J Hum Genet (2001) 46:48–49

SHORT COMMUNICATION

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

Novel single nucleotide polymorphisms of the human colony-stimulating factor 2 (*CSF2*) gene identified by sequencing the entire gene

Received: October 13, 2000 / Accepted: October 23, 2000

Abstract We describe three single nucleotide polymorphisms (SNPs) of the human colony-stimulating factor 2 (*CSF2*) gene and their allelic frequencies, as determined by direct sequencing of 48 alleles of the entire *CSF2* gene. Three polymorphisms were identified, at nucleotide positions 1816 (T/C), 2284 (C/T), and 3079 (G/A). These polymorphisms will be useful in genetic studies not only of hematologic disorders but also of disorders of bone metabolism.

Key words Colony-stimulating factor 2 · Single nucleotide polymorphism · Japanese population · Hematologic disorders · Bone metabolism

Introduction

Colony-stimulating factors (CSFs) are proteins necessary for the survival, proliferation, and differentiation of hematopoietic progenitor cells. They are named according to the cells they stimulate. Macrophage CSF is known as CSF1. Granulocyte-macrophage CSF (CSF2, also known as GM-CSF) stimulates both cell types, as well as enhancing osteoclast development. Multi-CSF is known as interleukin-3 (IL3).

To investigate a possible relationship between genetic variation at the human *CSF2* locus and hemalotologic disease, as well as osteoporosis, we searched for SNP-type sequence variation by sequencing the entire *CSF2* gene in a large panel of Japanese individuals.

M. Kajita · H. Iwasaki · N. Ota · Y. Shinohara · M. Kodaira · T. Nakajima · 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

Subjects and methods

Genomic DNA extraction. Blood samples were obtained, with informed consent, from 24 healthy Japanese individuals. Genomic DNA was prepared from each sample.

Polymerase chain reaction (PCR) conditions and primer design. PCR and sequencing primers were determined by analyzing the IFNG genomic sequence with DNAsis 3.0 software (Hitachi Software Engineering, Tokyo, Japan). The sequencing numbering was adjusted to the RNA transcription start site (A at the top of GenBank M13207.1 is now +1). Four pairs of PCR primers and three pairs of sequencing primers were chosen at approximately 1500-bp intervals, with approximately 200-bp overlap. Genomic DNA (10 ng) was used as a template for PCR in 10-μl reaction volumes containing 10 mM Tris HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 20 mM each of dNTPs, 1 μM each of oligonucleotide primers, and 0.5 U Taq DNA Polymerase (Roche Molecular Biochemicals, Mannheim, Germany), as described previously (Tsukamoto 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 and 72°C for 3 min; then 5 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s; and 25 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s.

DNA sequencing. Following affinity membrane purification, PCR products were subjected to cycle sequencing with the appropriate primer, as described previously (Tsukamoto and Emi 1998). All sequencing reactions were performed using the ABI dye terminator cycle sequencing reaction kit (PE Biosystems, Tokyo, Japan). Sequencing samples were then resuspended in formamide and separated on an ABI 377 sequencer (PE Biosystems) and analyzed with attached sequencing software. Differences of sequences in the 24 samples were regarded as SNPs.

Table 1. Novel polymorphisms in the human *CSF2* gene in the Japanese population

Gene region	Position	Nomenclature	Frequency
Intron 3	1816	1816 T/C	T (0.98), C (0.02)
Intron 3	2284	2284 C/T	C (0.37), T (0.63)
Intron 4	3079	3079 G/A	G (0.26), A (0.74)

Results and discussion

A total of 24 Japanese individuals were genotyped for sequence variations of the entire *CSF2* structural gene. A total of three SNPs were found in the gene by this method, as shown in Table 1. The location of these SNPs in relation to the genomic structure of the *CSF2* gene is shown in Fig. 1. Of the three SNPs found in the *CSF2* gene, two were located within the third intron, i.e., a T/C at nucleotide (nt) position 1816, and a C/T at nt position 2284. The other SNP was found in the fourth intron, i.e., a G/A substitution at nt position 3079. Table 1 shows a summary of the allele frequencies of the novel SNPs determined in the 24 Japanese individuals, consisting of 48 alleles.

Postmenopausal women are at an increased risk for osteoporosis because of the lack of the protective effects of estrogen. In mice, estrogen loss brought about by ovariectomy increased the number of colony-forming units for granulocytes and macrophages, enhanced osteoclast development in ex-vivo cultures of marrow, and increased the number of osteoclasts in trabecular bone (Jilka et al. 1992). These data suggest that the *CSF2* gene is a candidate involved in osteoclast development and, thus, in the regulation of bone mineral density in humans.

In conclusion, these polymorphisms will be useful for the investigation of a possible relationship between genetic variation at the human *CSF2* gene and human diseases, especially in association studies between specific polymorphisms and susceptibility to hematologic diseases and osteoporosis.

Acknowledgements This work was supported in part by a special Grant 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.

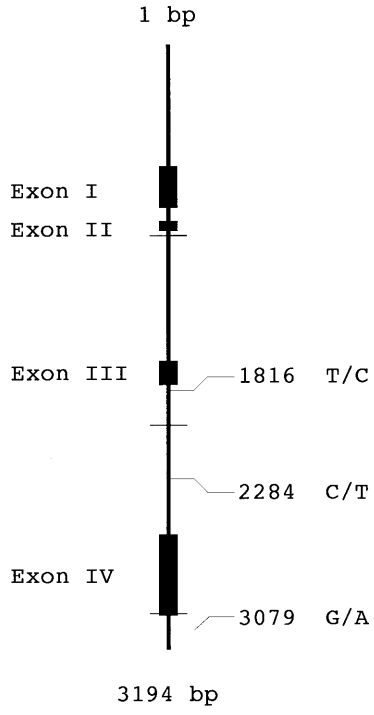

Fig. 1. Genomic structure and location of single nucleotide polymorphisms (SNPs) in the human *CSF2* gene

References

Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC (1992) Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science 257:88–91

Tsukamoto K, Haruta K, Shiba T, Emi M (1998) Isolation and mapping of a polymorphic CA repeat sequence at the human interleukin 6 locus. J Hum Genet 43:71–72

Tsukamoto K, Emi M (1998) A polymorphic CA repeat sequence at the human calcitonin locus. J Hum Genet 43:146–147