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BRIEF COMMUNICATION

Co-occurrence of mutations in *FOXP1* and *PTCH1* in a girl with extreme megalencephaly, callosal dysgenesis and profound intellectual disability

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Abstract

Heterozygous disruptions in *FOXP1* are responsible for developmental delay, intellectual disability and speech deficit. Heterozygous germline *PTCH1* disease-causing variants cause Gorlin syndrome. We describe a girl with extreme megalencephaly, developmental delay and severe intellectual disability. Dysmorphic features included prominent forehead, frontal hair upsweep, flat, wide nasal bridge, low-set, abnormally modelled ears and post-axial cutaneous appendages on the hands. Brain MRI showed partial agenesis of the corpus callosum and widely separated leaves of the septum pellucidum. Exome sequencing of a gene set representing a total of 4813 genes with known relationships to human diseases revealed an already known heterozygous de novo nonsense disease-causing variant in *FOXP1* (c.1573C>T, p.Arg525Ter) and a heterozygous novel de novo frameshift nonsense variant in *PTCH1* (c.2834delGinsAGATGTTGTGGACCC, p.Arg945GlnfsTer22). The composite phenotype of the patient seems to be the result of two monogenic diseases, although more severe than described in conditions due to disease-causing variants in either gene.

Introduction

FOXP1 (forkhead box protein P1; Online Mendelian Inheritance in Man (OMIM) 605515) is one of the four members of the FOXP subfamily (FOXP1–4) and acts as a transcriptional repressor [1]. The *FOXP1* gene is located on chromosome 3p13. Monogenic *FOXP1* pathogenic variants and more extensive 3p chromosomal deletions encompassing *FOXP1* have been reported previously [2–4]. Eventually, a *FOXP1* mutation-related phenotype (OMIM 613670) as a recognizable entity has been outlined with intellectual disability, specific language impairment with or without autistic spectrum disorder and dysmorphic features [3–5].

The *PTCH1* (patched homologue 1, OMIM 601309) gene on chromosome 9q22.3 encodes a transmembrane glycoprotein [6] which functions as a hedgehog (HH) receptor and regulates HH signalling at the primary cilium [6, 7]. Heterozygous germline *PTCH1* pathogenic variants cause Gorlin syndrome (OMIM 109400), an autosomal dominant disorder that predisposes affected individuals to developmental defects and tumourigenesis [6–8].

Megalencephaly is defined as an oversized brain with head circumference exceeding the age-related mean by two or more standard deviations [9]. A tendency to megalencephaly has been reported in both *FOXP1*- and *PTCH1*-related conditions [3–5, 7–9]. We describe here a girl with co-occurrence of heterozygous disease-causing variants in both *FOXP1* and *PTCH1* genes in association with extreme megalencephaly, unreported in mutations of either gene, dysmorphic features, abnormal corpus callosum, and profound intellectual disability.

Case report

The proband, a girl, was born from the second pregnancy at 36 weeks of gestation via vaginal delivery with a birth weight of 2870 g (+0.4 SD), length of 50 cm (+0.8 SD) and head circumference of 37 cm (+1.8 SD). Dysmorphic features,

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such as prominent forehead, frontal hair upsweep, arched eyebrows, strabismus, broad nasal bridge, short nose with broad tip, long philtrum, pointed chin, low-set, abnormally modelled ears and symmetrical post-axial cutaneous appendages (skin tags) on both hands were observed (Fig. 1a, b, d). Progressive growth of her head circumference was noted (Fig. 1c); it was 58 cm (+6.1 SD) at the age of 4 years, and 61 cm (+7.1 SD) at the age of 8 years. Her height was 129 cm (+0.2 SD) and weight 33 kg (+1.3 SD) at 8 years of age. Her motor and cognitive development was severely delayed. Brunet-Lézine test at the age of 2.5 years showed a developmental quotient of 40. On examination, generalized hypotonia and preserved deep tendon reflexes were observed.

Brain magnetic resonance imaging (MRI) revealed partial agenesis of the corpus callosum (Fig. 1e) and widely separated leaves of the septum pellucidum (Fig. 1f). Brain computed tomography was not performed. Abdominal and cardiac ultrasound, chest X-ray and dental radiography were normal.

Methods

Routine chromosomal analysis was performed by standard techniques. Genomic DNA was extracted from peripheral blood samples with the Puregene kit (Gentra) after obtaining informed consent. Copy number changes were investigated by Affymetrix 750K array. The Illumina Trusight One Exome Sequencing Panel (Illumina Inc., San Diego, CA, USA), covering the coding region of 4813 clinically relevant genes, was applied using Illumina MiSeq (Illumina Inc., San Diego, CA, USA). Variants were filtered based on severity and frequency against public variant databases including single-nucleotide polymorphism database (dbSNP), ClinVar, Exome Aggregation Consortium (ExAC), Exome Variant Server (EVS) and in-house clinical exome database of 140 unrelated Hungarian persons.

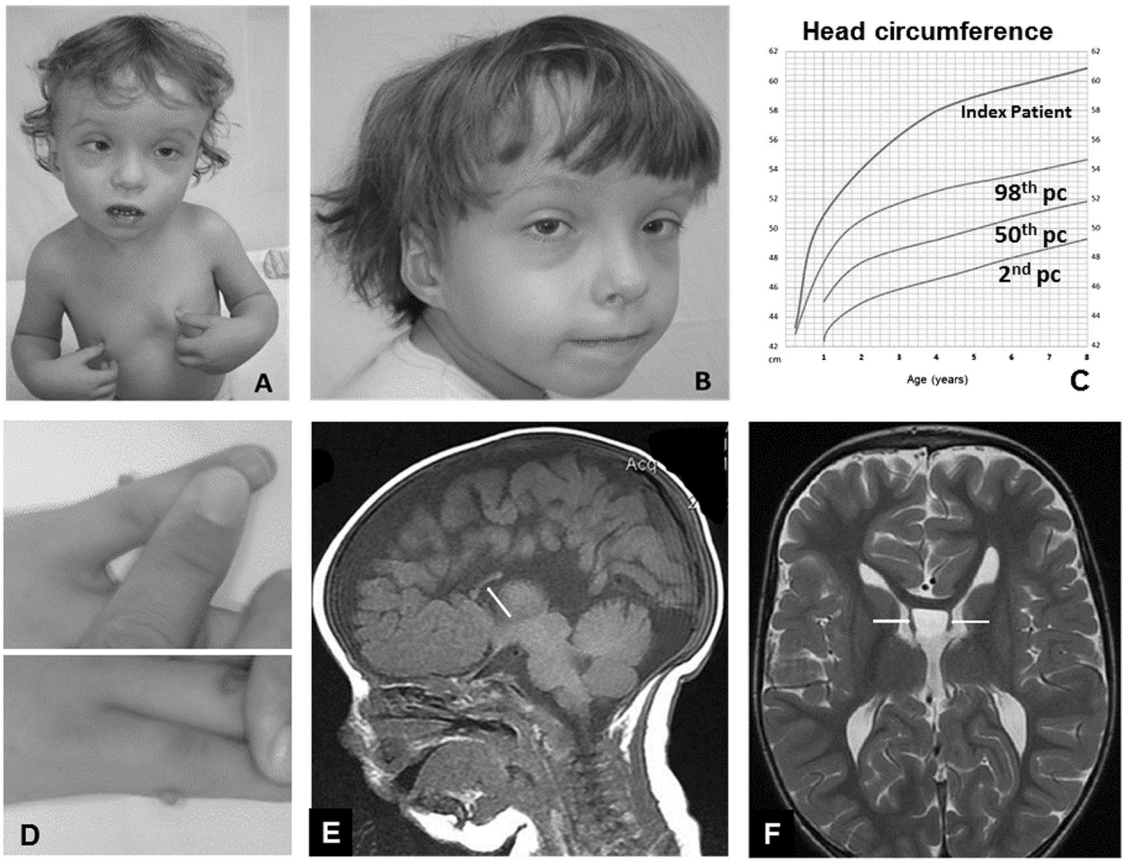

Fig. 1 Photographs, head circumference chart and brain MR images. **a**, **b** The patient at the age of 2 (**a**) and 6 (**b**) years. Note prominent forehead, arched eyebrows, strabismus, flat, wide nasal bridge, short nose with a broad tip, long philtrum, small lower jaw and low-set ears with abnormally modelled helices. **c** The chart shows the progressive growth of the head circumference. **d** Bilateral skin tags on the fifth fingers. **e** Sagittal T1-weighted MR image at the age of 3 months shows the partial agenesis of the corpus callosum with a thin remnant of the anterior part of the body (arrow). **f** Axial T2-weighted MR image at the age of 4 years demonstrates the thin anterior part of the corpus callosum, dilated ventricles and widely separated leaves (arrows) of the septum pellucidum

Results

Chromosomal analysis showed a normal 46,XX karyotype. Copy number changes were not found by Affymetrix 750K array. Clinical exome (Trusight One panel) sequencing revealed heterozygous variants in exon 18 of the *FOXP1* (NM_032682.5:c.1573C>T, NP_116071.2:p.Arg525Ter) and exon 17 of the *PTCH1* (NM_000264.4:c.2834delGinsAGATGTTGTGGACCC, NP_000255.2:p.Arg945GlnfsTer22) genes in the patient (Figs. 2 and 3). The *FOXP1* variant has been reported earlier as a nonsense pathogen mutation (rs112795301, RCV000005214), while the *PTCH1* variant (RCV000655932) has not been found in either dbSNP, ClinVar, ExAC or EVS databases or a cohort of 140 unrelated Hungarian controls. Sanger sequencing confirmed both variants as de novo mutations in the patient. They were not present either in the parents or in the patient's healthy brother (Figs. 2 and 3).

Discussion

We describe heterozygous disruptions in two genes, namely *FOXP1* and *PTCH1*. Megalencephaly has been described in both *FOXP1*- and *PTCH1*-related conditions; however, such an extreme rate of head growth, as seen in our patient, has never been reported in association with disease-causing variants in either *FOXP1* [3–5] or *PTCH1* [8, 10–13] genes. Although structural brain abnormalities have been found occasionally in *FOXP1*-related disorders, dysmorphic corpus callosum has been reported only in a single case [3, 4]. Brain malformations were also rarely reported in *PTCH1* mutation [10–12], notwithstanding corpus callosum agenesis, dysgenesis and hypoplasia occurred in a few patients [11, 13, 14]. It seems that the intellectual disability and language deficit were less severe in children with mutations only in one of these genes [3–5, 8, 10–13]. We suggest that the effects of multiple hits, disruptive disease-causing variants in two different genes simultaneously added and resulted in the serious composite clinical phenotype in our patient (Table 1).

Functional characterization of *FOXP1* variants [4, 15] provided evidence that the de novo heterozygous nonsense mutation, c.1573C>T, p.Arg525Ter, found in our patient, was pathogenic [4, 15]. FOXP proteins regulate gene expression by forming homo- and hetero-dimers with each other and they interact with other transcription factors forming a network involved in cortical development [16]. Studies on embryonic neural stem cells and mice with *Foxp1* down-regulation or deletion in the developing forebrain confirmed that an extensive signalling network regulated by Foxp1 is involved in important developmental processes such as neurogenesis, neuronal migration and differentiation [17–19].

Several hundred pathogenic variants in the *PTCH1* gene have been described [6, 7]. The novel de novo heterozygous variant c.2834delGinsAGATGTTGTGGACCC, p.Arg945GlnfsTer22, in our patient causes truncation of one of the extracellular loops of the PTCH1 leading to the loss of a significant part of the C-terminal protein, including five transmembrane domains [6]. The PTCH1 protein is a member of the HH receptor complex in the primary cilia. It represses Smoothened (SMO), which is a member of the G-protein-coupled receptor superfamily. The truncated PTCH1 protein probably fails to inhibit SMO activation resulting in dysregulation of the HH signalling pathway. We

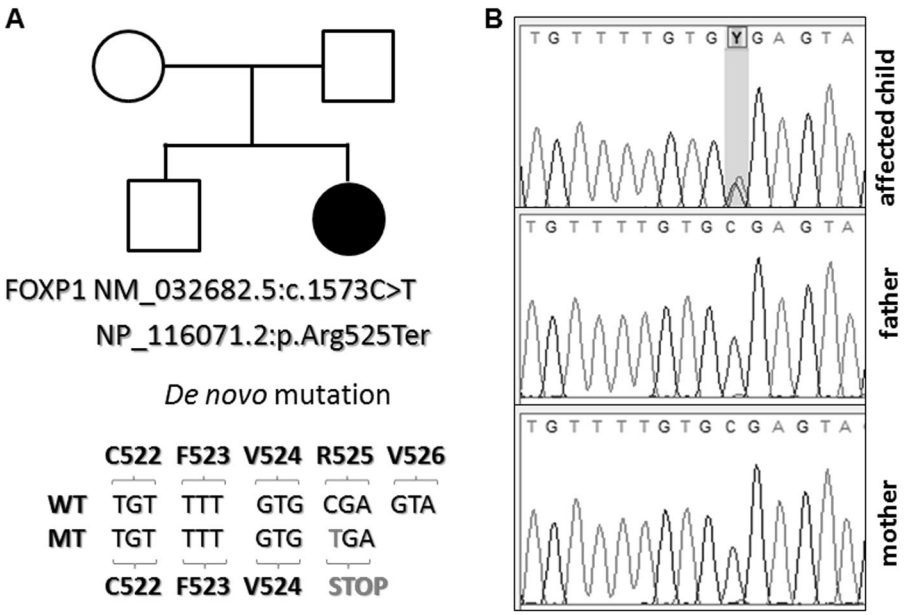

Fig. 2 *FOXP1* mutation. Mutation in *FOXP1* gene (NM_032682.5) at c.1573 position results in a STOP codon. The mutation is very likely de novo since it was absent in the parents (**a**). The mutated position is highlighted in the DNA sequence of the affected child. Standard IUPAC (International Union of Pure and Applied Chemistry) nucleotide ambiguity DNA code (Y = T/C) used (**b**)

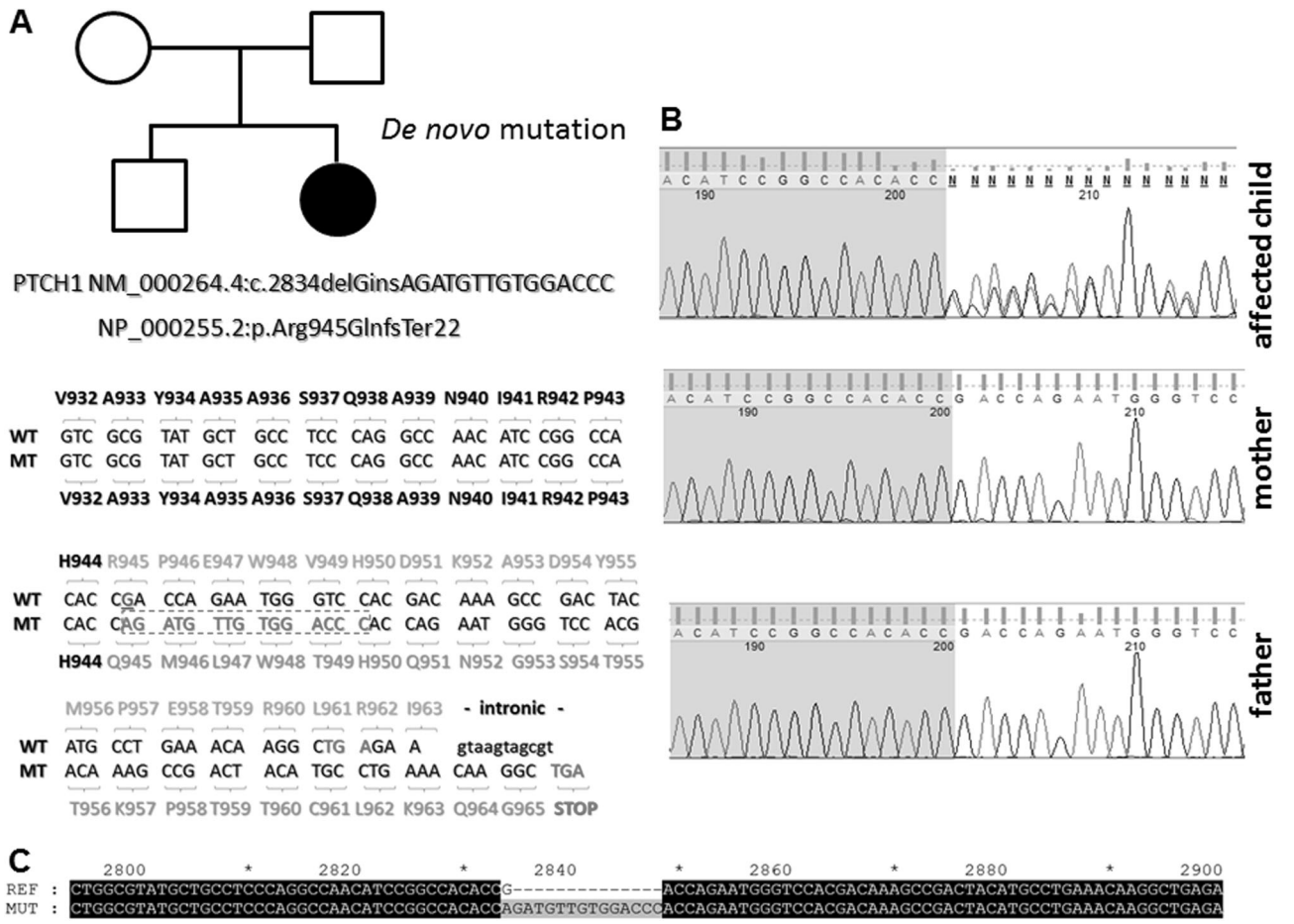

Fig. 3 *PTCH1* mutation. **a** The complex *PTCH1* variant caused by a single-nucleotide deletion and a 15 bp long insertion in exon 17 which causes a premature STOP codon (NP_000255.2:p.Arg945GlnfsTer22). The mutated portion of the sequence is highlighted in red. The mutation is very likely de novo since it was absent in the parents. **b** The presence of *PTCH1* (NM_000264.4) mutation at DNA level confirmed by Sanger sequencing in the affected child. It was absent in the parents. The complex mutation starts after the highlighted portion of the sequence (blue). **c** The sequence alignment of the reference and mutated *PTCH1* alleles

Table 1 Common versus distinct clinical features attributable to mutations in FOXP1 and PTCH1

	FOXP1 (refs. [2–5])	*PTCH1* (refs. [7, 8, 10–14])
Head size	Extreme megalencephaly (macrocephaly)	
Dysmorphic features	Prominent forehead Strabismus Broad nasal bridge	
	Frontal hair upsweep Down slanting palpebral fissures Short nose with broad tip Long philtrum Abnormally modelled ears	Highly arched eyebrows Low-set ears Symmetrical post-axial cutaneous appendages (skin tags) on both hands
Brain imaging		Partial agenesis of the corpus callosum Widely separated leaves of the septum pellucidum
	Enlarged ventricles	
Neurological signs	Global developmental delay Profound intellectual disability Generalized hypotonia	

can hypothesize that the extreme large volume of the brain tissue in our patient can partly be related to persistent HH pathway activation [20].

Further studies on possible functional interactions between FOXP1 and PTCH1 may clarify the molecular basis of this phenotype.

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Compliance with ethical standards

Ethical approval Written informed parental consent has been obtained. The study was approved by the Human Investigation Review Board at Albert Szent-Györgyi Clinical Centre, University of Szeged, Hungary.

Conflict of interest The authors declare that they have no conflict of interest.

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