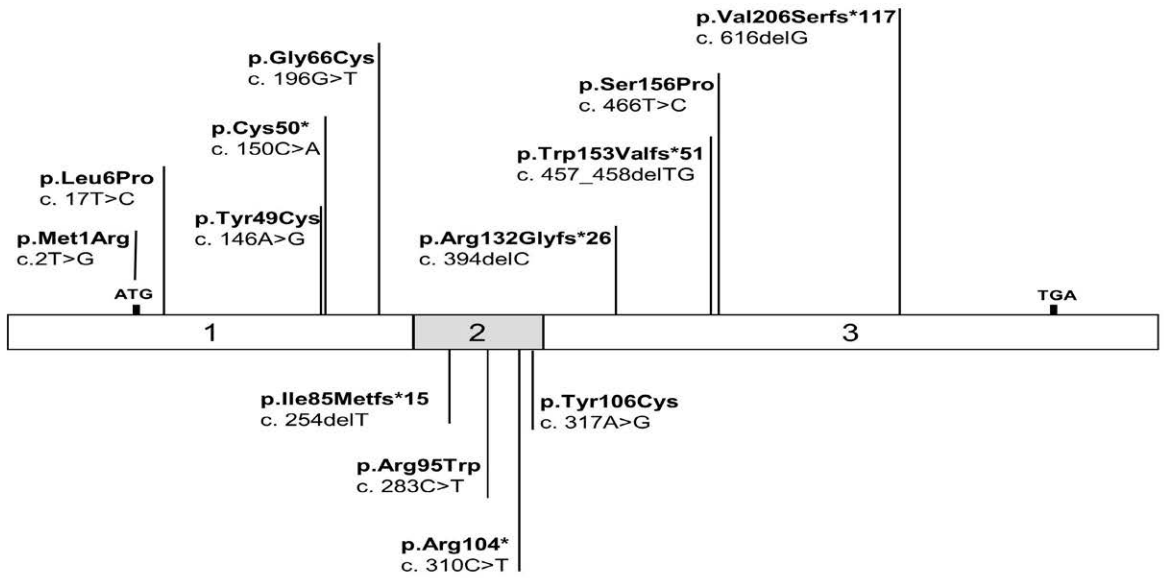


**Figure 5:** Mutations reported in *FGF3* to date. Each mutation was reported in only one family except for the mutations p.Arg95Trp and p.Arg104* described respectively in 2 and 3 independent families (indication in exponent in the figure). The mutation identified in this study is underlined.

Indeed, a very low risk genetic counseling could be advocated to the siblings for their offspring concerning the recurrence of this disabling disease that necessitate a specialized education for severe deafness (no cochlear implantation for obvious anatomical reasons) as well as dental implants in adulthood.

Clinically, our report confirmed the possibility of middle ear abnormalities in LAMM syndrome, described only once to date. Moreover, we described in detail the orodental phenotype observed in LAMM syndrome. This report highlights the importance of a global clinical examination of the patient behind a symptom seemingly isolated and especially the importance of dental examination as a highly valuable diagnostic guidance.

### Acknowledgement

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