# **Novel Insights from Clinical Practice**



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# New ANTXR1 Gene Mutation for GAPO Syndrome: A Case Report

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#### **Established Facts**

• GAPO syndrome manifestations were recently associated with putative loss-of-function mutations in the *ANTXR1* gene.

### **Novel Insights**

We describe a non-reported missense mutation in the ANTXR1 gene in 2 siblings with clinical manifestations of GAPO syndrome.

#### **Key Words**

 $\label{eq:analytical_continuous} A lopecia \cdot \textit{ANTXR1} \ mutation \cdot Growth \ retardation \cdot \\ Optic \ atrophy \cdot Pseudoanodontia$ 

rying a new missense mutation. This mutation produces the substitution of a glutamine in position 137 for a leucine (c.410A>T, p.Q137L). © 2016 S. Karger AG, Basel

## **Abstract**

GAPO syndrome is a very rare genetic disorder characterized by growth retardation, alopecia, pseudoanodontia and progressive optic atrophy (GAPO). To date, only 30 cases have been described worldwide. Recently, gene alterations in the *ANTXR1* gene have been reported to be causative of this disorder, and an autosomal recessive pattern has been observed. This gene encodes a matrix-interacting protein that works as an adhesion molecule. In this report, we describe 2 homozygous siblings diagnosed with GAPO syndrome car-

GAPO syndrome (OMIM 230740) is a very rare genetic disorder that has been described in ~30 patients worldwide since its first report in 1947 [Anderson and Pindborg, 1947]. The characteristic phenotype of this syndrome, which at the same time forms its name is: growth retardation, alopecia, pseudoanodontia, and progressive optic atrophy, among other variable features [Gagliardi et al., 1984; Tipton and Gorlin, 1984; Wajntal et al., 1990]. Patients affected with GAPO syndrome have

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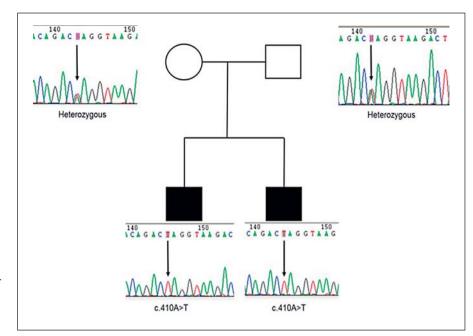
**Fig. 1.** Phenotypic features of GAPO syndrome. **a** Two siblings with dysmorphic facial features, diagnosed at 13 (left) and 14 (right) years of age. **b** Alopecia (13 years old). **c** Pseudoanodontia (14 years old).

a reduced life expectancy and usually die in their third or fourth decade of life due to generalized interstitial fibrosis and atherosclerosis [Anderson and Pindborg, 1947; Wajntal et al., 1990]. Although there has been evidence of an autosomal recessive pattern of inheritance, it was not until 2013, when by exome sequencing and fibroblast culture Stránecký et al. [2013] reported mutations in the anthrax toxin receptor 1 gene (*ANTXR1*) as causative of GAPO syndrome. In this report, we describe a novel mutation in the *ANTXR1* gene in 2 patients with clinical features of GAPO syndrome.

The 2 male siblings diagnosed with GAPO syndrome were 13 and 14 years of age. The parents denied consanguinity. The boys resembled typical cases of GAPO syndrome reported in the literature (fig. 1). Both patients presented with alopecia (fig. 1b), a saddle nose, thickened eyelids and thick lips, in addition to dwarfism, hypotrichosis, strabismus, shallow orbits, protruding auricles, prominent supraorbital ridges, high and bossed forehead, and a small face with dysplasia. Oral examination showed thickened upper and lower alveolar ridges in a buccolingual direction and lined with normal mucosa; pseudoanodontia was also present (fig. 1c). After obtaining signed informed consent, blood samples were taken from all family members (both children and par-

ents) and genomic DNA was isolated using the Easy-DNA<sup>TM</sup> kit from Invitrogen (Carlsbad, Calif., USA). PCR and Sanger sequencing of the coding region of ANTXR1 (18 exons) was performed. The results of bioinformatic analysis showed a novel homozygous mutation (c.410A>T, p.Q137L) in the ANTXR1 gene, located in exon 5. The base pair substitution was present in both patients, while both parents were heterozygous (fig. 2). This mutation is not described in dbSNP or in the Exome Aggregation Consortium database (http://exac.broadinstitute.org) and has not previously been reported to our knowledge. In silico function analyses were performed using online programs such as SIFT (sift.bii.astar.edu.sg, Bioinformatics Institute of Singapore) [Ng and Henikoff, 2001], MutationTaster (www.mutationtaster.org, NCB) [Schwarz et al., 2014], and PolyPhen-2 (genetics.bwh.harvard.edu, Harvard) [Adzhubei et al., 2010]. The SIFT program predicted an 'affect protein function' with a score of 0.02 and a median value of 3.04, while MutationTaster showed a 'disease-causing model' with p = 0.995 and an alteration in the splicing site affecting the translation process. Conversely, PolyPhen-2 analysis resulted in a 'benign mutation' with a score of 0.004 (sensitivity: 0.97; specificity: 0.59). Once proven that the mutation found had high probabilities of affecting the protein encoded, we searched in SNP databases, such as Exome Aggregation Consortium and dbSNP, to see if the mutation found had been previously reported. There was no information about the c.410A>T variant in either database. In summary, we report a new mutation of ANTXR1 that potentially affects protein function resulting in the GAPO syndrome phenotype.

The first evidence of genetic alterations related to the GAPO syndrome phenotype was reported by Stránecký et al. [2013]. They discovered 2 nonsense mutations and 1 substitution that create a new splicing site, resulting in a truncated isoform of the ANTXR1 protein, in 4 unrelated families (1 Czech family, 1 Sri Lankan family, and 2 Egyptian families). They also analyzed the loss of function of these variants using fibroblasts isolated from the patients. Later, Bayram et al. [2014] analyzed 5 GAPO patients from 3 unrelated Turkish families by exome sequencing, describing the presence of an indel mutation that produces a truncated protein, a missense mutation predicted to be a deleterious version of the protein, and a synonymous mutation that seems to modify a splicing site in ANTXR1. In our study, we identified a new missense mutation (c.410A>T, Q137L) in the ANTXR1 gene in a Mexican family. This genetic alteration was analyzed by 3 computational programs



**Fig. 2.** Family pedigree and segregation of the *ANTXR1* mutation. The affected siblings are homozygous for the c.410A>T variant.

online resulting that 2 of them predicted a non-functional protein, while 1 predicted a 'benign mutation' with no effect on the functionality of the encoded protein.

All mutations found in GAPO syndrome patients are located in the ANTXR1 gene, which encodes a type I transmembrane protein with a molecular weight of 85 kDa. The amino acid sequence of this receptor contains a von Willebrand factor type A domain, a domain that is important for protein-protein interaction; an Anthrax receptor extracellular domain (Anth\_Ig), and an Anthrax receptor C-terminus region (Ant\_C) [Bradley et al., 2001]. In addition to this one, 2 other isoforms have been described, variant 2, which is shorter than variant 1 in the cytoplasmic domain, and variant 3 (the last discovered), which is predicted to be secreted as it does not contain a transmembrane or cytoplasmic domain [Liu and Leppla, 2003]. This protein has been involved in cell attachment and migration; additionally, it allows the interaction of cells and several components of the extracellular matrix by binding extracellular ligands with the actin of cytoskeleton. Moreover, it has been shown that the ANTXR1 protein is a key player in cell spreading [Nanda et al., 2004; Hotchkiss et al., 2005]. The functions depicted above explain the dysmorphic phenotype observed once the protein is not present or has lost its function.

Although previous evidence has shown that mutations in *ANTXR1* are responsible for the GAPO phenotype,

further studies of function for the Q137L variant are needed to demonstrate that it produces the GAPO syndrome phenotype.

#### Statement of Ethics

The study was approved by the institutional review board of the Universidad de Monterrey, and complied with all the principles of the Helsinki Declaration.

#### **Disclosure Statement**

The authors have no financial or other conflicts of interest to declare.

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