NOTE

A Missense Mutation of C1659 in the Fibroblast Growth Factor Receptor 3 Gene in Russian Patients with Hypochondroplasia

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Abstract. To carry out the genetic screening for the common mutation in the first tyrosine kinase domain (TK1) of the fibroblast growth factor receptor 3 gene (*FGFR3*) in a Russian population, a cohort of 16 patients with hypochondroplasia diagnosed previously were studied, among them twelve familial cases and four sporadic cases. The heterozygous N540K *FGFR3* mutation was detected in 9 cases (56.3%) due to that C1659A substitution in 6 patients and C1659G substitution in 3 patients, respectively. The ratios of familial and sporadic cases among patients which carried *FGFR3* mutation were similar. Seven (43.7%) patients, negative cases of N540K mutation, were all familial cases. Our results support evidence of similar frequency of common type N540K mutation of *FGFR3* in Russian hypochondroplasia and of the genetic heterogeneity of hypochondroplasia, suggesting the need for further search for responsible molecular abnormalities for phenotypically similar hypochondroplasia patients negative for TK1 domain mutation in *FGFR3*, reported in hypochondroplasia.

Key words: Hypochondroplasia, Fibroblast growth factor receptor 3 (*Fgfr3*), *Fgfr3* gene (*FGFR3*)
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HYPOCHONDROPLASIA is an autosomal dominant inherited skeletal dysplasia characterized by micromelia, disproportional short stature, relative macrocephaly and lumbar lordosis, but its phenotype is much more moderate than in achondroplasia [1, 2]. Radiological changes in the skeleton in hypochondroplasia are similar to those

in achondroplasia, but are mild or moderate, in comparison with severe changes in achondroplasia. They include some shortening of tubular bones, the absence of a normal increase or reduced interpedicular distance in the lumbar spinal column, increased length of the fibulae, metaphyseal flaring, small ilial wings, and broad and short metacarpals and phalanges. The phenotypes of hypochondroplasia patients vary widely from mild forms, when the disorder is often overlooked in clinical practice, especially in early childhood, to severe forms, similar to achondroplasia [1, 2]. Skeletal disproportion may develop only in puberty and it often may be

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apparent only on measurement [3]. Usually growth retardation occurs from infancy and gradually worsens through childhood. Since the pubertal growth spurt is either absent or poor, final height in hypochondroplastic children is 140–150 cm.

In contrast, the molecular basis of a number of skeletal dysplasias has been recently described after identification of the role of different fibroblast growth factor receptors (Fgfr 1, Fgfr 2, Fgfr 3) in the processes of skeletogenesis [4–6]. The group of dysplasias, including achondroplasia, hypochondroplasia and thanatophoric dysplasia type II are tightly linked to the *FGFR3* mutations [4, 5, 7–10].

Especially hypochondroplasia has recently been reported to be reflected by the abnormality of the proximal tyrosine kinase (TK1) domain of the *FGFR3*, mapped to chromosome 4p16.3. N540K mutation in the *FGFR3* has been identified as a molecular etiology in many cases of hypochondroplasia [11–13]. Another reported *FGFR3* mutation in hypochondroplasia patients is Ile538Val [14].

In Russia, molecular-genetic searches for *FGFR3* mutation in hypochondroplastic patients have not yet been conducted. We therefore performed genetic screening for abnormalities in the TK1 domain of the *FGFR3* in children and adults with

familial and sporadic forms of hypochondroplasia, living in the Russian Federation, to evaluate the prevalence of the common N540K mutation in Russian patients.

Materials and Methods

Clinical study and hormonal analysis

Sixteen patients (8 males and 8 females) with hypochondroplasia, diagnosed in childhood on the basis of clinical, radiological and genealogical data were included in the study (Table 1). Most of the patients had been followed up from 5 to 15 years at the Central Institute of Traumatology and Orthopedics in Moscow. The hormonal study was performed at the Endocrinology Research Center, Moscow. There were twelve familial cases and four sporadic cases. Altogether seven familial cases were studied, including 5 affected families (pairs “child-parent” are represented by cases No 1–2, 3–4, 9–10, 13–14, and 15–16) and two cases (No 5, 6) which had one parent with clinical features which corresponded to hypochondroplasia, but were not examined. By the time of the molecular investigation the chronological age of the patients was 23.49 (3.43) years (Mean (SE)) and ranged from

Table 1. Clinical and hormonal *FGFR3* mutational data for patients with hypochondroplasia

No	Sex (m/f)	C.A.(yr.)	Familial /Sporadic	Height SDS	Upper:Lower segment ratio	B.A. (yr.)	Peak GH-level in Insulin/Clonidin Test (ng/ml)	<i>FGFR3</i> mutations
1.*	m	11.7	Familial	−4.64	1.26	9.0	48.4	–
2.*	f	41.3	Familial	−6.12	1.53	Closed	20.3	–
3.**	f	3.7	Familial	−3.11	1.80	2.5	27.5	C1659G
4.**	f	28.7	Familial	−3.62	1.36	Closed	ND	C1659G
5.	m	29.3	Familial	−5.14	1.34	Closed	ND	C1659A
6.	f	23.6	Familial	−3.43	1.50	Closed	ND	–
7.	f	11.7	Sporadic	−1.96	1.43	11.0	47.5	C1659A
8.	m	20.6	Sporadic	−3.91	1.45	Closed	ND	C1659A
9.***	m	30.0	Familial	−4.06	1.28	Closed	38.9	–
10.***	f	49.9	Familial	−2.00	1.39	Closed	ND	–
11.	m	20.2	Sporadic	−3.91	1.53	Closed	25.1	C1659G
12.	f	16.9	Sporadic	−4.2	1.39	16	12.5	C1659A
13.#	m	32.9	Familial	−4.15	1.57	Closed	ND	C1659A
14.#	m	0.4	Familial	+0.30	2.29	ND	ND	C1659A
15.##	m	14.5	Familial	−5.57	1.32	12	33.9	–
16.##	f	39.9	Familial	−5.95	1.45	Closed	28.0	–

C.A., chronological age; B.A., bone age; ND: Not done. *, **, ***, # and ##: cases from the same family. –: no *FGFR3* mutation.

0.44 to 49.9 years. All except three, patients had severe growth retardation, with height SDS in the entire group of -3.85 . Two patients (Nos. 7 and 10) were of moderately short stature, with a height SDS of -1.96 and -2.0 , respectively. One 4-month-old boy presented with normal height with a height SDS of 0.30 . Ten patients reached final height with a height SDS of -4.22 . The upper/lower segment ratio was increased in all patients. GH-stimulation tests (insulin, clonidin), performed in 10 patients, showed a normal peak GH-level in all cases examined.

IGF-I and IGF-BP3 levels in circulation were normal in all patients except patient No 3 (357.9 ng/ml, normal range: 54–333 ng/ml and $3.7 \mu\text{g/ml}$, normal range: 1.54–3.39 $\mu\text{g/ml}$, respectively).

FGFR3 mutation analysis

Genomic DNA from patients and their parents was extracted from peripheral leukocytes by the standard phenol-chloroform extraction method.

The DNA fragment (224 bp), covering exon 13 of the FGFR3, was amplified with PCR (Polymerase Chain Reaction) (Perkin Elmer, USA). PCR products were then purified with a QIAEX II Agarose Gel Extraction kit (QIAGEN, USA) and SUPREC-02 columns (Takara, Japan). Further PCR with Dye Terminator was performed with a Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer, USA). Subsequent CENTRI-CEP Column Purification (Princeton Separation Inc, USA) was performed to remove excess dye terminator. Direct DNA sequencing of both strands of PCR products was performed and analyzed with ABI PRISM 377 DNA Sequencer (Perkin Elmer, USA).

Finally, restriction enzyme analysis of PCR amplified genomic DNA was performed with *AluI* to confirm the point mutation of the target sequence.

Results and Discussion

The common N540K mutation in the TK1 of the FGFR3, in the heterozygous state, which converted asparagine to lysine (Asn540Lys), was detected in 9 out of 16 Russian hypochondroplastic patients (56.3%); including 5 familial and 4 sporadic cases

Table 2. Summary of patients with hypochondroplasia and N540K mutation of the FGFR3 studied

Cases	Number of patients	N540K mutation	
		C1659A	C1659G
Familial	12	3	2
Sporadic	4	3	1
Total	16	6 (37.5%)	3 (18.8%)

(Table 2). A C1659A substitution was revealed in 6 cases (37.5%), including 3 familial and 3 sporadic cases. A C1659G substitution was revealed in 3 (18.8%) cases, including 1 sporadic and 2 familial cases (Fig. 1). We evaluated the prevalence of N540K mutations in the TK1 domain of the FGFR3 from the standpoint of familial and sporadic cases. Our data showed that N540K mutations were present in 3 out of 7 independent kindreds, or in 5 (41.7%) patients out of 12 with the familial form of hypochondroplasia, but all sporadic cases had N540K mutations (4 out of 4). Restriction enzyme digestion analysis of amplified DNA fragment was performed in patients with C1659G mutation. A C to G change created a new *AluI* restriction site with elimination of 83 bp and 141 bp from the PCR product. As demonstrated in Fig. 2, the PCR products of DNA samples from patients Nos. 3, 4 and 11 were heterozygous for the *AluI* site, whereas there was not restriction site for *AluI* in the control samples. We also performed mutational analysis in parents of sporadic cases, harbouring FGFR3 mutation. The direct sequencing of PCR products of phenotypically normal parents' DNA did not show any mutations in TK1 of the FGFR3.

The prevalence of N540K mutation in the Russian cohort of hypochondroplasia patients was comparable with that seen in some other populations [11–13], but higher than reported by Grigelioniene *et al.* [14].

Both C1659A and C1659G mutations were detected in our patients, although the first mutation was twice as frequent as the second one. The relative distribution in familial and sporadic cases among the patients who carried the FGFR3 mutation was not changed. Hypochondroplasia is now considered to be a clinically and genetically heterogeneous disorder [13, 14], but it is worth noting that the clinical heterogeneity was not so apparent in our cohort of hypochondroplastic patients. Indeed, patients harboring the N540K

Fig. 1. DNA sequence of the exon 13 of *FGFR3*, codon 540. a) wild type, b) C1659A heterozygous mutation (arrow) in a patient with hypochondroplasia and c) C1659G heterozygous mutation (arrow) in a patient with hypochondroplasia.

mutation did not differ significantly in clinical severity from those negative for this mutation. The well known *FGFR3* mutations are: achondroplasia: G375C, G380R; and thanotophoric dysplasia type II: R248C, K650E, although our cases were all far from these diseases phenotypically. Therefore we only concentrated on the search for N540K substitution in Russian hypochondroplasia.

Fig. 2. Screening of N540K mutation in the *FGFR3* by restriction digestion analysis. Lane 1, DNA marker; Lane 2, control DNA; Lanes 3,4 and 5, hypochondroplasia patients' DNA with C1659G mutation.

Interestingly, seven out of 16 patients (43.7%) in the group of patients with hypochondroplasia studied were negative for N540K mutation, and all represented familial hypochondroplasia. Recent studies on *Fgfr3* knockout mice suggest the essential role of *FGFR3* in the inhibition of enchondral ossification [15], but no other dramatic changes were observed except the disturbance of inner ear development. Distinct from achondroplasia, other transmembrane domain mutations in *FGFR3* have been reported in some patients with Crouzon syndrome, an autosomal dominant inheritance characterized by craniosynostosis and previously associated with mutations, such as the immunoglobulin-like domain III of *FGFR2* [16]. There is still room for further clarification of different types of mutations of *FGFR* groups. We must therefore search for associated *FGFR3* abnormalities in enchondral bone cell signal transduction. Alternatively, other regions of the *FGFR3* or another candidate gene may be involved in the pathogenesis of the skeletal dysplasia. Generally speaking, activation of intrinsic FGF-R kinase involves different intracellular substrates

[17], among which the *FGFR3* signaling pathway has been recently shown to utilize GRB2-containing complexes Shc-GRB2-Sos and 80K-H-p66-GRB2-Sos [18]. The specific pathway downstream of *FGFR3* responsible for causing hypochondroplasia remains to be clarified.

Finally, besides recent advances in molecular analysis of skeletal dysplasia, we must pay much more attention to careful clinical investigation of

phenotypes of hypochondroplasia to further clarify the disease-genetic causative relationship.

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