Article

Molecular Basis for Hypochondroplasia in Japan

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Abstract: Hypochondroplasia is an autosomal dominant genetic disorder due to a heterozygous pathogenic variant of the FGFR3 gene. The early diagnosis of hypochondroplasia is necessary, since growth hormone is effective for improving adult height. The genetic test for the FGFR3 gene could help the early diagnosis. The detailed characteristics of FGFR3 genotypes have not been widely investigated in Japan, except for a common pathogenic variant, p.Asn540Lys. This study retrospectively analyzed the FGFR3 genotypes of 35 patients from 30 families with hypochondroplasia (age, range 0–6 years, median 1 year) in Japan. The pathogenic variants of FGFR3 were identified in all the patients: p.Asn540Lys in 23 probands (76.7%), p.Lys650Gln in 2 (6.7%), p.Leu324His in 2 (6.7%), p.Leu324Val, p.Ser351Cys, and p.Lys650Thr in 1 each (3.2%). The median age at diagnosis, height SD score at diagnosis, or the severity of radiologic findings was not significantly different between probands with p.Asn540Lys and those with other variants. Intellectual disability or epilepsy was identified in seven patients with p.Asn540Lys, but none with other variants. The genetic test of FGFR3 can be useful for assessing the potential risk of neurological sequela in children with hypochondroplasia.

Keywords: hypochondroplasia; FGFR3; genotype

1. Introduction

Hypochondroplasia (HCH) is an autosomal dominant genetic disorder of bone dysplasia, characterized by disproportionate short stature with rhizomelic shortening of the limbs. HCH is caused by a heterozygous pathogenic variant of FGFR3 that constitutionally activates the signaling pathway of fibroblast growth factor receptor 3 [1]. The early diagnosis of HCH is necessary, since growth hormone is effective for improving adult height [2]. The genetic test for FGFR3 could help the early diagnosis of HCH. However, the possible role of FGFR3 genotyping in the diagnosis of HCH has not been clarified.

Pathogenic variants in FGFR3 are responsible for a group of bone dysplasia, including thanatophoric dysplasia, achondroplasia, or HCH [3]. The genotype–phenotype relationship in the FGFR3-related disorders is clear. Each FGFR3 genotype corresponds to each
disorder without overlap [4]. p.Asn540Lys in FGFR3 is the most common pathogenic vari-
ant for HCH and is observed in 50–70% of the cases [1,5,6]. The variation and rarity of other
variants preclude detailed investigation. There is no comprehensive study determining
FGFR3 genotype in a large number of patients with HCH. Thus, the genotype–phenotype
relationship in HCH has not been fully understood. This study conducted a molecular
investigation on 35 Japanese patients with HCH to define the role of the genetic test in the
clinical management of HCH.

2. Materials and Methods

2.1. Study Subjects

This study retrospectively investigated 35 patients with HCH from 22 institutions in all
regions of Japan. Thirty-one were offered for genetic analysis of FGFR3 to the Department
of Pediatrics, Keio University School of Medicine, and four were provided from the database
of the Foundation for Growth Science. Four families with affected mothers and their
offspring were included. All the patients were clinically diagnosed as HCH based on
rhizomelic short stature and characteristic radiologic findings. The characteristic radiologic
findings were assessed by an expert radiologist (G.N.). Clinical information of the patients
was collected such as birth length and weight, development, epilepsy, or brain MRI. Three
patients (Cases 4, 5, and 9) were already reported previously [7,8]. The study collected
the clinical data of each patient including the age and height at diagnosis, the presence or
absence of intellectual disability or epilepsy, and brain MRI findings.

2.2. FGFR3 Genotype

We collected genomic DNA samples from the 31 patients who were offered for the
genetic analysis. Genomic DNA was extracted from peripheral white blood cells. We
examined all coding exons and flanking introns of FGFR3 by Sanger sequencing. Primer
sequences and PCR conditions are available upon request. This study was approved by
the Ethics Committee of Keio University School of Medicine and the Ethics Committee of
Tokyo Metropolitan Children’s Medical Center. We obtained written informed consent for
molecular studies from the parents. The FGFR3 genotypes of the other four patients were
provided directly from the database of the Foundation for Growth Science.

3. Results

Heterozygous missense variants of FGFR3 were observed in all the 35 patients with
HCH (Table 1). The variants were p.Asn540Lys in 23 families (76.7%), p.Lys650Gln in 2
(6.7%), p.Leu324Val in 1 each (3.2%), p.Ser351Cys in 1 each (3.2%), and p.Lys650Thr in 1 (3.2%).
These six variants were previously reported as pathogenic [9]. The severity of radiologic findings was not significantly different between probands with
p.Asn540Lys and those with other variants.

Table 1. Clinical and genetic characteristics of patients with hypochondroplasia.

<table>
<thead>
<tr>
<th>Case</th>
<th>Family</th>
<th>Sex</th>
<th>FGFR3 Genotype</th>
<th>Age at Diagnosis (Years)</th>
<th>Height SDS at Diagnosis</th>
<th>Intellectual Disability</th>
<th>Epilepsy</th>
<th>Temporal Lobe Dysgenesis</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>M</td>
<td>c.1948A&gt;C, p.Lys650Gln</td>
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<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>2</td>
<td>1</td>
<td>M</td>
<td>c.1948A&gt;C, p.Lys650Gln</td>
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<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>3</td>
<td>1</td>
<td>F</td>
<td>c.1949A&gt;C, p.Lys650Gln</td>
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<td>c.970C&gt;G, p.Leu324Val</td>
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<td>5</td>
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<td>c.1620C&gt;A, p.Asn540Lys</td>
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<td>NA</td>
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<td>-</td>
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<td>c.1052C&gt;G, p.Ser351Cys</td>
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The age and height SD score at diagnosis in probands were plotted in Figure 1. The median age at diagnosis in probands was 2 years, ranging from 0 to 6 years. The median height SD score at diagnosis in probands was $-3.1$, ranging from $-5.6$ to $-0.3$. The height SD scores at diagnosis were below $-2.0$ SD in all the patients, except in two cases diagnosed at 4 months of age or younger (Case 27, $-0.6$ SD at 4 months of age; Case 31, $-0.3$ SD in the neonatal period). The median age or height SD score at diagnosis was not significantly different between probands with p.Asn540Lys ($n = 23$) and those with other variants ($n = 7$) ($p = 0.635$ or 0.649, respectively).

At least seven patients had intellectual disability or epilepsy. Temporal lobe dysgenesis was confirmed in two out of four patients with intellectual disability and two out of four with epilepsy who underwent a brain MRI scan.

4. Discussion

This study reported FGFR3 genotypes of 35 Japanese patients who were radiologically and genetically confirmed as having HCH. All 33 patients diagnosed older than 4 months of age had a height SD score less than $-2.0$ SD, resembling the finding of achondroplasia that short stature becomes evident during infancy [10]. Radiologic findings become evident
even before decreased growth velocity. In fact, Saito et al. reported that radiological clues are useful to diagnose HCH in the neonatal period [8]. The early detection of patients was possibly explained by the expertise in diagnostic radiology. Despite a possible selection bias, these data can be helpful to reveal genotypic or phenotypic features of HCH in Japan.

p.Asn540Lys has been reported as the most prevalent pathogenic variant among different ethnicities [5,6]. The results from this study are consistent with those of previous studies, showing that p.Asn540Lys was observed in 76.7% of HCH. Rousseau et al. identified p.Asn540Lys in 8 of 16 familial cases (50.0%) and 13 of 13 sporadic cases (100.0%) [5]. Katsumata et al. reported p.Asn540Lys in 8 of 14 cases (57.1%) [6]. The proportions of p.Asn540Lys in HCH do not significantly differ between the previous and the present studies (chi-square test). The previous studies examined only the presence or absence of p.Asn540Lys. In contrast, this study identified other atypical variants than p.Asn540Lys. These atypical variants varied in terms of the position and substitution pattern of amino acid residue. This study did not find any significant differences in the radiological findings, age at diagnosis, or height SD score at diagnosis of patient groups between p.Asn540Lys and other variants. Further studies are warranted to delineate the relationship between genotype and phenotype among HCH.

Neurological sequelae has been reported only for HCH [11,12], not for achondroplasia. Linnankiv et al. reported neurocognitive difficulties in 8 of 13 HCH patients with p.Asn540Lys (61.5%) and a possible relationship between the neurological sequelae and temporal dysgenesis observed by brain MRI scan [13]. FGFR3 is expressed in the developing brain, but the mechanism underlying neurological sequelae is not clarified yet. This study found intellectual disability or epilepsy in at least four or six patients with p.Asn540Lys, respectively. In contrast, no such sequelae was reported in those with the atypical variants. The analysis with a small number of patients with atypical variants does not reach any conclusion. p.Asn540Lys might be a risk factor for neurological sequelae, supporting the possible role of the genetic test for HCH in predicting neurological prognosis.

In this study, we determined pathogenic variants of FGFR3 in all cases of HCH. Thus, the combination of rhizomelic short stature and characteristic radiologic findings can reach the accurate diagnosis of HCH with the help of radiologic experts. The genetic test of FGFR3 is probably useful for atypical cases of suspected HCH such as relatively severe or mild cases resembling achondroplasia or idiopathic short stature, respectively. Furthermore, these results suggest that the genetic test is useful for assessing a potential risk of neurological sequelae based on the presence of p.Asn540Lys.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Keio University School of Medicine (Protocol Code: 20170375 and date of approval March 30, 2018) and the Ethics Committee of Tokyo Metropolitan Children’s Medical Center (Protocol Code: H24-94).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

References