Short Report

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Identification of a New Mutation (L46P) in the Human *NOG* Gene in an Italian Patient with Symphalangism Syndrome

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Key Words
BMP7 · In silico · L46 · Mutation · *NOG* · Noggin · Proximal symphalangism

Abstract
Proximal symphalangism (SYM1) is a joint morphogenesis disorder characterized by stapes ankylosis, proximal interphalangeal joint fusion, skeletal anomalies and conductive hearing loss. Noggin is a bone morphogenetic protein (BMP) antagonist essential for normal bone and joint development in humans and mice. Autosomal dominant mutations have been described in the *NOG* gene, encoding the noggin protein. We analyzed an Italian sporadic patient with SYM1 due to a novel *NOG* mutation (L46P) based on a c.137T>C transition. A different pathogenic mutation in the same codon (L46D) has been previously described in an in vivo chicken model. An in silico model shows a decreased binding affinity between noggin and BMP7 for both L46D and L46P compared to the wild type. Therefore, this codon should play an important role in BMP7 binding activity of the noggin protein and consequently to the joint morphogenesis.

Proximal symphalangism (SYM1; OMIM 185800) is a joint morphogenesis disorder characterized by stapes ankylosis, proximal interphalangeal (PIP) joint fusion, skeletal anomalies and conductive hearing loss [Vesell, 1960]. SYM1 presents an autosomal dominant heritability with a minimal genetic heterogeneity. Most of the mutations have been described in the *NOG* gene and rarely in the *GDF5* gene [Wang et al., 2006]. Noggin, encoded by *NOG*, is a bone morphogenetic protein (BMP) antagonist essential for normal bone and joint development in humans and mice [Zimmerman et al., 1996; Brunet et al., 1998].

Here, we report on a 55-year-old male from Southern Italy who was referred to us for diagnosis of skeletal dysplasias, due to a novel heterozygous *NOG* missense mutation.

Case Report

Family history was non-contributory; particular focus was kept on family members showing signs of skeletal dysplasias. At 55 years of age, the proband was severely obese and was diagnosed as suffering from binge eating disorder. The eating disorder was not observed in other family members. His body weight had begun to increase since he was 4 years old. At the time of the visit,

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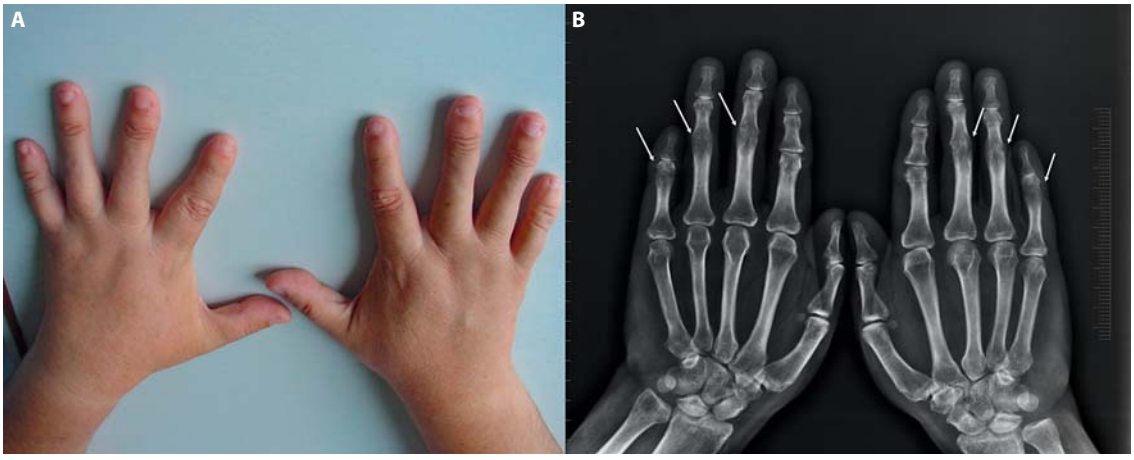

Fig. 1. Hand view (**A**) and hand radiograph (**B**) of the proband with PIP joint synostoses of the 3rd–5th fingers bilaterally (arrows) and 5th finger brachymesophalangy.

his height was 167 cm (10th–25th percentile) and his weight was 147 kg (>97th percentile). Hypergonadotropic hypogonadism, reduced volume of testes (at the bottom of the normal range), non-insulin-dependent diabetes mellitus and arterial hypertension were components of his clinical history. Intelligence quotient and audiogram tests were not performed. Mental retardation and hearing loss were not reported. Karyotype was 46,XY.

The proband showed an unusual facial appearance with bifid nasal tip and asymmetric ears, the right one being low set and 'cup' shaped. Limited extension at the right elbow, absence of skin creases over the PIP joints of the 3rd and 4th fingers, contiguous skin creases over proximal and distal PIP joints of the 5th finger, hypoplastic fingernails and cutaneous syndactyly of the 2nd and 3rd toes were demonstrated. Physical measurements showed a relative macrocephaly and brachydactyly of the hands (fig. 1A). A skeletal survey by posteroanterior and lateral skull, whole anteroposterior and lateral spine, anteroposterior pelvis as well as anteroposterior upper and lower limb, and hand and foot X-rays have been evaluated. Hand radiography showed a 5th finger brachymesophalangy and PIP joint synostoses of the 3rd–5th fingers (fig. 1B). The patient's feet are characterized by big trapezia and short halluces; PIP joint synostoses of the 2nd–5th toes were difficult to assess due to the hammer toe deformation. Skull radiography indicated a neurocranium smaller than viscerocranium. Moreover, an aplastic styloid process of the ulnae has been shown. Due to the clinical signs, proximal symphalangism was hypothesized, and molecular analysis of *NOG* and *GDF5* genes were performed. Written consent for the proband and his parents was obtained.

Results and Discussion

A mutation analysis of *NOG* and *GDF5* genes was carried out. Variants were confirmed by repeated sequencing of both DNA strands on a second PCR product and tested in 100 healthy Italian control persons. Sequence analysis of the *NOG* gene detected a de novo missense variant c.137T>C (NM_005450.4, NCBI) (fig. 2A), not described as an already known mutation nor polymorphism in any online database. The nucleotide substitution changes the conserved amino acid leucine to proline at the codon 46, L46P (NP_005441.1, NCBI). This position is located at the interface between noggin and BMP7 receptor structures (fig. 2B). To evaluate the new variant, the NCBI-SNP database and the conservation of the amino acids across mammalian species were consulted. Furthermore, to test the potential impact of the amino acid substitution of this variant on the structure and function of the noggin precursor protein, the web tools PolyPhen2 [Adzhubei et al., 2010] and MutationTaster [Schwarz et al., 2010] were used. Moreover, the binding affinity of the noggin protein to the BMP7 receptor was measured in silico for different noggin variants by means of standard modeling [Sali and Blundell, 1993] and the docking tool AutoDock Vina [Trott and Olson, 2010]. The crystal structure of the noggin-BMP7 complex was used as a reference (PDB accession code: 1M4U).

Moreover, a different amino acid change at the same position, L46D, has also been described by Groppe et al. [2002]. To probe the molecular interface between BMP7 and noggin, point mutations were generated and an in vitro binding affinity study was performed by the same group. Groppe et al. [2002] demonstrated that the BMP7 binding activity of noggin-L46D is abolished in comparison to the wild type protein. A subsequent in vivo ex-

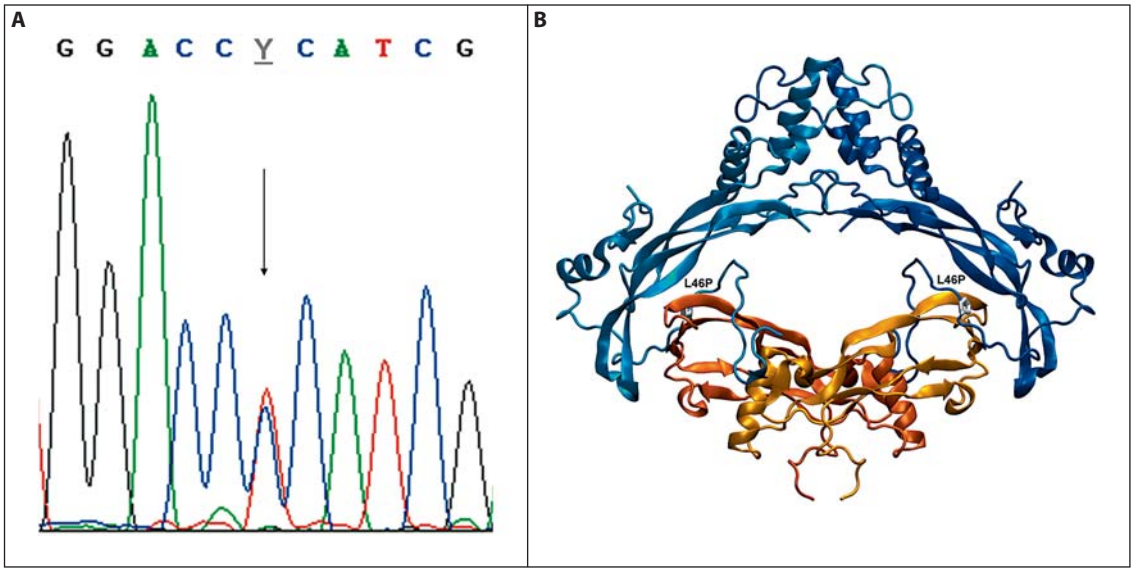

Fig. 2. A Sequencing of *NOG* showed heterozygosity for the mutation c.137T>C (arrow). **B** Cartoon diagram of noggin-BMP7 complex structure viewed with the 2-fold symmetry axis vertical. Noggin and BMP7 subunits are colored blue and orange/red, respectively. The L46P mutation, represented as thick white lines, is located at the interface between the 2 molecules.

periment has been carried out in the chicken limb bud, without any discernible effect on chondrogenic aggregation in chicken [Groppe et al., 2002]. Up to now, patients with changes in the same position (L46) have not been reported.

The prediction analysis for the novel variant L46P suggests it as deleterious (Condel score 0.879) [González-Pérez and López-Bigas, 2011]. Performing an in silico mutation analysis for the L46P and L46D amino acid substitutions in the noggin structure, both variants decrease the binding affinity to the BMP7 receptor by 0.8 kcal·mol^{-1}. Both variants at L46 position presumably interfere with the binding between noggin and BMP7 since it is consistent with the measured effect in vitro. A control test was also done in silico for the silent D39A variant which is known to keep the noggin-BMP activity unperturbed [Groppe et al., 2002]. Indeed, the measured binding affinity of this variant is slightly improved in comparison to the wild type by 0.2 kcal·mol^{-1}.

Features of our patient do not resemble Teunissen-Cremers or other *NOG*-related syndromes, except for isolated proximal symphalangism, since there is no hearing loss due to stapes ankylosis or broad thumbs and first toes or hyperopia. Up to now, there are no specific genotype-phenotype correlations in proximal symphalangism caused by *NOG* mutations [Usami et al., 2012]. Moreover, to our knowledge, the association between eating disorders and *NOG* mutations has never been reported. In this case, it was not possible to establish a hypothesis of genotype-phenotype correlation since there are no eating disorders or *NOG* mutations in other family members.

In conclusion, here we describe a new allele demonstrating its pathogenicity in 3 ways: (a) according to the predicting web tools; (b) showing the in silico reduced affinity between noggin-L46P and BMP7, and (c) reporting a patient with the L46P mutation and a proximal symphalangism due to the alteration of the chondrogenic pathway.

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