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Short Communication

# Frequency of the allelic variant c.1150T > C in exon 10 of the fibroblast growth factor receptor 3 (*FGFR3*) gene is not increased in patients with pathogenic mutations and related chondrodysplasia phenotypes

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## Abstract

Mutations in the *FGFR3* gene cause the phenotypic spectrum of FGFR3 chondrodysplasias ranging from lethal forms to the milder phenotype seen in hypochondroplasia (Hch). The p.N540K mutation in the *FGFR3* gene occurs in ~70% of individuals with Hch, and nearly 30% of individuals with the Hch phenotype have no mutations in the *FGFR3*, which suggests genetic heterogeneity. The identification of a severe case of Hch associated with the typical mutation c.1620C > A and the occurrence of a c.1150T > C change that resulted in a p.F384L in exon 10, together with the suspicion that this second change could be a modulator of the phenotype, prompted us to investigate this hypothesis in a cohort of patients. An analysis of 48 patients with FGFR3 chondrodysplasia phenotypes and 330 healthy (control) individuals revealed no significant difference in the frequency of the C allele at the c.1150 position (p = 0.34). One patient carrying the combination `pathogenic mutation plus the allelic variant c.1150T > C' had a typical achondroplasia (Ach) phenotype. In addition, three other patients with atypical phenotypes showed no association with the allelic variant. Together, these results do not support the hypothesis of a modulatory role for the c.1150T > C change in the *FGFR3* gene.

*Keywords*: FGFR3, F384L, hypochondroplasia, skeletal dysplasia.

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The FGFR3 protein is encoded by a gene of the same name that has been extensively studied because of its role in the regulation of linear bone growth. Mutations in this gene cause the phenotypic spectrum of FGFR3 chondrodysplasias that classically include hypochondroplasia (Hch), achondroplasia (Ach), thanatophoric dysplasia types I and II (TDI and TDII) and SADDAN (Severe Achondroplasia with Developmental Delay and Acanthosis Nigricans) (Warman *et al.*, 2011).

The mutations c.1620C > A and c.1620C > G in exon 13 of the *FGFR3* gene both lead to the p.N540K substitution, a mutation found in 50-70% of individuals with Hch (Bellus *et al.*, 2000). Other mutations in the *FGFR3* gene associated with Hch account for ~2% of the cases (Castro-Feijóo *et al.*, 2008). For ~30% of individuals with the Hch phenotype no mutations have been found in the *FGFR3* gene, suggesting that this condition is clinically and genetically heterogeneous (Bonaventure *et al.*, 1996). The diagnosis of Hch is usually suspected or established at school age when short stature plus clinical and radiological changes become evident (Spranger *et al.*, 2012). However, in cases where the phenotype is more severe, the diagnosis can be made already in the neonatal period (Karadimas *et al.*, 2006).

The identification of a severe case of Hch diagnosed in the neonatal period associated with a combination of the typical mutation (c.1620C > A) in exon 13 and the c.1150T > C change (p.F384L) in exon 10, together with the suspicion that the change in exon 10 could modulate the phenotype (Trujillo-Tiebas *et al.*, 2009), prompted us to investigate this hypothesis in a cohort of patients with FGFR3 chondrodysplasia phenotypes.

We studied 48 patients with FGFR3 chondrodysplasia phenotypes. All subjects provided formal informed consent prior to enrollment, and this study was approved by an institutional Committee for Ethics in Research (protocol no. 992/2007). Blood samples were collected and genomic DNA was extracted using a standard phenol-chloroform method. The molecular diagnosis was done by direct sequencing after PCR amplification of the exons related to the respective phenotypes. The amplified products were purified and sequenced using an ABI3500XL® sequencer

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in conjunction with BigDye v.3.1 sequencing kits (Applied Biosystems, Foster City, CA, USA). The data were processed using ABI software and analyzed using CodonCode Aligner 4.1.1. Exon 10 was sequenced in all cases. In addition, exons 7, 13 and 15 related to the TDI, Hch and TDII phenotypes, respectively, were sequenced in patients with the respective phenotypes. Patients with Ach had no other exon sequenced since the hot spot for this phenotype is located in exon 10. Table 1 shows the eight pathogenic mutations identified in the 48 patients studied.

Two of the 48 patients presented the c.1150T > C change in addition to the pathogenic mutation. One of these two patients had Hch because of the c.1620C > A mutation and was diagnosed in the first months of life because of the severity of his phenotype (birth length of 44 cm plus corporal disproportion with rhizomelic shortening of limbs at birth). As his diagnosis was initially doubtful (mild Ach or severe Hch), exons 10 and 13 were analyzed. The second patient had a typical Ach phenotype (c.1138G > A). In both cases, the c.1150T > C change was inherited, but without apparent phenotypic consequences in the respective parents. Paternal inheritance was confirmed in the patient with Hch and maternal inheritance in the patient with Ach. Molecular cloning was used to determine whether this change was *cis* or *trans* in relation to the pathogenic mutation. In the Ach patient, the c.1150T > C change was in *trans* with the c.1138G > A mutation. A haplotype analysis was not feasible in the patient with Hch because of the distance (1,447 bp) between the two changes in the *FGFR3* gene.

With regard to phenotype, we identified four atypical patients: one with Ach (c.1138G > A) associated with corporal asymmetry, mental retardation and acanthosis nigricans, two with severe Hch (one with c.1620C > A and the other with c.1620C > G, and clinical and radiological manifestations in the neonatal period) and one with TD1 (c.742C > T) and long-term survival. Except for the patient with Hch already described, no other patient among these three atypical individuals had the allelic variant c.1150T > C.

For comparison, we also sequenced exon 10 of the *FGFR3* in 330 healthy control subjects. These control samples consisted of DNA extracted from the cord blood of healthy newborns. The c.1150T > C change was found in four individuals. As Hch is usually not apparent in the newborn period, for all these four controls with the c.1150T > C change the Hch hot spots (exons 3, 5, 7, 9, 12, 13 and 15) were also examined and no mutation was found. Table 2 shows the frequencies of the C allele in the control group and patients, as well as the *p* value (Fischer test) for the comparison between the two groups.

Trujillo-Tiebas *et al.* (2004) reported the presence of a c.1150T > C change in two families. In one family, the change was found in a patient with severe but unspecified skeletal dysplasia. Although unaffected, the patients father and grandmother displayed subtle symptoms. No other information about the patients specific mutation was provided. In the other family, the change was found in a patient with Hch, along with the most common mutation (c.1620C > A), and in his mother who was unaffected by the skeletal dysplasia but presented some skeletal signs. An analysis of 194 chromosomes from individuals of the general population detected this change in one chromosome (0.5%). These authors suggested an additive pathological effect of this change in the FGFR3 receptor and reported another affected individual found in a series of prenatal cases. The father carried the same change (c.1150T > C in *FGFR3*) as the abortus, but with no apparent clinical sign (Trujillo-Tiebas *et al.*, 2009).

In the present study, the presence of a c.1150T > C change in two patients in a relatively small sample appears to be a stochastic event. In addition, there was no significant difference in the frequency of the C allele between the controls and patients. The frequency of the C allele in the control group (0.6%) was the same as that found in a British population (0.6% or one C allele in 178 chromosomes) according to the 1000 Genomes Project (Abecasis *et al.*, 2012). Interestingly, the frequency of the C allele was 3% (three C alleles in 100 chromosomes) among individuals of

**Table 1** - Mutations related to their respective phenotypes in 48 patients with the FGFR3 phenotype.

| Clinical diagnosis | Number of patients | Mutation | |
|---|---|---|---|
| | | Nucleotide change | Amino acid change |
| Ach | 27 | c.1138G > A* | G380R |
| | 1 | c.1138G > C | |
| Hch | 5 | c.1620C > A* | N540K |
| | 2 | c.1620C > G | |
| TDI | 7 | c. 742C > T | R248C |
| | 2 | c. 746C > G | S249C |
| | 2 | c.1118A > G | Y373C |
| TDII | 2 | c.1948A > G | K650E |

*One patient also carried the c.1150T > C change. Ach – achondroplasia, Hch – hypochondroplasia, TDI and TDII – thanatophoric dysplasia types I and II, respectively.

**Table 2** - Frequency of the c.1150T > C change in both - control individuals and patients with pathogenic mutations in the *FGFR3*.

| | c.1150T > C change (p.F384L) | | Frequency of the mutated allele (%) | p |
|---|---|---|---|---|
| | Positive | Negative | | |
| Controls | 4 | 326 | 0.6 | 0.34 |
| Patients | 2 | 46 | 2.2 | |

Turkish descent but was uncommon among those of German descent (no C alleles in 100 chromosomes) (Golla *et al.*, 1997).

Although the c.1150T > C mutation leads to a phenylalanine for leucine substitution at position 384 in the amino acid sequence, this change apparently does not affect receptor function. Both of these amino acids are nonpolar and their substitution does not change the molecular characteristics of the transmembrane domain nor the hydrophobicity of this structure (Adzhubei *et al.*, 2010). Moreover, leucine is present in the bovine *FGFR3* homolog, which indicates a normal FGFR3 protein function (Golla *et al.*, 1997).

In conclusion, the data here presented do not support the hypothesis of a modulatory role for the c.1150T > C change in the *FGFR3* gene. The more severe phenotype of the patient with Hch reported here was apparently related to the known phenotypic heterogeneity of this condition rather than to any modulatory effect of the c.1150T > C change. We suggest that this phenotypic heterogeneity occurs by chance or via another, as yet unknown, modulatory mechanism.

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