Clinical and genetic heterogeneity of hypochondroplasia

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Abstract

Hypochondroplasia (HCH) is an autosomal dominant condition characterised by short stature, micromelia, and lumbar lordosis. In a series of 29 HCH probands (13 sporadic cases, 16 familial cases), we tested their DNA for the N540K recurrent mutation previously described in the proximal tyrosine kinase domain of the FGFR3 gene on chromosome 4p16.3, and we detected this mutation in 21/29 HCH patients. Interestingly, three familial cases were clearly unlinked to chromosome 4p16.3. Reviewing the clinical and radiological manifestations of the disease a posteriori, we observed that the N540K mutation was associated with relative macrocrania with a high and large forehead and short hands. By contrast, in the three pedigrees inconsistent with linkage to chromosome 4p16.3, the clinical phenotype was milder, macrocephaly and shortening of the long bones was less obvious, the hands were normal, and no metaphyseal flaring was noted. This study supports the view that HCH is a clinically and genetically heterogeneous condition.

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Hypochondroplasia (HCH, MIM 146000) is an autosomal dominant condition characterised by short stature, micromelia, and lumbar lordosis.¹ Clinical symptoms, radiological features, and histopathological aspects of the growth plate cartilage are similar to but milder than those seen in achondroplasia (ACH).²⁻⁴ ACH and HCH have long been regarded as allelic disorders based on (1) inter- and intrafamilial clinical variability of HCH with some cases resembling ACH, and (2) the observation of severe dwarfism similar to homozygous ACH in the offspring of an ACH father and an HCH mother.⁵

In the last two years, the ACH gene has been mapped to chromosome 4p16.3^{6,7} and a common mutation (G380R) in the transmembrane domain of the fibroblast growth factor receptor 3 (FGFR3) gene has been identified in more than 98% of ACH patients.⁸⁻¹⁰ On the other hand, family studies supported allelism of ACH and HCH^{7,11} and a recurrent mutation in the proximal tyrosine kinase (TK1) domain of the FGFR3 gene has been identified in HCH (N540K).^{12,13} Yet other HCH cases were clearly unlinked to chromosome 4p16.3.^{14,15}

Studying a series of 29 patients, we report here on the clinical and genetic heterogeneity of HCH and show that the clinical severity of HCH ascribed to FGFR3 mutations is greater than that of HCH unlinked to chromosome 4p16.3.

Patients and methods

Twenty nine HCH probands (13 sporadic cases and 16 familial cases) with at least two affected subjects over at least two generations were included in the study. Minimal criteria for inclusion were a disproportional short limb dwarfism (height below -2 SD) and x ray evidence of shortening of the long bones with reduced interpedicular distances or an absence of normal increase in lumbar spinal column (front view) and short pedicles (lateral view).²⁻⁴

For linkage analyses in familial HCH, an EDTA blood sample (20 ml) was collected from each family member and DNA was prepared from lymphocyte pellets by SDS lysis, proteinase K digestion, phenol chloroform extraction, ethanol precipitation, and Tris-EDTA resuspension. For genotyping with the hypervariable microsatellite DNA markers of chromosome 4, genomic DNA (50 ng) was amplified using 1 U *Taq* polymerase in a buffer containing 50 pmol of each primer, 6.25 nmol of each deoxynucleotide, 50 mmol/l KCl, 10 mmol/l Tris HCl, pH 8, 1.5 mmol/l MgCl₂, and 0.1% gelatin in a final volume of 50 l. *Taq* polymerase was added after the first step of denaturation (10 minutes, 95°C) followed by 30 cycles of denaturation (94°C, 40 seconds), annealing (55°C, 30 seconds), and elongation (72°C, 40 seconds), with a final elongation step (72°C, 10 minutes). An aliquot of amplified DNA was mixed with the loading buffer. Samples were denaturated for 10 minutes at 94°C and loaded on a 6% polyacrylamide denaturing gel. After blotting, membranes were fixed in 0.4 mol/l sodium hydroxide and hybridised for two hours with (CA)₁₂ (³²P) labelled probes. Blots were washed once in 2 × SSC, 0.1% SDS for 10 minutes at room temperature and autoradiographed. DNA of CEPH subject 1347-02 was included in each experiment as control of allele size. Allele frequencies were based on the genotypes of parents of a subset of the CEPH panel. To calculate pairwise lod scores, we used the M-LINK program of the LINKAGE package (version 5.1). HCH was tested assuming that the disease is transmitted as an autosomal dominant trait (gene frequency $f = 1/30\,000$) with full penetrance. For mutation studies, a combination of single

Figure 1 Evidence of genetic heterogeneity in hypochondroplasia. Three HCH pedigrees excluding linkage to chromosome 4p16.3 are shown.

Table 1 Phenotype-genotype correlation in HCH

		Short interpedicular distance	Macrocrania	Short phalanges	Short femoral neck	Shortened long bones	Elongated fibulae
Familial HCH	16						
N540K mutation	8	+	++	++	++	++	++
Unlinked to 4p16.3	6	+	+	+/-	+/-	+/-	-
Uninformative to 4p16.3	2	+	+	++	++	++	++
Sporadic HCH	13						
N540K mutation	13	+	++	++	++	++	++
Total	29						

strand conformation polymorphism (SSCP) analysis and direct sequencing of amplification products was used (automatic DNA sequencer ABI 370 A).

Results

We first tested HCH for allelism to ACH at the FGFR3 locus, as ACH has been previously ascribed to mutations in the FGFR3 gene at locus D4S98 on chromosome 4p16.3. The order tel – (D4S227, PDEβ) – 0.01 – (D4S111, IDUA) – 0.02 – (D4S98, FGFR3) – 0.01 – D4S1614 – 0.001 – D4S412 – 0 – D4S43 – 0.020 – D4S432 – 0.01 – D4S2925 – cen has been established by analysis of CEPH reference data (genetic distances in parentheses). Among our 16 familial HCH cases, 11/16 were informative for linkage analysis and 5/16 were available for mutation analysis only. Surprisingly, when the 11 informative HCH families were tested, non-significant results were obtained for linkage of the disease gene to several polymorphic loci of chromosome 4p ($Z_{\max} = 0.8$ for locus

D4S111 at $q = 0.1$). Haplotype analyses provided direct evidence of exclusion of chromosome 4p16.3-pter in three HCH pedigrees (fig 1) while the other eight families were consistent with linkage to the ACH locus ($Z_{\max} = 2.5$ at locus D4S227 at $q = 0$).

Our strategy, therefore, was to look for the N540K FGFR3 mutation¹² in our HCH families which showed no recombination with chromosome 4p16.3 markers (eight families), in uninformative HCH families (five families), and in sporadic cases (13 cases). Two abnormal patterns of migration indicative of sequence variations in the TK1 domain were observed in 8/16 familial forms and 13/13 sporadic cases of HCH (fig 2). Sequence analyses showed heterozygosity for the C→A or the C→G transitions at nt1620, changing asparagine 540 into lysine (N540K) in all sporadic cases and in 8/16 familial cases, but not in the three families unlinked to chromosome 4p (fig 3, table 1).

Considering the genetic heterogeneity of HCH, the clinical and radiological profiles of the patients were reviewed a posteriori. Based

on the results of our genetic analysis, HCH patients were split into two groups. In the group of HCH patients harbouring the N540K mutation (group 1, 23 patients), we observed relative macrocrania (+1 SD) with a high and large forehead and short hands (fig 4). Radiographs disclosed broad and short long bones with metaphyseal flaring and overmodelling and an increased length of the fibulae. Femoral heads were slightly enlarged with short femoral necks. Metacarpals and phalanges were short and stubby (fig 5). In two uninformative families, no FGFR-3 mutation was identified but affected subjects had very

short stature (-3, -4 SD), relative macrocephaly, and x ray findings similar to those observed in patients carrying the N540K mutation. Finally, in the three pedigrees inconsistent with linkage to chromosome 4p16.3 (group 2, six patients), relative macrocephaly was also present (fig 4), but shortening of the long bones and iliac wings was milder, the hands were normal, and no metaphyseal flaring, overmodelling, or elongated fibulae were noted (fig 5).

Discussion

Hypochondroplasia is a difficult condition to diagnose, especially in the newborn period. Indeed, the short stature is usually only recognised after 2 years and relative macrocephaly is present in only 57% of cases.²⁻⁴ Narrowing of interpedicular distances with short pedicles and shortened tubular bones are minimal inclusion criteria.^{3,4}

The present study supports the view that HCH is a clinically and genetically heterogeneous condition. Indeed, while the majority of HCH cases were allelic to ACH and ascribed to the previously described N540K mutation in the FGFR3 gene,^{12,13} several pedigrees clearly excluded linkage of the disease gene to chromosome 4p in our series, as in other reports.^{14,15} Haplotype analyses in the three unlinked families provided evidence of exclusion of chromosome 4p in pedigree A. Although variable expressivity is a possibility in pedigrees B and C, clinical examination of the two recombinant subjects failed to disclose any clinical features of hypochondroplasia, such as short stature (subjects B II.1, 168 cm, and C II.2, 165 cm), micromelia, or macrocephaly.

Interestingly, when patients were divided into two groups based on the detection of FGFR3 mutations (group 1) or exclusion of the 4p16.3 region in our series (group 2), it appeared that the N540K mutation was associated with a severe phenotype resembling ACH

Figure 2 Single strand conformation polymorphism analysis of the tyrosine kinase domain of FGFR3 gene in three HCH patients (lanes 3, 5, and 6) and controls (lanes 1, 2, and 4). Arrows indicate abnormal bands in the probands.

Figure 3 Sequence analysis of the tyrosine kinase domain of the FGFR3 gene in HCH.

Figure 4 Variable clinical phenotypes in HCH. (A) Sporadic case harbouring the N540K FGFR3 mutation at the age of 12 years; (B) familial case unlinked to chromosome 4p16 (pedigree B, III.2 at the age of 4 years). Note micromelia and relative macrocephaly with high forehead.

Figure 5 X rays of the spinal column, hands, long bones, and femoral necks in a case of HCH harbouring the NS40K mutation (type 1, panel A) or unlinked to chromosome 4p16.3 (type 2, panel B).

(overmodelling of the long bones, metaphyseal flaring, increased length of the fibulae, small iliac wings, short femoral necks and tibiae, broad and short metacarpals and phalanges). By contrast, in HCH unlinked to chromosome 4p16.3, x ray anomalies were milder, the hands and long bones were normal, and no metaphyseal flaring was noted. In this latter group, other diagnoses such as dyschondrosteosis or metaphyseal dysplasia type Schmid were clearly excluded by radiographic examinations at different ages.

These results are at variance with those reported by Bellus *et al*,¹² who found no significant clinical or radiological differences among their patients regardless of the mutant geno-

type. HCH should be regarded therefore as a clinically and genetically heterogeneous condition, with one clinical subtype being allelic to ACH. This heterogeneity contrasts with the clinical and genetic homogeneity of ACH, whose invariable clinical severity is accounted for by a single mutation in the transmembrane domain of the FGFR3 gene in 98% of patients (G380R). The clinical variability of HCH has been previously noted by Maroteaux and Falzon,² Wynne Davies *et al*,³ and Hall and Spranger.⁴ Hitherto, however, the genetic basis of clinical variability in HCH has remained unknown. The demonstration of genetic heterogeneity in HCH provides a rationale for the clinical variability of this condition and should help in directing molecular studies both in sporadic cases and in uninformative HCH families.

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