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Letter to the Editor

A new missense mutation in FGF23 gene in a male with hyperostosis–hyperphosphatemia syndrome (HHS)

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ABSTRACT

Hyperostosis–hyperphosphataemia syndrome (HHS) is a rare autosomal recessive metabolic disorder, characterized by recurrent painful swelling of long bones, periosteal new bone formation and cortical hyperostosis or intramedullary sclerosis, hyperphosphatemia and low intact fibroblast growth factor 23 (FGF23) protein levels. It is caused by mutations in 2 genes, N-acetylgalactosaminyltransferase 3 (GalNAc-transferase; GALNT3) and FGF23. We have performed mutation analysis of the GALNT3 and FGF23 genes in a patient with HHS and detected a homozygous mutation in exon 3 of FGF23 gene (NM_020638.2: c.471C>A) which results in amino acid change from phenylalanine 157 to leucin (p.F157L) in receptor interaction site.

Hyperostosis–hyperphosphataemia syndrome (HHS) is a rare autosomal recessive metabolic disorder, characterized by recurrent painful swelling of long bones, periosteal new bone formation and cortical hyperostosis or intramedullary sclerosis (Mikati et al., 1981). Hyperphosphatemic familial tumoral calcinosis (HFTC) is another rare recessive disorder with biochemical abnormalities similar to HHS, but different musculoskeletal phenotypes (Ichikawa et al., 2005). Both HFTC and HSS are associated with hyperphosphatemia resulting from increased renal tubular reabsorbtion of phosphate, inappropriately normal or only slightly elevated levels of serum 1,25-dihydroxy vitamin D, normal calcium and alkaline phosphatase, low/low normal parathyroid hormone levels, and low intact fibroblast growth factor 23 (FGF23) protein levels (Joseph et al., 2010). The two diseases have been found to result from mutations in the same genes, N-acetylgalactosaminyltransferase 3 (GalNAc-transferase; GALNT3) and FGF23 (Frishberg et al., 2005; Garringer et al., 2008; Ichikawa et al., 2010). GALNT3 has been shown to mediate o-glycosylation of FGF23, a potent phosphaturic protein, to protect FGF23 from proteolysis (Ichikawa et al., 2005). Mutations in Klotho gene encoding an FGF23 co-factor involved in FGF receptor activation have been shown to cause HFTC (Ichikawa et al., 2010). However, Klotho mutations have not been associated with HHS yet.

In the present report, we performed mutation analysis of the GALNT3 and FGF23 genes in a patient with HHS, initially diagnosed as

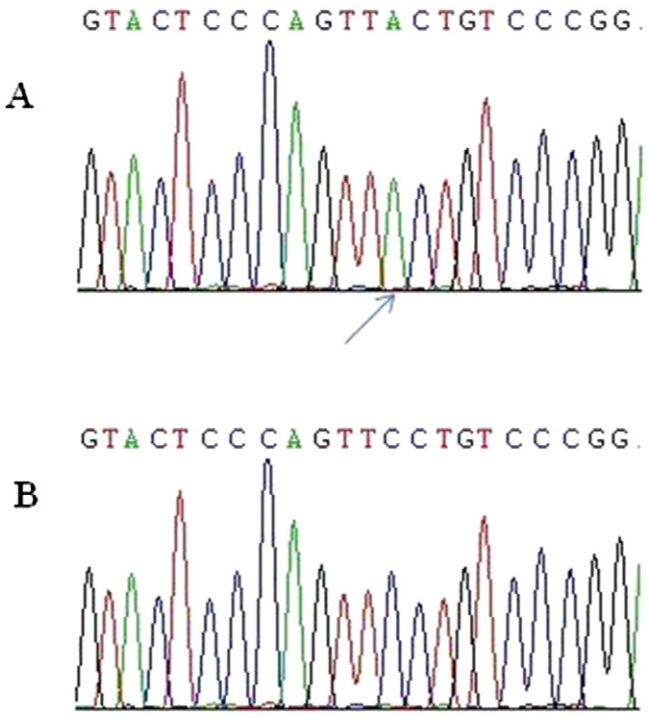

Fig. 1. A. Mutation detected in patient. B. Normal sequence.

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osteopetrosis. A 4 year old male from the southern west of the Islamic Republic of Iran, the first child to healthy consanguineous parents was referred for evaluation of genovalgus and management of hyperphosphatemia (8.8 mg/dl; normal: 2.7–4.5 mg/dl). Additional serum biochemistries revealed normal calcium (9.3 mg/dl, normal: 8.5–10.4 mg/dl), PTH (32 pg/ml, normal: 9–64 pg/ml), and 25(OH) vitamin D (45 pg/ml, normal: 15–60 pg/ml) concentrations. No additional skeletal disease was detected in physical examination. In the next 2 years, he suffered from reduced visual acuity, facial nerve palsy and stenosis of aortic valve due to the calcium deposition. Radiological examinations indicated diaphyseal hyperostosis and large vessel calcification.

Written informed consent was obtained from his parents prior to participation in the study according to the protocol approved by local institutional review board. Blood sample was collected from the patient. DNA was isolated using the standard salting out method. All exons and

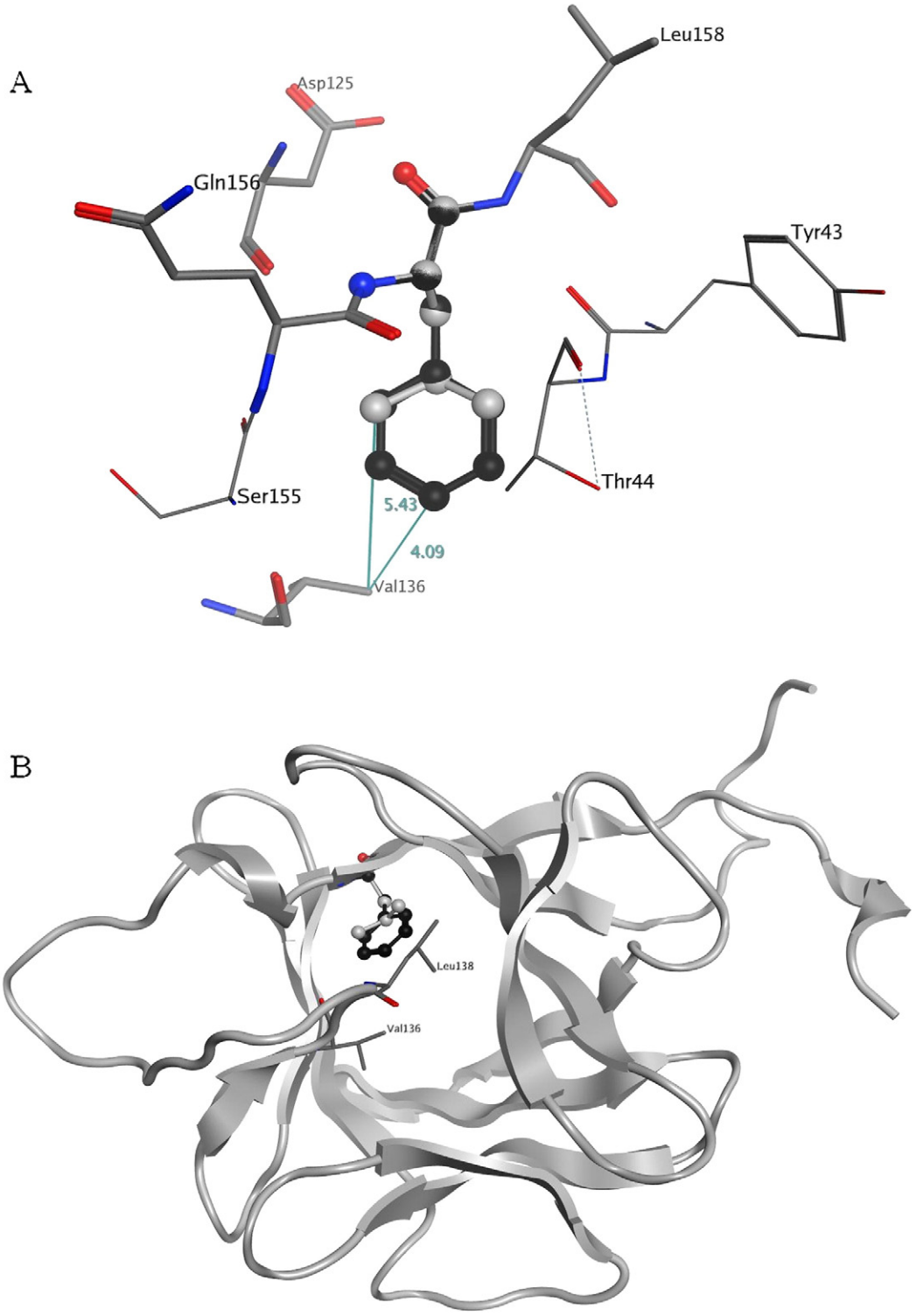

Fig. 2. A. Residues surrounding F157 (in black ball and sticks) and L157 (in light gray ball and sticks). B. The whole structure of FGF23 is represented with ribbons; F157 and L157 are in black and gray ball and sticks respectively.

exon–intron boundaries of GALNT3 and FGF23 genes were amplified according to Ichikawa et al. and Garringer et al. respectively (Garringer et al., 2007; Ichikawa et al., 2005). PCR products were sequenced using the ABI Prism3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). No nucleotide change was observed in GALNT3 exons and exon–intron boundaries. However, a homozygous mutation was detected in exon 3 of FGF23 gene (NM_020638.2: c.471C>A) (Fig. 1). The nucleotide change results in amino acid change from phenylalanine 157 to leucin (p.F157L) in receptor interaction site. In order to analyze the effects of observed missense variant PolyPhen-2 was used. This software shows that the mutation leads to substitution of a highly conserved amino acid and is predicted to be probably damaging with a score of 1.000. With this software, values nearer 1 are more confidently predicted to be deleterious. Using the 2P39.pdb file, which is a crystal structure of FGF23, the mutation was also produced in silico with MOE.2012.10 (Molecular Operating Environment (MOE), 2012.10; Chemical Computing Group Inc.). Interactions of F157 with its neighbor residues occur via its backbone carbonyl with D125, and its side chain could interact with aliphatic residues such as V136 (Fig. 2A). When L157 is present, the backbone interaction may not differ, but due to a smaller volume of the side chain, interactions with aliphatic residues would be impaired: the shortest distance between a carbon atom of L157 with V136 is 5.43 Å, compared with 4.09 Å for F157 (Fig. 2A). When the whole structure of FGF23 is considered, it may be suggested that the F157L mutation (which occurs at the beginning of the β-12 strand) may affect the positioning of the β-10 strand where V136 and L138 are present (Fig. 2B). The segment containing β-10 to β-12 strand is one of the heparin (ligand) binding sites of FGF23 (Goetz et al., 2007).

Previous researches have proposed that FTHC and HHS are clinical variants of the same disease (Joseph et al., 2010). This study, in accordance with previous studies shows that FGF23 mutations can cause HHS.

Conflict of interest

No conflict of interests exists.

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