Genotype phenotype correlation in achondroplasia and hypochondroplasia

Y. Matsui, N. Yasui, T. Kimura, N. Tsumaki, H. Kawabata, T. Ochi
From Osaka University) Medical school and Osaka Medical centre
and Research Institute for Maternal and Child Health, Japan

Abstruct Recent studies of the fibroblast growth factor receptor 3 (FGFR3) gene have established that achondroplasia and hypochondroplasia are allelic disorders of different mutations. To determine whether the genotype could be distinguished on the basis of the phenotype, we analysed height, arm span, and skeletal radiographs from 23 patients with achondroplasia and the G380R mutation of FGFR3 and eight with hypochondroplasia and the N540K mutation. Both conditions share the classical pathological features of micromelic short stature, reduced or unchanged interpedicular distances in the lumbar spine, disproportionately long fibulae, and squared and shortened pelvic ilia. These were significantly more severe in the G380R patients than in the N540K patients. Our findings have shown a firm statistical correlation between the genotype and the phenotype, although there were a few exceptional cases in which there was phenotypic overlap between the two conditions.

Achondroplasia (ACH) is an autosomal dominant disorder characterised by 12micromelic short stature and a unique facial appearance.1,2 Recent reports have confirmed that more than 98% of patients with ACH have an identical glycine-to-arginine substitution at residue 380 (G380R) of fibroblast growth factor receptor 3 (FGFR3)3-6. This exceptional genotypic homogeneity is likely to contribute to the phenotypic homogeneity of ACH. Hypochondroplasia (HCH) is another disorder with short-limbed short stature and autosomal dominant inheritance. Many of its features resemble those of ACH, but the abnormalities are less severe overall and the face is almost completely normal. About 60% of patients with HCH have been reported to have an asparagine-to-lysine substitution at residue 540 (N540K) of FGFR3.10,11 Although the genotypic background of the remaining cases of HCH has not been clarified, the N540K substitution seems to be responsible for a significant proportion of cases of this condition.12-14 In spite of a long history of 50 observations suggesting allelism between ACH and HCH15, it has been claimed that the clinical and radiological features of ACH and HCH overlap.16,17 We have compared the phenotype of genotyped populations to clarify whether or not each of these two genotypes has a distinctive phenotype.

Patients and Methods

Blood samples were collected from patients clinically diagnosed as having ACH and HCH, and the FGFR3 genotype was determined as previously described. There were 23 patients with ACH and the G380R substitution, 11 boys and 12 girls with a mean age of 10.0 years (5 to 18). Of the eight HCH patients with the N540K substitution, there were five boys and three girls with a mean age of 11.6 years (7 to 18), all of whom had sporadic mutations. We also assessed 30 genetically normal individuals as a control group. There were 17 boys and 13 girls with a mean age of , 11.1 years (3 to 17). We analysed height, arm span and skeletal radiographs. Height was evaluated by the height standard deviation score which was obtained from standard growth curves for the Japanese population. Arm span was examined by calculating the span to height ratio percentage. We studied three of the pathological features of ACH and HCH.19-21 We assessed the ratio of the interpedicular distances at the

first and fourth lumbar vertebrae (L 1/L4 ratio) irrespective of whether they were reduced or not. We also determined the ratio of the length of the fibula to that of the tibia (F/T ratio) to ascertain whether the fibular length was disproportionate. Lastly, we measured squaring and shortening of the pelvic ilia by calculating the ratio of the interteardrop distance to the pelvic width (pelvic index). The first two indices have already been reported 17,22 and we devised the third to quantitate the skeletal changes. Statistical analysis used the unpaired t-test for the height standard deviation score and the span to height ratio percentage or analysis of variance and Scheffe's post-hoc test for the three radiological indices.

Results

Both groups of patients had severe short stature. The mean height standard deviation score of the G380R patients was -5.3 ± 1.2 which was lower than that of the N540K patients (-4.2 ± 1.5) although there was no statistical significance (p = 0.0502;). The mean span to height ratio percentage of the G380R patients was 90.4 ± 3.6 which was significantly lower than that of the N540K patients (96.9 ± 2.4; p < 0.0001;f).

The lumbar interpedicular distance was considerably reduced in both groups in comparison with the normal controls.23 In the G380R patients the mean L1/L4 ratio was l.18 ± 0.10 which was significantly higher than that of the N540K patients (1.02 ± 0.09; p = 0.0006;), but the latter was itself significantly higher than that of the normal group (0.89 ± 0.05; p = 0.0032). The mean F/T ratio in the G380R patients was 1.12 ± 0.03 which was significantly higher than that of the N540K patients (1.07 ± 0.03; p = 0.0002), but again the latter was significantly higher than that of the control group (1.01 ± 0.02; p = 0.0002;). The mean pelvic index of the G380R patients was 0.42 ± 0.03 which was significantly higher than that of the N540K patients (0.37 ± 0.04; p = 0.0026;). There was no significant difference in the pelvic index between the N540K patients and the control group (0.37 ± 0.03; p = 0.8908).

Discussion

We have shown that patients with ACH (G380R genotype) and HCH (N540K genotype) shared the classical skeletal changes (i.e., phenotype) to different degrees. According to the height standard deviation score and the span to height ratio percentage, the micromelic short stature was present in both conditions, but was more severe in ACH. Measurement of the L1/L4 ratio, the F/T ratio, and the pelvic index showed that the reduction of the interpedicular distances in the lower lumbar spine, the disproportionally long fibulae, and the squared iliac bones were more pronounced in ACH than in HCH. Both conditions carry a distinct mutation of FGFR3, which is a negative regulator of bone growth.24 25 Different levels of activation of FGFR3 should account for the differences in phenotypic severity between ACH and HCH.26~28 Statistical analysis of our results clearly separated the two conditions, showing that the skeletal changes in the G380R patients were more severe than those in the N540K patients. There were, however, four exceptional patients with the G380R mutation (a 5-year-old girl, a 7-year-old girl, and two 12-year-old boys) who showed milder phenotypes in all of the three radiological indices than the most severe case of the N540K mutation, demonstrating a phenotypic overlap between the two genetically distinct conditions. In these patients the radiological measurements may fail to distinguish the genotype and therefore genotyping is required for the differential diagnosis.

A short and broad femoral neck is another characteristic of ACH and HCH.19-20 In spite of our efforts to quantitate the deformity of the femoral neck, none of the radiological measurements could represent the appearance of the films. The hip sustains more mechanical loading than other joints and thus the coxa planovarus becomes more severe as growth continues. Comparison of the uniformity of the femoral neck in young G380R patients with that in older N540K patients would therefore confuse the clinical diagnosis . The L1/L4 and F/T ratios were less affected by the age of the patients. Genetically diagnosed ACH and HCH share common skeletal changes to different degrees, with a firm statistical correlation between the genotype and the phenotype. A few exceptional cases showed phenotypic overlap between the two conditions.

References

1. Langer LO Jr, Baumann PA, Gorlin RJ. Achondroplasia. Am J Roentgoenol Radium Ther Nucl Med 1967;100: 12-26.

2. Maroteaux P, Lamy M. Achondroplasia in man and animals. Clin Orthop 1964;33:91-l03.
3. Bellus GA, Hefferon TW, Ortiz de Luna RI, et al. Achondroplasia is defined by recurrent G380R mutations of FGFR3. Am J Hum Genet 1995;56:368-73.
4. Rousseau F, Bonaventure J, Legeai-Mallet L, et al. Mutations in the gene encoding fibroblast growth factor recept-33 in achondroplasia. Nature 1994;371:252-4.5. Shiang R, Thompson LM, Zhu YZ, et al. Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. Cell 1994;78:335-42.
6. Stoilov I, Kilpatrick MW, Tsipouras P. A common FGFR3 gene mutation is present in achondroplasia but not in hypochondroplasia. Am J Med Genet 1995;55:127-33.
7. Beals RK. Hypochondroplasia: a report of five kindreds. J Bone Joint Surg [Am] 1969;51-A:728-36. 8. Hall BD, Spranger J. Hypochondroplasia: clinical and radiological aspects in 39 cases. Radiology 1979;133:95-100.
9. Walker BA, Murdoch JL, McKusick VA, Langer LO, Beals RK. Hypochondroplasia. Am J Dis Child 1971 ;122:95-l04.
10. Bellus GA, Mclntosh I, Smith EA, et al. A recurrent mutation in the tyrosine kinase domain of fibroblast growth factor receptor 3 causes hypochondroplasia. Nat Genet 1995;lO:357-9.
11. Prinos P, Costa T, Sommer A, Kilpatrick MW, Tsipouras P. A common FGFR3 gene mutation in hypochondroplasia. Hum Mol Genet 1995;4:2097- 101 .
12. Bonaventure J, Rousseau F, Legeai-Mallet L, et al. Common mutations in the fibroblast growth factor receptor 3 (FGFR3) gene account for achondroplasia, hypochondroplasia, and thanatophoric dwarfism. Am J Med Genet 1996;63:148-54.
13. Rousseau F, Bonaventure J, Legeai-Mallet L, Schmidt H, Weissenbach J. Clinical and genetic heterogeneity of hypochondroplasia. J Med Genet 1996;33:749-52.
14. Prinster C, Carrera P, Del Maschio M, et al. Comparison of clinical-radiological and molecular findings in hypochondroplasia. Am J Med Genet 1998;75: 109-12.
15. McKusick VA, Kelly TE, Dorst JP. Observations suggesting allelism of the achondroplasia and hypochondroplasia genes. J Med Genet l 973; I O: 11 -6.
16. Oberklaid F, Danks DM, Jensen F, Stace L, Rosshandler S. Achondroplasia and hypochondroplasia: comments on frequency, mutation rate, and radiological features in skull and spine. J Med Genet 1979;16: 140-6.
17. Wynne-Davies R, Walsh WK, Gormley J. Achondroplasia and hypochondroplasia: clinical variation and spinal stenosis. J Bone Joint Surg [Br] 1981;63-B:508-15.
18. Matsui Y, Kimura T, Tsumaki N, et al. A common FGFR3 mutation functions as a diagnostic marker for achondroplasia-group disorders in the Japanese population. J Orthop Sci 1996;1 : 130-5.
19. Horton WA, Hecht JT. The chondrodysplasias: In: Royce PM. Steinmann B, eds. Connective tissue and its heritable disorders. New York: Wiley-Liss, 1993:641-75.
20. Rimoin DL, Lachman RS. Genetic disorders of the osseous skeleton. In: Beighton P, ed. Heritable disorders of connective tissue. St Louis: Mosby, 1993:557-689.
21. Spranger JW, Langer LO Jr, Wiedemann HR. Bone dysplasias. In: An atlas of constitutional disorders of skeletal development. Philadelphia, etc: WB Saunders, 1974:305-12.
22. Nehme AM, Riseborough EJ, Tredwell SJ. Skeletal growth and development of the achondroplastic dwarf. Clin Orthop 1976;ll6: 8-23.
23. Hinck VC, Clark WM, Hopkins CE. Normal interpediculate distances (minimum and maximum) in children and adults. Am J Roentgenol Radium Ther Nucl Med 1966;97: 141-53.
24. Colvin JS, Bohne 'BA, Harding GW, McEwen DG, Ornitz DM. Skeletal overgrowth and deafness in mice lacking fibroblast growth factor receptor 3. Nat Genet 1996;12:390-7.
25. Deng C, Wynshaw-Boris A, Zhou F, Kuo A, Leder P. Fibroblast growth factor receptor 3 is a negative regulator of bone growth. Cel l 996;84:9 1 1 -2 1 .
26. Naski MC, Wang Q, Xu J, Ornitz DM. Graded activation of fibroblast growth factor receptor 3 by mutations causing achondroplasia and thanatophoric dysplasia. Nat Genet 1996;13:233-7.
27. Webster MK, Donoghue DJ. Constitutive activation of fibroblast growth factor receptor 3 by the transmembrane domain point mutation found in achondroplasia. EMBO J 1996;15:520-7.
28. McEwen DG, Ornitz DM. Regulation of the fibroblast growth factor receptor 3 promoter and intron I enhancer by SPI family transcription factors. J Biol Chem 1998;273:5349-57.