J Hum Genet (2001) 46:32–34

SHORT COMMUNICATION

**Hironori Iwasaki · Nobutaka Ota · Toshiaki Nakajima
Yasutomo Shinohara · Mina Kodaira · Mitsuko Kajita
Mitsuru Emi**

Five novel single-nucleotide polymorphisms of human interferon gamma identified by sequencing the entire gene

Received: September 18, 2000 / Accepted: October 2, 2000

Abstract Interferon gamma (IFNG) plays important roles in the regulation of bone remodelling. We describe here six single-nucleotide polymorphisms (SNPs) in the *IFNG* gene, five of which are novel, and their allelic frequencies in the Japanese population, as determined by sequencing 48 alleles of the entire gene. Four of these polymorphisms were identified inside the third intron, at nucleotide (nt) positions 2459 (A/G), 2671 (T/C), 3177 (T/G), and 3273 (G/A). In exon 4, SNPs were identified at nt positions 5199 (A/T) and 5272 (A/G). These polymorphic sites will be useful for genetic studies of disorders that affect the inflammatory process or calcium metabolism.

Key words Interferon gamma · Single-nucleotide polymorphism · Japanese population · Inflammation · Calcium metabolism

Introduction

The reduction in bone mass per unit volume that is characteristic of osteoporosis is not accompanied by any qualitative abnormalities in mineral content or organic matrix. Therefore, in this disease, the rate of bone resorption must exceed that of bone formation, and, in fact, the rate of bone formation does tend to be lower than normal in patients with osteoporosis, especially in postmenopausal women. Among a large number of risk factors for the development of osteoporosis (Ershler et al. 1997), genetic variation, especially of genes whose products regulate bone formation or resorption, could influence the heterogeneity in bone mass observed within population samples.

H. Iwasaki · N. Ota · T. Nakajima · Y. Shinohara · M. Kodaira · M. Kajita · M. Emi (✉)
Department of Molecular Biology, Institute of Gerontology, Nippon Medical School, 1-396 Kosugi-cho, Nakahara-ku, Kawasaki 211-8533, Japan
Tel. +81-44-733-5230; Fax +81-44-733-5192
e-mail: memi@nms.ac.jp

The process of bone remodeling involves complex interactions between the osteoclast, the primary bone-resorbing cell, and other cells in its microenvironment. These interactions can regulate bone resorption by affecting either the number of osteoclasts present at a given site, or the bone-resorbing capacity of individual osteoclasts (Roodman 1993). One of the most likely candidates for determining bone mass is interferon gamma (IFNG), a molecule known to influence both osteoclasts and osteoblasts (Ershler et al. 1997). To investigate a possible relationship between osteoporosis and genetic variation at the human *IFNG* locus, we searched for single-nucleotide polymorphisms by sequencing the entire *INFG* gene in 48 chromosomes from the normal Japanese population.

Subjects and methods

Extraction of genomic DNA. Blood samples were obtained, with informed consent, from 24 healthy Japanese individuals. Genomic DNA was prepared from each sample according to procedures described previously (Emi et al. 1999).

Polymerase chain reaction (PCR) conditions and primers. PCR and sequencing primers were determined by analyzing the *IFNG* genomic sequence with DNAsis 3.0 software (Hitachi Software Engineering, Tokyo, Japan). The numbering of sequences was adjusted to conform to the RNA transcription-start site (A at the top of GenBank J00219 is now +1). Four pairs of PCR primers and sequencing primers were selected, at approximately 1500-bp intervals, with overlaps of about 200 bp. Each genomic DNA (20 ng) was used as a template for PCR in a 10-μl reaction volume containing 10 mM Tris HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 20 mM of each dNTP, 1 μM of each oligonucleotide primer, and 0.5 U Taq DNA Polymerase (Roche Molecular Biochemicals, Switzerland), as described previously (Tsukamoto et al. 1998; Watanabe et al. 1998). PCR amplification was performed with a Gene Amp PCR 9600 System (Perkin Elmer Cetus, Norwalk, CT, USA) under

the following conditions: 94°C for 3 min, 72°C for 3 min, then 5 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 90 s, followed by 25 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 90 s.

DNA sequencing. After purification on affinity membranes, PCR products were subjected to cycle-sequencing (Tsukamoto and Emi 1998) with appropriate primers. All sequencing reactions were performed using the ABI dye terminator cycle-sequencing reaction kit (PE Biosystems, Tokyo, Japan). The samples were then resuspended in formamide, separated on an ABI 377 sequencer (PE Biosystems), and analyzed with the attached software. Differences among *IFNG* sequences from the 24 DNA samples were regarded as SNPs.

Results and discussion

The entire genomic structure of the *IFNG* gene was sequenced in all 48 alleles from 24 healthy Japanese individuals, and we found a total of six SNPs. The genomic structure and locations of confirmed SNP sites are shown in Fig. 1. Four of the six SNPs were intronic: an A/G at nucleotide (nt) position 2459, a T/C at nt position 2671, a T/G at nt position 3177, and a G/A at nt position 3273. The other two SNPs were present in exon 4, an A/T substitution at nt position 5199 and an A/G substitution at nt position 5272. Table 1 summarizes the SNPs and their allele frequencies in our population sample.

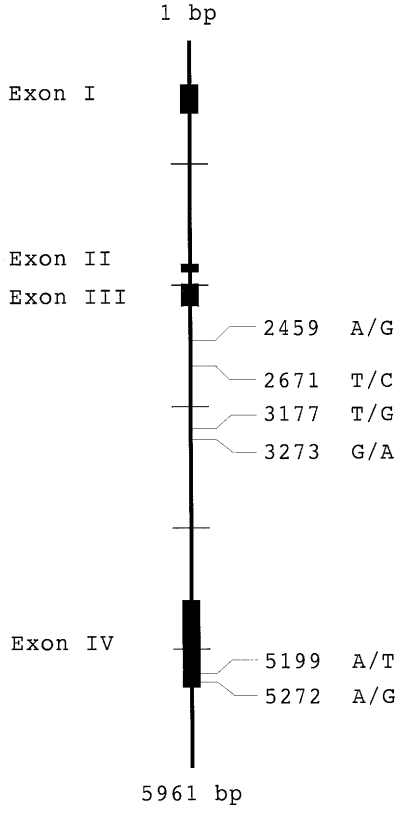

Fig. 1. Genomic structure and locations of single-nucleotide polymorphisms (SNPs) in the human *IFNG* gene. *Black boxes* represent coding regions (exons I–IV)

Of the six SNPs, five were novel; the T/C polymorphism at position 2671 was previously described in a Caucasian population (Bream et al. 2000). Although sequencing only 48 alleles might have limited the sensitivity of our experiments sufficiently to account for different results in the two studies, we suspect that differences in SNP distribution and allele frequencies in the *IFNG* gene must exist among different ethnic groups. We recently observed major differences between Japanese and Caucasian populations with regard to distribution and allele frequencies of SNPs in several other genes that are under investigation (manuscript in preparation).

Calcium levels in serum are kept in homeostasis through balanced interactions among calcitonin, parathyroid hormone, vitamin D, steroid hormones, and cytokines, and their receptors and modulators. IFNG is one of the most likely candidates for determining bone mass, because it is known to influence both osteoclasts and osteoblasts (Ershler et al. 1997). The powerful biological activities of IFNG are under strict control at both the transcriptional and the post-transcriptional levels (Cippitelli et al. 1995, 1996; Sica et al. 1997).

With respect to other cytokine genes, SNPs in cis-acting regions have been shown to alter transcriptional activity (Bailly et al. 1996; Gebhardt et al. 1999) and/or to be associated with the expression of specific phenotypes (Pociot et al. 1992; Rosenwasser and Borish 1997). For instance, in the *IFNG* promoter of the Albino Oxford (AO) rat, a single-base insertion results in disruption of a consensus binding site for E4F1 transcription factor that coincides with a significantly decreased capacity to produce IFNG protein (Pravica et al. 1997). In light of recent progress in the understanding of the biological functions of INFG, the polymorphic sites characterized in the present study will serve as useful markers for examining the potential role of IFNG in bone metabolism and the pathogenesis of osteoporosis in Japanese patients

Acknowledgments This work was supported in part by special grants for Strategic Advanced Research on "Cancer" and "Genome Science" from the Ministry of Education, Science, Sports, and Culture of Japan; by a Research Grant from the Ministry of Health and Welfare of Japan; and by a Research for the Future Program Grant of The Japan Society for the Promotion of Science.

Table 1. Polymorphisms in the human *IFNG* gene in the Japanese population

Gene region	Position	Nomenclature	Frequency
Intron 3	2459	2459 A/G	A (0.53), G (0.47)
Intron 3	2671[a]	2671 T/C	T (0.42), C (0.58)
Intron 3	3177	3177 T/G	T (0.98), G (0.02)
Intron 3	3273	3273 G/A	G (0.98), A (0.02)
Exon 4	5199	5199 A/T	A (0.05), T (0.95)
Exon 4	5272	5272 A/G	A (0.98), G (0.02)

[a] Also identified by Bream et al. (2000) in Caucasians

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