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A novel *FGF8* mutation in a female patient with isolated congenital anosmia

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Dear Editor,

Isolated congenital anosmia (ICA) is a rare condition, with an incidence of 1 in 10,000, which rarely presents in an autosomal pattern of inheritance: it has incomplete penetrance, while very few genetic causes have been associated with it [1-4]. Even though congenital anosmia can be isolated, there are many syndromes associated with it, including Kallmann syndrome (KS) (OMIM #308700), CHARGE syndrome (OMIM #214800), Bardet-Biedl syndrome (OMIM #209900), congenital insensitivity to pain (OMIM #243000), Leber congenital amaurosis (OMIM #611755),and Refsum disease (OMIM #266500). Even though the pathophysiology of the disease has been previously studied, analyses of the genetic background of congenital anosmia have been limited, with few genes linked to it, such as *PROKR2, PROK2* [5], *CNGA2* [6], *SCN9A* [7], and *TENM1* [8]. In a recent study by Alkelai et al. [9], whole exome or genome sequencing in eight families with multiple affected family members sought to identify a genetic link to congenital anosmia. Even though hypothesis-free analysis did not show any strong single candidate variant in any of these families, 3 KS genes (*FGFR1, SEMA3A*, and *CHD7*) were found to be enriched in patients with anosmia.

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Authors' contributions SIM conceived part of the study, participated in its design and coordination, participated in the molecular genetics studies, and drafted the manuscript; PL participated in coordination of the project, carried out the molecular genetic studies, and participated in the sequence alignment; KV participated in coordination of the project and carried out part of the molecular genetic studies; AGT examined the patient, performed all hormonal and imaging tests and set the final diagnosis, referred the patient for genetic testing, and monitored the patient in the pediatric endocrinology unit; GAN conceived the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

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KS is a rare heritable disorder, characterized by the combination of anosmia with hypogonadotropic hypogonadism, and is caused mainly by neurodevelopmental defects in GnRH neuronal migration from the olfactory placode to the hypothalamus, leading to isolated GnRH deficiency (IGD) with olfactory defects [10, 11]. Apart from anosmia, KS is also characterized by other non-reproductive features, such as unilateral renal agenesis (URA), midline defects, dental agenesis, synkinesia, and hearing loss, such that genetic prioritization has been proposed based on such additional non-reproductive features [12, 13]. Importantly, congenital anosmia has been detected in family members of affected patients with KS who are reproductively normal [14]. Thus, KS genes are valuable candidates for elucidation of congenital anosmia. Moya-Plana and colleagues [5] have previously reported rare sequencing variants (RSVs) in known IGD genes, e.g., prokineticin receptor 2 (*PROKR2*), harbored by patients with ICA and other KS genes, such as SEMA3A, CHD7, and FGFR1, which have been found to be enriched in patients with ICA [9]. Here, we report the case of a female patient presenting with ICA, high-arched palate and low-set ears, who carries a novel rare RSV in the neurodevelopmental pathway gene of fibroblast growth factor 8 (*FGF8*).

In our study, we analyzed the genetic background of a female patient, who was referred to the 4th Department of Pediatrics, Medical School, Aristotle University of Thessaloniki, Greece, at the age of 9 years and 9 months for evaluation of ICA. Birth and past medical history were unremarkable. The physical examination showed the midline defect of the high-arched palate and low-set ears. Her breast development was Tanner stage I–II and pubic hair growth Tanner stage I. The response to the GnRH test is shown in Table 1. Her bone age correlated with her chronological age and her renal US was normal. Her olfaction was tested by use of the Greek version of the University of Philadelphia Smell Identification Test (UPSIT), which revealed anosmia (scoring 1/12), and the MRI of the brain showed bilateral olfactory bulb aplasia. Given that non-reproductive features of KS are the only features evident before pubertal timing, genetic screening for genes implicated not only in congenital anosmia, but also in KS, was performed, as detection of rare sequencing variants (RSVs) implicated in the disease could be crucial for early detection and prompt treatment in the case of a diagnosis of hypogonadism. Importantly, while genetic testing was being performed in the patient, we were informed that she achieved *spontaneous menarche* at the age of 13 years. Informed consent was obtained from the proband and both of her parents after full explanation of the purpose and nature of all procedures used. Of note, olfactory and reproductive function in both parents was intact, with normal scores in UPSIT (12/12). Neither of her parents had any midline defects.

Whole exome sequencing was performed by the Genomics Platform at the Broad Institute. Even though priority was given to the genes that are known to cause KS, rare sequence variants (RSVs) were sought in all 37 known IGD genes, including *AXL* (OMIM ID: 109135), *CHD7* (OMIM 608892), *DUSP6* (OMIM ID: 602748), *FEZF1* (OMIM ID: 613301), *FGF17* (OMIM ID: 603725), *FGF8* (OMIM 600483), *FGFR1* (OMIM 136350), *FLRT3* (OMIM ID: 604808), *GNRH1* (OMIM 152760), *GNRHR* (OMIM 138850), *HS6ST1* (OMIM 1604846), *IL17RD* (OMIM ID: 606807), *ANOS1* (OMIM 308700), *KISS1* (OMIM 603286), *KISS1R* (OMIM 604161) *LEP* (OMIM ID: 164160), *LEPR* (OMIM ID: 601007), *NSMF* (OMIM 608137), *OTUD4* (OMIM ID: 611744), *PCSK1*

(OMIM ID: 162150), *PNPLA6* (OMIM ID: 603197), *POLR3A* (OMIM ID: 614258), *POLR3B* (OMIM ID: 614366), *PROKR2* (OMIM 607212), *PROK2* (OMIM 607002), *RNF216* (OMIM ID: 609948), *SEMA3A* (OMIM ID: 603961), *SEMA3E* (OMIM ID: 608166), *SOX10* (OMIM ID: 602229), *SOX2* (OMIM ID: 184429), *SPRY4* (OMIM ID: 607984), *STUB1* (OMIM ID: 607207), *TAC3* (OMIM 162330), *TACR3* (OMIM 162332), *WDR11* (OMIM ID: 606417), *KL* (OMIM ID: 604824), and *DMXL2* (OMIM ID: 612186). Sanger sequencing was used to confirm the detected RSVs. The PCR primers and amplification conditions for each gene have been previously published [15, 16]. Since intragenic *ANOS1* deletions have been associated with KS, we also performed multiple ligand probe amplification (MLPA) for detection of *ANOS1* dosage variation. To identify other RSVs in genes associated with congenital anosmia, we screened the patient's whole exome sequencing data for RSVs in additional genes associated with congenital anosmia, including SCN9A, TENM1, and CNGA2.

By performing this thorough genetic analysis, we discovered a novel missense RSV in *FGF8* p. S235G. On verification, the mutation was absent from three different public databases: the Exome Variant Server, the NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) [February 2016], the 1000 Genomes Project [http://www.1000genomes.org/home] [17], and the non-Finnish European population of the Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.org) [February 2016]. The amino acid is conserved across multiple species, including human, mouse, and rat, as shown in Supplement Fig. 1. [18, 19]. The RSV was predicted to be possibly damaging, with a score of 0.759 (sensitivity 0.85; specificity 0.92) by Polyphen [20] and damaging by SIFT with a prediction score of 0.05 [21].

As shown in Fig. 1, the novel *FGF8* RSV S235G was confirmed by Sanger sequencing. This novel mutation was discovered in a female patient with congenital anosmia and facial dysmorphic features, including high-arched palate and low-set ears. *FGF8* is a neurodevelopmental gene that controls the migration of GnRH neurons from the olfactory epithelium into the hypothalamus. Mutations in *FGF8* are found in patients with KS and are typically inherited in an autosomal dominant way with incomplete penetrance [22]. Genetic changes in the FGF signaling pathway have frequently been associated with other non-reproductive features, like dental agenesis and midline defects [13]. Thus, in our case, the combination of congenital anosmia with high-arched palate and other dysmorphic features can be associated with the disruption of the FGF effect on the development and migration of the olfactory tracts into the hypothalamus.

The occurrence of spontaneous menarche and, thus, the presence of a functional GnRH neuronal network in this patient, who completely lacked olfactory bulbs, could be explained in various ways, including (i) possible differences in the degree of the neurodevelopmental defects in congenital anosmia compared to KS; (ii) involution of the olfactory bulbs and tracts occurring after the GnRH neuronal migration to the hypothalamus in ICA but not in KS; (iii) a potential future diagnosis of an adult form of hypogonadotropic hypogonadism, which is rarely seen in patients with KS, requiring close follow-up of our patient until an older age [23]; (iv) the possibility of a familial case of KS with variable expressivity

and incomplete penetrance, with the reproductive defects to be expected in the descending generations should the RSV be inherited; (v) the possible role of oligogenicity (i.e., two or more genes implicated in the expression of the disease) in the expression of the KS phenotype compared to ICA; and, finally, (vi) the possible presence of a novel gene implicated in ICA that is yet to be discovered. Even though there are several KS genes implicated in congenital anosmia, this is the first time this novel RSV in FGF8 has been associated with this disease. In our study, menarche occurred spontaneously in the patient and, therefore, close follow-up of children with KS-associated features is suggested to monitor for spontaneous puberty. Finally, describing the genetic basis of ICA will also shed light on the olfactory and GnRH neuronal biology and complex neuronal pathways and networks involved in the pathophysiology of such genetically complex diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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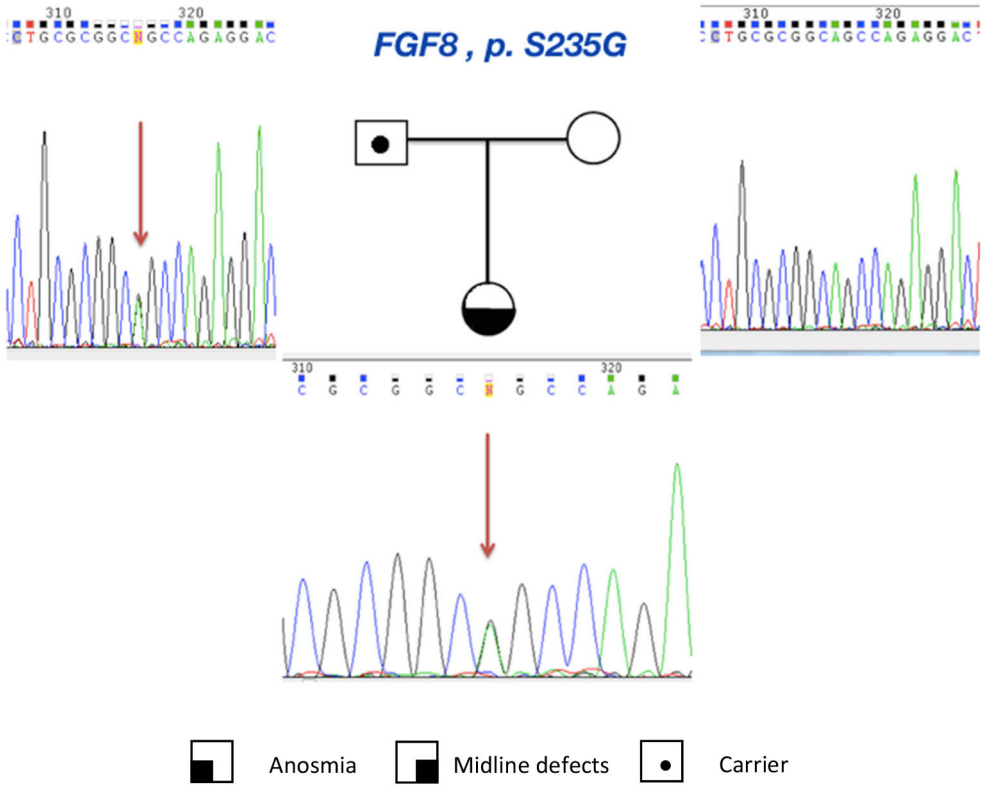

Fig.1.

Detection of a novel FGF8 mutation in a female patient with ICA. Sanger sequencing tracks and the segregation of the heterozygous novel mutation in the gene of *FGF8*, carried by a female with ICA, midline defects, and low-set ears. The mutation is inherited from her unaffected father, whereas the proband's mother was negative for the mutation. KS, Kallmann syndrome

Table 1

GnRH stimulation test results

Time	0 min	30 min	60 min
FSH (mIU/ml)	1.57	7.17	11.9
LH (mIU/ml)	< 0.10	2.94	2.84
E2 (pg/ml)	35.1	29	20

Response of FSH, LH, and E2 to GnRH stimulation revealed a prepubertal pattern with LH/FSH < 1 and no response of E2