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BRIEF REPORT — POLYMORPHISM REPORT

Atsushi Ogawa · Shigenori Yamamoto
Masaki Takayanagi · Toshiaki Kogo · Masaki Kanazawa
Yoichi Kohno

An Ile/Val polymorphism at codon 1464 of the *ATP7A* gene

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Abstract An isoleucine/valine polymorphism was observed at codon 1464 of the *ATP7A* gene, which is thought to encode a copper transporting adenosine triphosphatase (ATPase). The frequency of Val1464 was estimated to be 5.7% in the Japanese population. This polymorphism may be useful in genetic studies of Menkes disease.

Key words Menkes disease · *ATP7A* · *MNK* gene · Polymorphism · Copper

Introduction

The *ATP7A* gene (previously designated as the "*MNK*" gene) encodes a protein predicted to be a P-type cation-transporting adenosine triphosphatase (ATPase) (Vulpe et al. 1993; Chelly et al. 1993; Mercer et al. 1993) based its similarity to a bacterial form of copper-transporting ATPase, and the presence of a putative metal-binding motif at the N-terminus (Vulpe et al. 1993). Recently, we reported mutations in the *ATP7A* gene in three unrelated Japanese patients with classical Menkes disease (Ogawa et al. 1999). During screening for possible disease-causing mutations by sequencing of reverse transcription-polymerase chain reaction (RT-PCR) products, we identified a polymorphism in the *ATP7A* gene. Here we describe the isoleucine/valine (Ile/Val) polymorphism in the *ATP7A* gene in the Japanese population.

A. Ogawa (✉) · S. Yamamoto · T. Kogo · M. Kanazawa · Y. Kohno
Department of Pediatrics, Chiba University School of Medicine,
1-8-1 Inohana, Chuou-ku, Chiba-shi, Chiba 260-8670, Japan
Tel. +81-43-226-2144; Fax +81-43-226-2145
e-mail: aogawa@pediat3.m.chiba-u.ac.jp

M. Takayanagi
Division of Metabolism, Chiba Children's Hospital, Chiba, Japan

T. Kogo · M. Kanazawa
Division of Pediatrics, National Sanatorium Shimoshizu Hospital, Chiba, Japan

Methods

Primers for the polymerase chain reaction (PCR)

Forward: 5′-TGTTGGAATAGATGATACCTCAAG-3′
Reverse: 5′-GTTTATCAGACAGTAGTGAGTGTA-3′

As the polymorphism did not cause modification of a restriction site in the *ATP7A* gene, an additional base substitution was artificially introduced into the region around the site. Using the reverse oligonucleotide primer, *an Rsa* I site was introduced into the Val1464 allele (**G**TAC; where the substitution in the reverse primer sequence is underlined and the polymorphic nucleotide position, A4535G, is shown in bold type), but not into the Ile1464 allele.

PCR conditions

To detect the Ile/Val polymorphism, PCR was carried out in a total volume of 50μl, containing 200ng of genomic DNA, 200μM dNTPs, 1μM of each primer, and 1.0U polymerase from the Expand High Fidelity PCR system (Boehringer Mannheim, Mannheim, Germany). Cycle conditions were 95°C for 3min, then 35 cycles of 95°C for 30s, 52°C for 30s, and 72°C for 1min, with a final extension step of 5min. PCR products were digested with *Rsa* I and subjected to electrophoresis on 3% NuSieve 3:1 agarose gels (FMC BioProducts, Rockland, ME, USA).

Informed consent for this study was obtained from the parents of all the individuals tested.

Polymorphism and allele frequency

Rsa *I polymorphism*. Digestion with restriction endonuclease *Rsa* I produced a 104-bp fragment from the Ile1464 allele PCR product, which lacked the artificial *Rsa* I site, and 81- and 23-bp fragments from the Val allele PCR product, which contained the recognition site (Fig. 1).

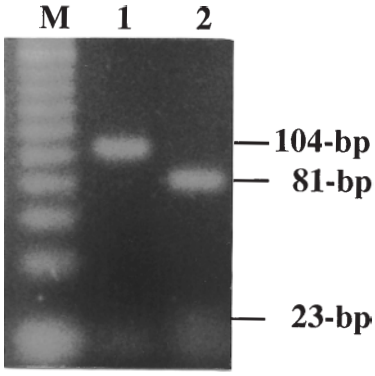

Fig. 1. *Rsa* I digestion of the PCR products. *Lane 1*, Ile1464 allele hemizygote that lacks the *Rsa* I site; *Lane 2*, Val1464 allele hemizygote that contains the *Rsa* I site; *Lane M*, 20-bp DNA ladder marker

Allele frequency. Six Val1464 alleles were detected in 104 X-chromosomes of unrelated male Japanese individuals. The observed frequency of the Val1464 allele was 5.7%.

Chromosomal localization. The human *ATP7A* gene has been mapped to Xq13.3 (Verga et al. 1991; Tümer et al. 1992).

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