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Short Communication

The c.1138G>A Variant of Fibroblast Growth Factor Receptor 3 is a Common Cause of Achondroplasia in Pakistan

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Authors' Contribution
NM and SN presented the concept of the study. NM and SY planned the methodology, curated and analysed the data. NM, SN and SY wrote the manuscript. ZF, NA and MF helped in investigation, methodology and writing. SN supervised the study.

Key words
Hypochondroplasia, *FGFR3*, Rhizomelic dwarfism, Skeletal dysplasia

ABSTRACT

Achondroplasia is an autosomal dominant disorder of rhizomelic dwarfism. It is predominantly caused by *de novo* mutations in *FGFR3*. This study was aimed to determine the common variants of *FGFR3* in one inherited, and eighteen sporadic cases of achondroplasia from Pakistan. Sanger sequencing analysis of *FGFR3* exon 9 revealed that more than 90% cases had the c.1138G>A p.(Gly380Arg) variant. Our results suggest that c.1138G>A variant is the most common cause of achondroplasia in Pakistan, a finding which is similar to that reported for achondroplasia patients from other countries.

Achondroplasia is the most common autosomal dominant skeletal dysplasia (Achondroplasia, OMIM# 100800). Its prevalence rate is 1: 25,000 to 1: 30,000 of live births worldwide (Pauli, 2019). Phenotypically, achondroplasia is characterized by disproportionate dwarfism, shortened rib cage, macrocephaly, brachydactyly, and short femoral necks. Achondroplasia is predominantly caused by *de novo* variants of *FGFR3,* though dominant inheritance is also observed (Nahar *et al*., 2009).

FGFR3 is comprised of 19 exons and encodes fibroblast growth factor receptor, a receptor tyrosine kinase. The variants c.1138G>A and c.1138G>C p.(Gly380Arg) in exon 9 of *FGFR3* are a major cause of achondroplasia worldwide including India, Japan and Turkey (Katsumata *et al*., 2000; Nahar *et al*., 2009; Pehlivan *et al*., 2003; Rousseau *et al*., 1994). On the basis of incidence of achondroplasia, the c.1138 nucleotide in *FGFR3* is among the most highly mutable single nucleotides in the human genome (Shiang *et al*., 1994).

Fibroblast growth factor (FGF) binds to the extracellular ligand binding domain of receptor to initiate FGF/FGFR signaling. This activates expression of cell cycle suppression genes to negatively regulate bone development (Pauli, 2019). However, mutated FGFR3 is constitutively activated which causes over-expression of FGF signaling.

Material and methods

In this study a family ACH8 with four affected members (Fig. 1a), and a group of eighteen non-familial cases (ACH1, ACH3-7, ACH9-13, ACH19-23, HCP5, ACH-NA10) with a diagnosis of achondroplasia were studied. Patients were recruited from Sindh, Khyber Pakhtunkhwa and Punjab provinces of Pakistan. Ethical approval to conduct this study was obtained from Institutional Review Board, School of Biological Sciences, University of the Punjab, Lahore. Written informed consent was obtained from all participants and blood samples were collected. Ages of the participants varied from 5 to 40 years.

All affected individuals had rhizomelic dwarfism, normal sized trunks, prominent large foreheads and short extremities. They had heights less than the third percentile for their age. Their fingers were short and pointed away from each other. Few of the patients had large sized heads with prominent foreheads.

Genomic DNA was extracted from leukocytes by a standard protocol including sucrose lysis and salting out.

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Two sets of primers were designed using Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/) based on genomic sequence of *FGFR3* (NM_000142.4) to amplify exons 9 c.1138G>A or c.1138G>C variants. PCR products were sequenced using Big Dye Terminator v.3.1 (ABI Thermo Fisher).

Results

Eighteen sporadic patients and all four affected members of the recruited family had typical manifestations of achondroplasia. Seventeen of the eighteen non-familial cases and all four affected members of the recruited family exhibited the c.1138G>A;p.(Gly380Arg) or the c.1138G>C; p.(Gly380Arg) *rs28931614* variant in *FGFR3* (Fig. 1b). Four affected members of the recruited family had the common c.1138G>A variant. The same variant c.1138G>A was found in fifteen of seventeen sporadic cases of achondroplasia. However, two patients had the c.1138G>C transversion of *FGFR3*. Thus 97% of achondroplasia patients had a transition of c.1138G>A while 2% achondroplasia cases were heterozygous for the transversion variant c.1138 G >C. These two *FGFR3* variants were not detected for one affected individual ACH-NA10.

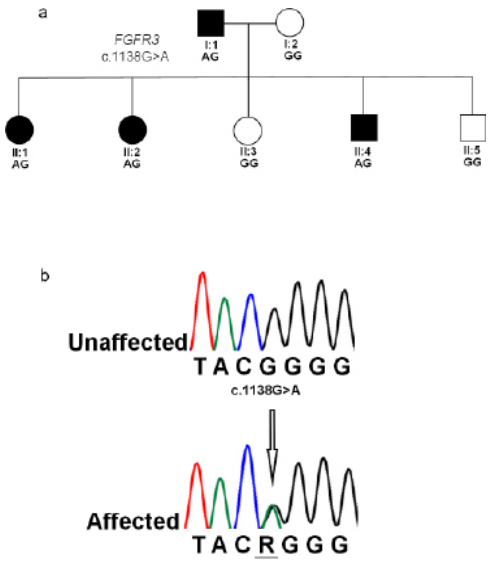

Fig. 1. (a) Pedigree of the family ACH8 in which members exhibited dominantly inherited achondroplasia. (b) Chromatogram for FGFR3 selected region showing the most common transition c.G1138>A variant. The arrow indicates the point of mutation.

Discussion

Health issues of achondroplasia patients include cardiac diseases, neurological problems, spinal problems, leg abnormalities, obesity, respiratory issues and hearing impairments (Fredwall *et al*., 2020). The finding of the variants detected in sporadic cases of patients with achondroplasia is consistent with occurrence of *de novo* mutations in *FGFR3*. About 80 % cases of achondroplasia occur as *de novo* mutations (Nahar *et al*., 2009). One of the main reasons hypothesized for prevalence of achondroplasia is increased paternal age. Offspring of fathers of older age have a higher rate of this disorder (Pauli, 2019). Most of the affected participants collected in this study had fathers in the age range of 35-40 at the time of conception. In the single familial case of achondroplasia presented here, the phenotype was inherited dominantly as expected.

Rare genetic skeletal dysplasia is not registered or documented in a developing country like Pakistan. Public facility for appropriate genetic testing is also not available (Ahmad *et al*., 2019). Previously, a familial case of achondroplasia with three affected individuals (one parent and two offspring) was reported in Pakistan with p.(Gly380Arg) variant in FGFR3 (Ajmal *et al*., 2017). The present study is the first report from Pakistan in which a large number of subjects with achondroplasia were studied. It can be concluded that achondroplasia in Pakistan is most commonly caused by the substitution of p.(Gly380Arg) in FGFR3. Our results agree with the world-wide distribution of p.(Gly380Arg) mutation among achondroplasia cases. However, we did not identify the two variants resulting in the p.(Gly380Arg) substitution for one patient with achondroplasia. Future studies are warranted by sequencing of the entire *FGFR3* gene in order to characterize the genetic underpinnings of his disorder.

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Statement of conflicts of interest

The authors have declared no conflict of interest.

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