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A homozygous loss-of-function mutation in *PTPN14* causes a syndrome of bilateral choanal atresia and early infantile-onset lymphedema PTPN14 mutation in lymphedema-choanal atresia

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ABSTRACT

A homozygous truncating mutation in nonreceptor tyrosine phosphatase 14 (*PTPN14*) has recently been associated with an extremely rare autosomal recessive syndrome of congenital posterior choanal atresia and childhood-onset lymphedema. *PTPN14* has been shown to interact directly with the vascular endothelial growth factor receptor 3 (*VEGFR3*), a receptor tyrosine kinase essential for lymphangiogenesis. Here we present an Iranian family with a single child affected by high-arched palate, congenital hypothyroidism, dysmorphic face, bilateral choanal atresia and infantile-onset lymphedema. Screening of the *PTPN14* revealed a novel homozygous frameshift mutation in exon 4 predicted to result in premature truncation of the polypeptide product, which segregated with the disease phenotype. To our knowledge, this is the second family with "choanal atresia and lymphedema syndrome" to be reported worldwide. In contrast to the first reported family that showed lymphedema in late childhood, the patient described here displays lymphedema in her lower limbs at early infancy associated with growth delay, mild facial swelling, congenital hypothyroidism and some minor developmental abnormalities. This report confirms the causality of *PTPN14* loss-of-function mutations and further expands the clinical phenotype of this rare genetic syndrome.

1. Introduction

An extremely rare autosomal recessive syndromic form of choanal atresia with childhood-onset lymphedema, high-arched palate and variable developmental abnormalities (OMIM#613611) in a consanguineous Yemenite kindred was first described in 1986 (Sheikh et al., 1986). Recently this condition was shown to be associated with a homozygous mutation in *PTPN14* in nine affected members of this family (Au et al., 2010). *PTPN14* encodes nonreceptor tyrosine phosphatase protein which has been suggested to be required for lymphangiogenesis (Au et al., 2010). The mutation defined was an intragenic homozygous 2016-bp deletion encompassing intron 6 to intron 7 of the *PTPN14* resulting in the loss of exon 7, frameshift (p.Ser194Argfs*19), and premature truncation (Au et al., 2010). All nine patients in the kindred displayed choanal atresia and by the age of 4–6 years, six out of the nine had developed hard, non-pitting, lower limbs lymphedema (Har-El et al., 1991; Au et al., 2010). Around half of the patients presented a high-arched palate as well as other abnormalities including small nipples, pericardial effusions and pectus excavatum (Qazi et al., 1982; Har-El et al., 1991). To our knowledge, this is the only family reported worldwide with this clinical phenotype. Patients with choanal atresia typically present with other congenital

Abbreviations: PTPN14, protein tyrosine phosphatase, non-receptor type 14; *VEGFR3*, vascular endothelial growth factor receptor 3; *FOXC2*, forkhead box C2; *CCBE1*, collagen and calcium binding EGF domains 1; *SOX18*, SRY-box 18; *KIF11*, kinesin family member 11; *IKBKG*, inhibitor of nuclear factor kappa B kinase subunit gamma; *PTPN11*, protein tyrosine phosphatase, non-receptor type 11; *GATA2*, GATA binding protein 2; *FAT4*, FAT atypical cadherin 4; NICU, neonatal intensive care unit; CT-scan, computed tomography scan; dbSNP, The Database of SNP; GME, The Greater Middle East; SNP, Single nucleotide polymorphic; IUGR, Intrauterine growth restriction

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anomalies, the most common being coloboma, cardiac disease, mental and physical development and growth delay, genital hypoplasia and craniofacial abnormalities (e.g. CHARGE syndrome) (Burrow et al., 2009). Besides *PTPN14*, mutations in several other genes, including *FOXC2, CCBE1, SOX18, KIF11, IKBKG, PTPN11*, *GATA2* and *FAT4* have been identified to cause syndromes with lymphoedema as an associate feature (Fang et al., 2000; Zonana et al., 2000; Tartaglia et al., 2001; Irrthum et al., 2003; Alders et al., 2009; Ostergaard et al., 2011; Ostergaard et al., 2012; Alders et al., 2014). The majority of their encoded proteins cluster within the signalling pathway of *VEGFR3* (Mendola et al., 2013).

While a mouse model of *PTPN14* deficiency did not display choanal atresia, around 14% of the mutant animals showed forelimb and/or hindlimb swelling of the limb extremities or periorbital edema which started after 5 months of age (Au et al., 2010). These mice also displayed hyperplasia of lymphatic capillaries of the ears (Au et al., 2010). Mutations in *PTPN14* have also been shown to be involved in tumorigenesis and have been associated with colorectal cancer, basal cell carcinoma and neuroblastoma (Laczmanska and Sasiadek, 2011; Schramm et al., 2015; Wang et al., 2015; Bonilla et al., 2016). Furthermore, it is proposed that *PTPN14* may regulate angiogenesis and/or arteriovenous specification implicated as abnormal in pathology of hereditary haemorrhagic telangiectasia (Benzinou et al., 2012; Letteboer et al., 2015). Although suggestions have been made regarding the biological role of *PTPN14* in the regulation of the lymphatic system in mouse, the physiologic relevance of the protein to choanal development as one of the two main features of this syndrome, as well as high-arched palate in humans, is still poorly understood.

2. Material and methods

2.1. Patients phenotype analysis

This study, including clinical data collection and genomic analysis, was approved by the local institutional ethics committee of the Iran University of Medical Sciences, and all participants provided their written informed consent. The medical records of the patient were reviewed and the patient was examined by the clinical team of the Shahid Akbar-Abadi Hospital. Detailed patient family history was collected and pedigree was drawn accordingly (Fig. 1). Blood samples from the proband and her parents were collected for genetic testing.

2.2. Genetic study genotype analysis

Genomic DNA was extracted from each participant's blood sample using the Qiagen Gentra Puregene DNA isolation kit (Valencia, CA). Mutation analysis was performed by PCR amplifying all exons and exon-intron boundaries of the *PTPN14*, using twenty-two pairs of primers. Primer sequences and screening conditions are available upon request. All the resulting amplicons underwent bidirectional dideoxy sequencing using an Applied Biosystems 3730XL DNA Analyzer, and sequencing data was analysed using Geospiza's FinchTV Software.

3. Results

3.1. Clinical report

Herein, we report on a two year old Iranian girl born at full-term with a history of intrauterine growth restriction (IUGR), weighing 1850 g, and measuring 39.5 cm in length with a head circumference of 32 cm under 10th centile. No history of medication (e.g. carbimozale), infectious diseases and drug/alcohol abuse was reported by mother during pregnancy. She was born by normal vaginal delivery to healthy first-cousin parents. She was admitted to the neonatal intensive care unit (NICU) of Shahid Akbar-Abadi Hospital (Tehran). She suffered from severe respiratory distress at birth and was immediately

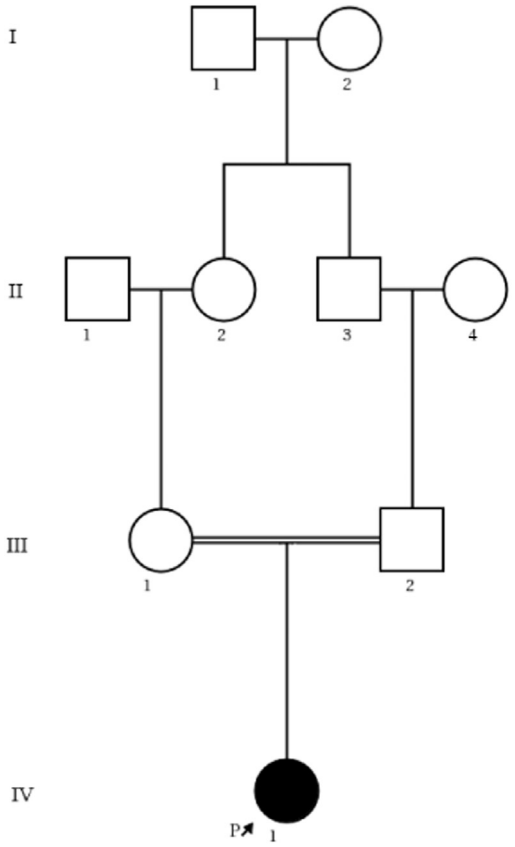

Fig. 1. Pedigree of the proband IV:1 born to a first cousin parents III:1 and III:2.

intubated, placed on mechanical ventilation and surfactant was administered. She was diagnosed with bilateral choanal atresia soon after birth and had seven corrective surgeries in the first five months after birth. She was dysmorphic with a “lymphedematous facial appearance” with hypertelorism, broad forehead, smooth philtrum, unilateral low set ear, high-arched palate and small nipples (Fig. 2).

Subtle infraorbital swelling was observed below the right eye soon after birth. She displayed transient congenital hypothyroidism during the first year of her life, but at the age of one year this had resolved. On ultra-sound scan (at 18 months) the thyroid gland was in its normal location and its size appeared to be normal for her age. Systolic murmur (grade 3/6) was noted at the lower left sternal border and following cardiac assessment, a diagnosis of a small muscular ventricular septal defect with a good ejection fraction was made. At the age of two months she developed swelling of both feet up to her ankles.

On examination at 22 months, the edema was firm, fibrotic and with pitting. This was unchanged at the latest follow-up (Fig. 2). The diagnosis of lymphedema was confirmed by lymphoscintigraphy. The radiotracer was injected in dorsal aspect of the feet. Neither the lymphatic vessels, nor the lymph nodes were seen, which means these vessels are not developed in the proband (aplasia or complete obstruction) (Fig. 3).

The main lymphatic tracts of the lower limbs cannot be clearly visualised and there is no inguinal lymph node uptake at 60 min, representing a functional aplasia of the lower limb lymphatic system. There was no evidence of lymphedema in the other parts of her body. The parents did not have any history or signs of lymphedema.

At follow-up at 24 months the growth parameters were as follow: weight: 8.5 kg, height: 77 cm (below the 3rd centile) and the head circumference was 46 cm (10th centile). The parent's height was also below the 3rd centile (The father's height: 155 cm; his weight: 60 kg and mother's height: 145 cm; her weight: 52 kg).

She had mild psychomotor developmental delay, which may be the