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RFLP Report

DETECTION OF MspI RFLP IN HUMAN THY1 GENE BY THE POLYMERASE CHAIN REACTION

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THY1 gene encodes a cell surface glycoprotein predominantly expressed in brain and peripheral nerves. Human THY1 gene region on chromosome 11q23 has been implicated in susceptibility to type 1 diabetes (Wong *et al.*, 1991). Two primers derived from the sequences flanking the polymorphic MspI site in intron 2 of the human THY1 gene (Gatti *et al.*, 1988) were selected for RCP to amplify a 566 bp fragment that spans the MspI polymorphism. Polymorphism was detected by MspI digestion of the PCR product.

Key Words THY1 gene, insulin-dependent diabetes mellitus (IDDM), polymorphism, PCR-RFLP

PCR primers. THY1F 5′-ACGTCACAGTGCTCAGAG-3′
THY1R 5′-CTCACACTTGACCAGTTTG-3′

Polymorphism. A1 allele that lacks the polymorphic MspI site generates fragments at 524 and 42 bp on MspI digestion due to non-polymorphic MspI site within the PCR products. A2 allele that contains the polymorphic MspI site generates fragments at 406, 118, and 42 bp on MspI digestion (Fig. 1).

Frequency. Estimated from 155 unrelated Japanese individuals.

A1: 0.10 A2: 0.90

Chromosomal localization. The THY1 gene has been assigned to chromosome 11q22.3 (Seki *et al.*, 1988).

Menderian inheritance. Codominant inheritance was observed in two families.

Other comments. The reaction was carried out in a volume of 10 μl containing 20 ng genomic DNA, 2.5 pmol of each primer, 500 μM of dNTP, 1 μl 10× buffer (1 mM $MgCl_2$) and 0.5 U Taq polymerase (Takara) for 35 cycles as follows: 94°C for 1 min, 52°C for 2 min, 72°C for 3 min. The PCR products were digested with

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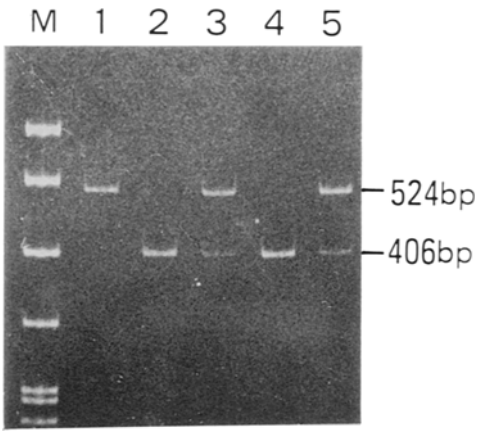

Fig. 1. Detection of a MspI polymorphism in the human THY1 gene by PCR-RFLP. Lane 1, homozygote of A1; lanes 2 and 4, homozygotes of A2; lanes 3 and 5, heterozygotes of A1 and A2; M, molecular weight marker (pBR322 MspI digest).

MspI and analyzed on a 5% polyacrylamide gel. The size of allele was determined by comparison with MspI digested pBR322.

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