MOLECULAR BIOLOGY OF THE Ah RECEPTOR AND Ah RECEPTOR-SEPENDENT SIGNALING

SEQUENCE POLYMORPHISMS OF THE HUMAN ARYLHYDROCARBON RECEPTOR

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Introduction

The arylhydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates the biological and toxicological effects of halogenated aromatic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

At least four alleles of the murine *Ahr* locus have been identified; one allele, Ahr^{d}, encodes an AhR with a 10 fold reduction in affinity for some ligands (e.g. TCDD), and no response to others (e.g. 3-methylcholanthrene). Expression of the Ahr^{d} allele dramatically effects the organisms susceptibility to certain environmental contaminants as compared to expression of the high affinity alleles Ahr^{b-1}, Ahr^{b-2}, or Ahr^{b-3}. Cloning of the murine AhR gene has revealed that a single nucleotide alteration, resulting in arginine to valine substitution at codon 375, is sufficient to confer reduced ligand affinity of the Ahr^{d} allele[1].

In humans relatively little is known about genetic variation in the AhR. An AhR polymorphism which results in replacement of 554Arg by 554Lys has been identified in a Japanese population (allele frequency 0.43), however no correlation between genotype and lung cancer susceptibility could be demonstrated[2]. Recently, Smart and Daly have demonstrated that the variant AhR-554Lys allele is readily detectable in African-Americans (allele frequency 0.41) but occurs much less frequently in Caucasians (allele frequency 0.11) and reported a second human AhR allele, V570I, in African-American populations[3].

The aim of this study was to search for AhR variants in human populations where some information already exists on receptor phenotype. We determined the apparent affinity (K_d) of TCDD binding to cytosolic AhR for two populations, and examined in detail individuals at the extremes of the distribution. We have also determined the frequency of AhR-554Lys and AhR-570Ile variants in several ethnic groups and report the finding of a third AhR polymorphism.

Methods

Collection of human tissues and DNA samples

Genomic DNA was isolated from human placentas collected from cesarean deliveries at Mount Sinai Hospital in Toronto (Toronto), and from vaginal births in Denver, Colorado (Denver), Kuujjuaq, Quebec (Inuit), and Sept Ile, Quebec (French Canadian). Genomic DNA samples from Chinese, German and Canadian Native Indian were obtained from peripheral blood lymphocytes. Genomic DNA from African subjects was also obtained (Ivory Coast African, African, and Caribbean African).

Cytosol preparation and binding of [^{3}H]TCDD to AhR.

Cytosol was prepared from placentas collected in Toronto and Denver and assessed for binding of [^{3}H]TCDD exactly as described previously[4].

Single strand conformational polymorphism analysis for codon 554.

The polymorphism at codon 554 is a G to A substitution which results in the amino acid substitution R554K. Single strand conformational polymorphism (SSCP) used to detect this polymorphism was adapted from Orita *et al*, using 5'-ACC AGC CTC AGG ATG TGA AC-3' (forward primer) and 5'-GAA TCT TGG ACA TAC GTC AG-3' (reverse primer)[5].

Restriction fragment length polymorphism analysis of codon 570..

The polymorphism at codon 570 is a G to A substitution resulting in the amino acid substitution V570I. This substitution eliminates a recognition site for the restriction endonuclease *Hinc*II. Genomic DNA was amplified using forward primer, 5'-ACC AGC CTC AGG ATG TGA A-3' and reverse primer 5'-TGC TGT GGA CAA TTG AAA GG-3', to generate a 457 bp product. Incubation of WT sequence with *Hinc*II gives rise to two fragments whereas the polymorphic sequence is not susceptible to digestion and the PCR fragment remains intact.

Restriction fragment length polymorphism analysis of codon 517.

A polymorphism at codon 517 results from a C to T substitution resulting in the amino acid substitution P517S. This substitution eliminates a recognition site for the restriction endonuclease *Bsu*36I. Genomic DNA was amplified using forward primer, 5'-TTT CCT GCC ATA ATG GAT CC-3' and reverse primer 5'-TGC TGT GGA CAA TTG AAA GG-3', to generate a 743 bp product. Incubation of WT sequence with *Bsu*36I gives rise to two fragments whereas the polymorphic sequence is not susceptible to digestion and the PCR fragment remains intact.

Results and Discussion

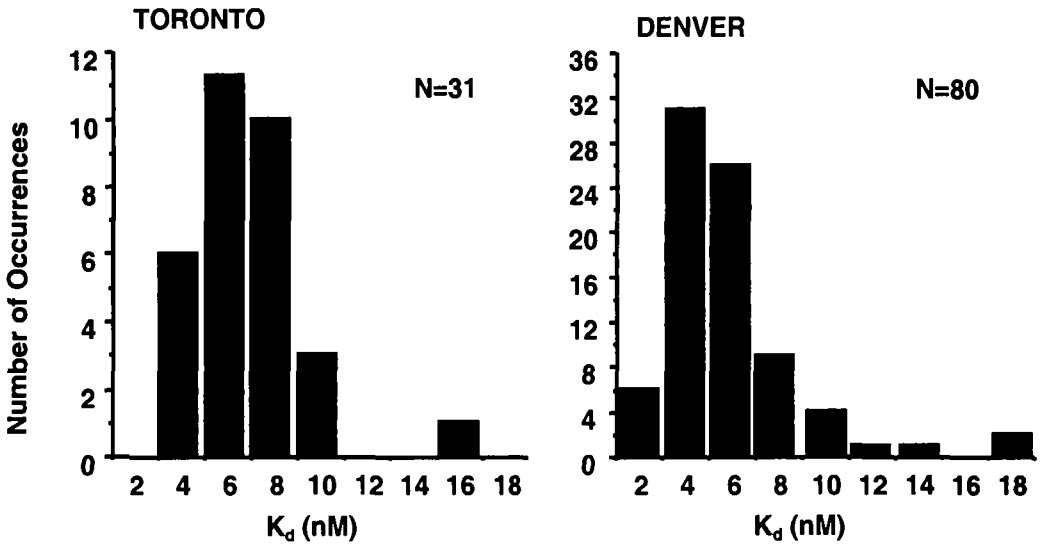
The ligand binding phenotype of AhR in the Toronto and Denver samples was determined by sucrose density gradient assays using radiolabelled [^{3}H]TCDD. Specific binding to AhR was readily detectable in cytosol from all placentas assayed, and an apparent K_d was estimated for each sample using Scatchard plots and Woolf plots. The individual K_ds determined for human AhR binding to TCDD ranged from 0.4 to 18 nM (Figure 1), and included the K_ds determined for the murine high affinity *Ahr*$^{b-1}$ allele (approximately 1 nM) and murine low affinity *Ahr*^{d} allele, (approximtely 16 nM), suggesting the possibility of a polymorphism in the ligand-binding domain of the human AhR [6]. We chose subjects classified as having either "higher" or "lower" affinity for TCDD, i.e. $K_d \leq$ to 1 nM or $K_d \geq 15$ nM from the individuals who had been phenotyped by the TCDD ligand-binding assay. Sequencing of the entire cDNA of the AhR coding region of these individuals did not reveal any sequence variations. We also directly sequenced exon 9 in all members of the phenotyped Toronto population to determine whether variation in codon 381 (the human counterpart to murine codon 375) could explain the low binding affinities found in some of our samples. Direct sequencing of AhR-codon 9 revealed no deviations from wild type sequence.

Thus, although the range of apparent K_d for ligand binding determined for our placental samples would suggest the existence of a sequence polymorphism analogous to that found in mice, direct sequencing of representative subjects, and direct sequencing of exon 9 in all individuals did not reveal any such polymorphism.

Single strand conformational polymorphism analysis

Single strand conformational-polymorphism was used to rapidly screen for mutations in the coding region of the human AhR. The organisation of human *Ahr* made it an ideal candidate

Figure 1. Distribution of Apparent K_d of [^{3}H]TCDD-binding to AhR from Human Placenta



for SSCP analysis since, with the exception of exons 10 and 11, all of the remaining 9 exons are under 300 bp in length and within the optimal window for SSCP detection. For exon 10, primers were designed to divide the exon into 6 overlapping regions with the average size of each region around 300 bp, the coding region in exon 11 is only 146 nt, hence multiple primer sets were not necessary.

We used SSCP to scan the entire AhR open reading frame of individuals from the Toronto, Denver, French Canadian, and Inuit samples and detected no sequence polymorphism other than at codon 554 and codon 570 (see below).

Single strand conformational polymorphism analysis for codon 554.

In an earlier study, Kawajiri *et al* identified an AhR polymorphism in a Japanese population resulting in the replacement of Arg554 with Lys[2]. This polymorphism occurred with a frequency of 43%. In our study, the frequency of the codon 554 polymorphism was markedly lower in populations that were predominantly Caucasian (0.11 and 0.12 in the North American and 0.07 in German subjects) in contrast to our Chinese population (0.32) and African populations (0.57 in both Ivory Coast and African population and 0.53 in Caribbean African population). The frequency was also low in Canadian Native Indian (0.14) and Inuit (0.09) populations. Similar findings for Caucasian and African-American populations have been recently reported by Smart and Daly[3].

This SSCP analysis also detected one individual with a T to C substitution in codon 550, whether this is a true polymorphism remains to be determined.

RFLP Analysis for codon 570 polymorphisms.

In a recent SSCP study Smart and Daly identified a novel *Ahr* allele that was very rare in Caucasians but more common in African-Americans (0.05)[3]. Using an RFLP assay, we found that the allelic frequency for V570I was only 7% in Ivory Coast African, 7% in African and 3%

Table 1. AhR Allele Frequency in Samples of Various Ethnic Origin

Population	n	Codon 554		Codon 570		Codon 517	
		Arg	Lys	Val	Ile	Pro	Ser
Ivory coast African	58	0.43	0.57	0.93	0.07	0.965	0.035
African	20	0.47	0.53	0.93	0.07	0.95	0.05
Caribbean African	55	0.60	0.40	0.97	0.03	1.00	0
Canadian Chinese	41	0.68	0.32	1.00	0	-	-
Native Indian	47	0.86	0.14	-	-	-	-
Denver (mixed)	17	0.88	0.12	1.00	0	-	-
French Canadian	20	0.88	0.12	1.00	0	-	-
Toronto (mixed)	28	0.89	0.11	0.97	0.03	-	-
Canadian Inuit	22	0.91	0.09	1.00	0	-	-
German	78	0.93	0.07	1.00	0	-	-

in Caribbean African samples. One heterozygous individual was identified in the Toronto sample, but information regarding ethnic origin was not available. This polymorphism was not detected in non-African populations.

RFLP Analysis for codon 570 polymorphisms.

During the course of our analyses we identified a novel AhR polymorphism, a proline to serine substitution at codon 517, in subjects of African background. The allelic frequencies for Ser 517 were low, 5% in Ivory Coast African, 3.5 % African populations, but not detected in Caribbean African population.

When examining the distribution of the three AhR alleles within our samples we noticed that having at least one variant allele of codon 554 appeared to be a prerequisite for possessing a variant allele for codon 570, and having at least one variant codon 570 appeared to be a prerequisite for possessing a variant allele at codon 517. The restriction endonuclease pattern for different combinations of allele localisation (linked vs not-linked) was determined, and together with direct sequencing was used to analyse the genotype of the three individuals heterozygous for all three polymorphisms. Our analysis indicates that all three polymorphisms lie on the same strand of DNA, i.e. all three polymorphisms occur within less than 200 bp of exon 10. The functional characteristics of an such an AhR remains to be determined.

References:

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