Jpn J Human Genet **40**, 347–349, 1995

Mutation Report

PREDOMINANCE OF THE MUTATION AT 1138 OF THE cDNA FOR THE FIBROBLAST GROWTH FACTOR RECEPTOR 3 IN JAPANESE PATIENTS WITH ACHONDROPLASIA

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Summary Fibroblast growth factor receptor 3 (FGFR3) has recently been identified as a putative gene for achondroplasia. Since a guanine to adenine mutation at 1138 of the cDNA for FGFR3 had been identified in most of the patients in Western population, we examined 13 Japanese patients to see if they also share the same mutation. Specific endonuclease digestion of the amplified coding sequence for the transmembrane domain of the FGFR3 revealed that the 12 patients have the G to A change at 1138, while the other had the G to C substitution at the same point, both of which result in G380A substitution. As far as we studied, the homogeneity of the point mutation at 1138 is also authentic to Japanese patient as well as Western patients.

Key Words achondroplasia, point mutation, fibroblast growth factor receptor 3

Achondroplasia (ACH, MIM 100800), the most common cause of chrondrodysplasia, is an autosomal dominant disorder with typical clinical features including short limb dwarfism, megalocephaly, low nasal bridge, and caudal narrowing of spinal canal (Jones, 1988; Gorlin *et al.*, 1990). While the incidence of the trait is estimated one per 26,000 live birth in Western population (Jones, 1988), Kuroki *et al.* (1994) showed the similar incidence of one in 22,329 live birth in Japanese population. In 1994, point mutations in the coding sequence for the transmem-

Received August 28, 1995; Revised version accepted October 16, 1995.
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braneous domain of the fibroblast growth factor receptor 3 (FGFR3), which is mapped to 4p16.3, were found in patients with ACH (Rousseau *et al.*, 1994; Shiang *et al.*, 1994). Interestingly, more than 90% of the mutations were G to A substitutions at 1138 resulting in glycine to arginine conversion at residue 380. According to one of the most recent report (Mulvihil, 1995), the G380R substitution account for 193 of 194 identified cases, five of which are due to G to C substitution at 1138, yet several unidentified mutations are left to be disclosed (Shiang *et al.*, 1994; Rousseau *et al.*, 1994; Bellus *et al.*, 1995). It is quite exceptional to have a unique mutation with a great predominance in such a popular genetic disorder. However, these studies including the latest one by Stoilov *et al.* (1995) examined patients whose ethnic backgrounds were mostly Caucasian origin but not Asian. We investigated 13 unrelated Japanese patients with ACH to identify the mutations in the FGFR3 gene to see if they share the same mutation in common.

The patients were ascertained to have ACH phenotype at the pediatric clinic of Hokkaido University Hospital or referred by the associated hospitals. All of them were considered as sporadic cases and none of them was suspected to be an ACH homozygote. The sequence for the transmembrane region of the FGFR3 was amplified by polymerase chain reaction (PCR) using the following primers (Rousseau *et al.*, 1994); the forward primer: 5′-TGACGAGGCGGGCAGTG-3′ and the backward primer: 5′-AGCGGGAAGCGGGAGAT-3′. Genomic DNA (200 ng), which was extracted from leukocytes, was incubated (94°C for 40 sec, 52°C for 20 sec, 72°C for 20 sec/30 cycles) with 50 mM KCl, 10 mM Tris-HCl, pH8.3, 1.5 mM $MgCl_2$, 25 pmol primers, 10% DMSO, 200 μM of each deoxynucleotide triphosphate, 0.5 U Taq polymerase in 50 μl of total reaction. The 159 bp fragment of the PCR products were, then, subjected to be digested either with the restriction enzyme *Sfc*I and *Msp*I, since the novel restriction site (G to A transition for *Sfc*I and G to C change for *Msp*I) yields 117 bp and 42 bp fragments. Electrophoresis of the *Sfc*I digests revealed these mutant fragments as well as uncut 159 bp fragment in 12 of the 13 patients, while *Msp*I digestion yielded the same restriction pattern in a patient whose *Sfc*I digestion gave no mutant restriction pattern. No homozygosity or compound heterozygosity of the mutant allele was found in any of the ACH patients. These results confirm the findings of previous studies (Rousseau *et al.*, 1994; Shiang *et al.*, 1994; Bellus *et al.*, 1995; Stoilov *et al.*, 1995) with an evidence that the mutation of a unique position account for almost all the occurrence of ACH in Japanese. As far as we know, except for one case of G375C mutation cited by Mulvihil (1995), FGFR3 G380R substitution is the only mutation that has been identified to cause the ACH phenotype. Adding our data to the previous studies, G380R mutation accounts for 226/229 (98.7%) due to predominant G to A mutation (220/226, 97.3%) at 1138. As discussed by Bellus *et al.* (1995), there is a few variation in the clinical spectrum of the manifestations in patients with ACH. This may be because of the homogeneity of the underling genetic mutation. In Japan, prevalence of ACH is similar to that in

the Western population, suggesting that similar tendency of the recurrent point mutation at the unique site account for the basic disturbance in both groups. The rate of mutation at the guanine base is much higher than anticipated from a calculation based upon the risk for introduction of mutation at the methylated cytosine in the CpG island on the opposite strand (Bellus *et al.*, 1995). Despite that several genetic diseases are known to have variety in its type of genetic mutation, ACH may not be the case though the reason for the predominance of point mutations on the unique site remains to be disclosed.

Acknowledgment The authors acknowledge to Dr. N. Katsumata, National Hospital for Children Tokyo, and Dr. Y. Fukushima, Shinshu University School of Medicine for kind advices.

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