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BRIEF REPORT

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Human aryl hydrocarbon receptor nuclear translocator gene (*ARNT*) D/N511 polymorphism

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Abstract We found a novel A → C change in codon 511 of the *ARNT* gene, which predicted the substitution of Asn (AAC) for Asp (GAC) at this position. Amplification using mismatched primers allowed the *ARNT* D/N511 polymorphism to be detected by digestion with endonuclease *Tth111*I. The frequency of the *ARNT* N511 allele was 0.019 in Caucasians and 0.026 in Africans. Because of the importance of the *ARNT* gene product in the metabolism of xenobiotics, this polymorphism may be useful in the study of associations with metabolic phenotypes and in pharmacogenetic studies.

Key words Pharmacogenetics · Xenobiotics · Cytochrome P450 · Lipodystrophy

Introduction

The aryl hydrocarbon receptor nuclear translocator (ARNT) belongs to a distinct subclass of basic helix-loop-helix transcription factors (Johnson et al. 1993; Reisz-Porszasz et al. 1994; Basci and Hankinson 1996). ARNT forms a complex with the aryl hydrocarbon receptor (AHR), which mediates carcinogenesis by certain pollutants, such as halogenated aromatic hydrocarbons and polycyclic aromatic hydrocarbons. After binding to such ligands in the cytosol, AHR translocates to the nucleus, where it heterodimerizes with ARNT. The AHR-ARNT heterodimer constitutes a transcription factor referred to as the transformed AHR complex, which stimulates the synthesis of CYP1A1 and other proteins involved in xenobiotic metabolism (Johnson et al. 1993; Reisz-Porszasz et al. 1994; Basci and Hankinson 1996).

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The *ARNT* gene has been mapped to chromosome 1q21 (Johnson et al. 1993) and is within a region that has shown linkage to interesting metabolic disorders, such as partial lipodystrophy (Anderson et al. 1999) and familial combined hyperlipidemia (Pajukanta et al. 1998). Thus, the availability of a marker for the *ARNT* gene would be useful for the study of inter-individual differences in xenobiotic metabolism and also for analysis of its possible association with distinctive metabolic phenotypes related to insulin resistance and lipid metabolism. In the course of DNA sequencing all of the coding regions and the 5′- and 3′-untranslated regions of the *ARNT* gene, we found a novel G → A change involving the first nucleotide of codon 511, which predicted the replacement of Asp (GAC) by Asn (AAC) at this position (D/N511).

Polymorphism and allele frequency

Primers for the polymerase chain reaction (PCR). For PCR, we used the following primers:
ARNT-511F 5′-TATTTGTCTTGTCAACTGGCCTTT-GAC-3′
ARNT-511R 5′-CTGGCCAGTCCATCTCTTCCTGGG-AC-3′

*Tth111*I *polymorphism.* The PCR fragment size was 90 bp. Digestion of the common D511 allele produced two smaller fragments, with sizes 63 and 27 bp. Digestion of the less common N511 allele produced a single 90-bp fragment.

Chromosomal localization. The human *ARNT* gene has been localized to chromosome 1q21 (Johnson et al. 1993).

Mendelian inheritance. Mendelian inheritance was confirmed in two large families.

Other comments. The polymorphism was detected through direct sequencing of all coding regions of the *ARNT* gene in Caucasian subjects with partial lipodystrophy. Codon 511 is

Table 1. Allele frequencies of *ARNT* D/N511 polymorphism as detected by *Tth111*I

Allele	Nucleotide	Fragments (bp)	Frequency	
			Caucasian (n = 267)	African (n = 74)
D511	G	63, 27	0.981	0.974
N511	A	90	0.019	0.026

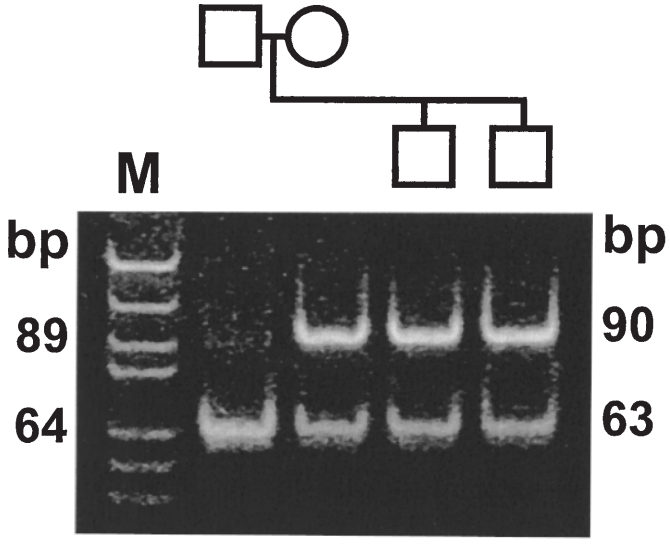

Fig. 1. *Tth111*I restriction fragment length polymorphism (RFLP) detecting *ARNT* D/N511 polymorphism. *Tth111*I digests were electrophoresed in 10% polyacrylamide gels. *Pedigree structure* indicates familial relationships between samples. *M* indicates molecular weight marker. *Numerals on left side* correspond to molecular weight marker band sizes. *Numerals on right side* correspond to fragment sizes. The 27-bp fragment could not be visualized on this gel

in exon 16. The D/N511 polymorphism in *ARNT* exon 16 was typed with a mismatched PCR primer, in which the third base from the 3′-end of primer ARNT-511R was changed from T to G. Target DNA was amplified using an initial melting temperature of 94°C for 5 min, followed by 30 cycles of 94°C for 20 s, 55°C for 20 s, and 72°C for 20 s. A final 72°C extension step for 10 min terminated the process. The fragments were visualized in 10% polyacrylamide gels.

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