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SNP Communication

An Evaluation of Single Nucleotide Polymorphisms in the Human Aryl Hydrocarbon Receptor-Interacting Protein (AIP) Gene

J. Craig Rowlands[1,*], Jonathan D. Urban[2], Daniele Staskal Wikoff[2] and Robert A. Budinsky[1]

[1]*The Dow Chemical Company, Toxicology and Environmental Research and Consulting, Michigan, USA*
[2]*ToxStrategies, Texas, USA*

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Summary: The human aryl hydrocarbon receptor (AHR) is a protein for which there is little evidence of polymorphic variability of functional consequence. It has been hypothesized that potential variability in dioxin sensitivity may be due to polymorphisms in AHR-associated proteins, such as the human AHR-interacting protein (AIP). There are limited data on AIP single nucleotide polymorphisms (SNPs) with potential functional consequences. We sequenced 103 human DNA samples within the open reading frames of the *AIP* locus using samples from six ethnic populations to further characterize AIP SNPs. Eight exonic SNPs were identified at the *AIP* locus, including three novel SNPs: T48T, L212L, and V302V. Combined with prior reports, there are now a total of 14 exonic SNPs that have been identified within AIP. Of these, six are non-synonymous and are therefore of potential functional importance, though only two of these (Q228K and A276V) were detected in the current study. The functional consequences of Q228K and A276V are unknown, although functional evidence from *AIP* SNPs associated with congenital pituitary tumors suggests that such amino acid changes are likely to have no effect or to decrease, rather than increase, sensitivity to dioxins. To date, no non-synonymous SNPs have been detected in the AHR-binding region of AIP.

Keywords: Ah receptor-interacting protein; polychlorinated dibenzo-*p*-dioxins; TCDD; human genetics; single nucleotide polymorphism

Introduction

Dioxin-like compounds (DLC), which include the most extensively studied congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, are aryl hydrocarbon receptor (AHR) agonists, which upon binding promote the translocation of the AHR to the nucleus where it dimerizes with aryl hydrocarbon receptor nuclear translocator (ARNT) protein. The AHR-ARNT protein complex functions as a transcription factor, regulating expression of target genes involved with xenobiotic metabolism and potentially toxic responses. In the absence of ligand, however, AHR is sequestered in the cytosolic cellular compartment where it is constitutively bound to heat shock protein 90 (HSP90), p23, and the aryl hydrocarbon receptor-interacting protein (AIP).[1,2)]

The *AIP* locus is approximately 8 kb in length and maps on chromosome 11q13. The gene comprises 6 exons that encode a 330-amino-acid protein transcription and translation. The AIP protein, also known as ARA9 and XAP2, stabilizes the AHR ligand-binding complex, retains ligand-free AHR complex in the cytoplasmic subcellular compartment by altering the ability of AHR to be recognized by importin-beta, and prevents ubiquitination and proteasomal degradation of the unliganded AHR complex.[3–5)] In addition to binding AHR, AIP binds to phosphodiesterase (PDE), affecting PDE-mediated cyclic adenosine monophosphate (cAMP) hydrolysis in a PDE isomer-dependent manner.[6,7)] AIP has also been reported to interact with survivin, thereby promoting protein stability and preventing apoptosis.[8)] AIP contains several accessible serine sites phosphorylated by protein kinase CK2, but this does not affect AHR function.[9)]

In vivo laboratory studies have demonstrated that AIP plays a significant role in physiological development.[10–12)] Improper expression of AIP is also known to have detrimental consequences in humans, especially in pituitary maturation and function.[13,14)] There have been as many as 33

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**To whom correspondence should be addressed:* Dr. J. Craig Rowlands, *The Dow Chemical Company, 1803 Building, Midland, MI 48574, USA. Tel. +1-989-636-0935, Fax. +1-989-638-9305, E-mail: jcrowlands@dow.com*
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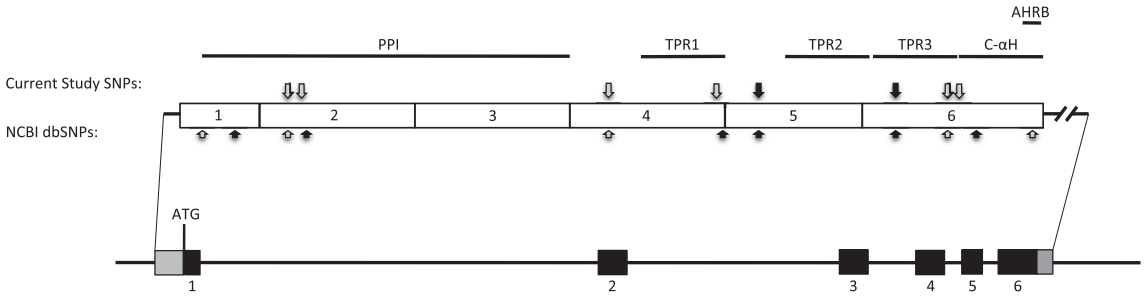

Fig. 1. An approximate representation of the human *aryl hydrocarbon receptor-interacting protein (AIP)* locus based on information presented in the literature[1,28,29] and the ENSEMBL database: http://uswest.ensembl.org/Homo_sapiens/Transcript/ProteinSummary?db=core;g=ENSG00000110711;r=11:67250505-67258578;t=ENST00000279146

The genome sequence is shown at the bottom with the exons denoted as numbers and gray boxes representing untranslated sequences and darkened boxes representing translated sequences. Introns are depicted as intervening horizontal lines. The deduced cDNA or mRNA is shown at the top. The domains are peptidyl-prolyl cis-trans isomerase FKBP-type domain (PPI), tetratricopeptide repeat domains (TPR), C-terminal α helix (C-αH), and aryl hydrocarbon receptor binding domain (AHRB). Black arrows are non-synonymous single nucleotide polymorphisms (SNPs), and grey arrows are synonymous SNPs identified in this study and as reported in the National Center for Biotechnology Information (NCBI) dbSNP database.

mutations identified in the *AIP* gene that have been associated with familial or sporadic pituitary adenomas.[15] These include nonsense, insertion, and deletion mutations, as well as missense mutations not observed in the general population. Functional assays designed to study the impact of many of these mutations suggest that AIP acts as a tumor suppressor in most tissues, although dysfunctional AIP has been shown to affect pituitary development and function only.[15,16]

The presence of single amino acid polymorphisms at key functional domains in the AIP protein could affect AHR protein integrity and/or signaling. In the normal population, genetic variants of human AHR were hypothesized to potentially sensitize carriers to the toxicities associated with exposures to DLCs.[17] Although several AHR alleles have been detected within the human population,[18] functional studies of these polymorphisms have failed to support this idea.[19,20] An extension of this hypothesis is that polymorphisms in genes that code for proteins associated with the AHR pathway could potentially confer dioxin susceptibility. Available data indicate that at least 11 single nucleotide polymorphisms (SNPs) have been identified in the human *AIP* coding sequence (**Fig. 1**), although the population frequencies of only five of these SNPs had been characterized prior to the current study.

The objective of the current investigation was to survey a subset of individual human DNA samples for novel AIP SNPs. DNA was obtained from immortalized human lymphocytes derived from six ethnic populations and sequenced in an effort to further identify SNPs within the open reading frame of the *AIP* locus. An in silico analysis was conducted to estimate the impact of novel SNPs on regulation of splice variation. The identification of polymorphisms is an important step in characterizing the potential sensitivity variation, particularly when placed in the context of evaluating human health risk associated with exposure to DLCs.

Methods

The methods used in this study have been described previously.[18] Briefly, 103 human genomic DNA samples from immortalized lymphoblast cell lines were obtained from the Coriell Cell Repositories (Camden, New Jersey, USA). These DNA samples originated from 29 Caucasians, 26 African-Americans, 18 Chinese, 13 Japanese, 10 Mexicans, and 7 Southeast Asians (not of Japanese or Chinese origin). The DNA samples were dissolved and stored in water and kept in the freezer until analysis. Sample concentrations were approximately 25 ng/µl, and 1 µl of each sample was used for the PCR reaction.

SNP discovery within exons of the human *AIP* locus was performed at Functional Biosciences, Inc. (Madison, Wisconsin, USA). The following sequences obtained from GenBank were used as reference sequences of AIP: NT_167190.1 (genomic), NM_003977.2 (mRNA). Each *AIP* exon from each DNA sample was amplified using PCR. PCR primers for each exon are listed in **Table 1** (synthesized at Integrated DNA Technologies; 1 µl for each direction at primer concentrations of 10 pmol/µl for exons 1, 4, and 5, and 2 pmol/µl for exons 2, 3, and 6). The PCR reaction mixtures included 0.25 µl Epicentre Failsafe PCR Enzyme Mix and 12.5 µl Epicentre Failsafe PCR Premix H (Madison, Wisconsin, USA) for a total reaction mixture volume of 24.5 µl under the following conditions: (1) 95°C for 2 min, (2) 95°C for 30 s, (3) 60°C for 30 s, (4) 72°C for 1 min and 30 s, (5) go back to step 2 and repeat 34 times, and (6) terminated at 4°C. One negative control for each primer set was processed for each sample run. All samples were cycled on a MJ Tetrad Thermal Cycler (Bio-Rad Laboratories, California, USA). Each PCR reaction was purified using an exonuclease I/shrimp alkaline phosphatase (exo/SAP) master mix and protocol: 3 µl of exo/SAP mix [49 µl SAP (1 Unit/µl) and 36 µl 10× SAP buffer (Promega, Wisconsin,

Table 1. Primer sequences utilized for the analysis of human aryl hydrocarbon receptor-interacting protein

Exon	PCR primers		Sequencing primers	
	Direction	Sequence	Direction	Sequence
Exon 1	F	GCCGCAGTCCCAATCAATTC	F	GCCCTTCCTCCTTGACAGG
	R	GTTCTCCGGCTTTGCACGTC	R	AAACCCAGATACCCGAGGAC
Exon 2*	F	*TGTAAAACGACGGCCAGT*TGGCTTGCTTTCTTCCTTACTCCC	F	CTCTTGCCCTGCTGTTTCC
	R	*CAGGAAACAGCTATGACC*GCATCATGGCGGGTGTTTGT	R	CGGGTGGCAGTCTAGCAGAGG
Exon 3*	F	*TGTAAAACGACGGCCAGT*TGGGCCTGTAAGGACCAGGG	F	TGGTAGGCATGTGTCTGTGG
	R	*CAGGAAACAGCTATGACC*TCAGCAGCAGACCTGACCCA	R	CAGACATCACACACCAGCAG
Exon 4–5	F	CGCACCTTGAAGTCCCTTG		
	R	CTTGCCCCGCTTGAAGTAG		
Exon 4			F1	GGCTTTTCACCGTCTTGTTC
			F2	GCTCTGCTGCTGGTGTGTGATG
			R	TAGGCCTTGACGTTGTCTGC
Exon 5			F1	GGCTTTTCACCGTCTTGTTC
			F2	GATGCCATTGCCTGCCTCAA
			R	TAGGCCTTGACGTTGTCTGC
Exon 6*	F	*TGTAAAACGACGGCCAGT*TGGCATCCTCAGGTCAGGGA	F	GGGCTCTCTCTCCCCTGTG
	R	*CAGGAAACAGCTATGACC*ACAGCCACCCAAGTACCAGGAA	R	CACCCAAGTACCAGGAATGC

F, forward; R, reverse.
*Italicized bases are m13 tails, which were not used for sequencing. Initially PCR primers for all exons were designed with m13 tails; however, the initial PCR primers for exons 1, 4, and 5 did not meet optimization standards, so they were redesigned without the tails.

USA); 25 µl exonuclease I (20 Units/µl; Epicentre Biotechnologies, Wisconsin, USA); 216 µl Nanopure water] were introduced to 10 µl PCR reaction product, and then cycled at (1) 37°C for 30 min, and (2) 85°C for 15 min. Purified PCR products were then sequenced using standard BigDye Terminator v3.1 cycle sequencing reagents and protocol (Applied Biosciences, California, USA).

The detection and sequencing of SNPs was conducted using Mutation Surveyor Software v. 3.10 (SoftGenetics, Pennsylvania, USA) and was confirmed by opposite strand sequencing. Results were compared with the human AIP SNPs documented in the National Center for Biotechnology Information (NCBI) dbSNP database: http://www.ncbi.nlm.nih.gov/SNP. Those SNPs not listed in the NCBI dbSNP for the *AIP* locus were characterized as novel. In an effort to thoroughly interpret the current study findings, SNPs that were previously reported in the NCBI dbSNP database, but not identified in the current dataset, were also discussed. The SNP detection data for each sample was used to calculate study-specific SNP genomic and allelic frequencies.

In addition, an in silico analysis was conducted to predict the impact of novel SNPs on the regulation of the AIP gene. The online bioinformatics tools FastSNP and Human Splicing Finder (HSF) were utilized based on their capacity to evaluate novel SNPs.[21] FastSNP is an integrated server (http://FASTSNP.ibms.sinica.edu.tw) that follows the decision tree principle with external Web service access to ESEfinder, RESCUE-ESE, and FAS-ESS, which predict whether a coding mutation alters exonic splice enhancer (ESE) or silencer (ESS) sites, respectively, of a gene.[22] A risk score of 0–5 is given to reflect the predicted impact on protein structure and function, with 0 signifying no effect, and 5 corresponding to very high effect. HSF (http://www.umd.be/HSF/) calculates consensus values for potential variant-affected splice sites.[23] Consensus value variation in canonical splice-site use and potential activation of cryptic splice-sites in the presence of mutations was evaluated by analysis of the novel SNP-containing exon sequences using default settings.

Results

This analysis identified eight unique SNPs at the human *AIP* locus among the 103 samples evaluated, three of which were novel (*i.e.*, were not listed in the NCBI dbSNP database) (**Table 2**). Most of the SNPs were synonymous; only two of the eight SNPs detected in the current study were non-synonymous. Prior to this study, there were 11 known AIP SNPs, six of which were not detected in the DNA samples examined in the current study. The current analysis identified SNPs in all but the 1st and 3rd exons. The eight SNPs identified in exons 2, 4, 5, and 6 are discussed below in the context of known AIP protein domains or regions. Of the 103 human samples evaluated, 42 had at least one SNP, 12 had two SNPs, and one sample had three SNPs.

Table 2. Summary of aryl hydrocarbon receptor-interacting protein variations reported in the NCBI dbSNP and the frequency of SNPs found in this study (including three novel variations)

Domain	Exon	dbSNP ID	SNP AA	Minor allele frequency of SNP in this study
PPI	1	rs79662690	G12G	NI
PPI	1	rs116940576	G23E	NI
PPI	2	rs11822907	D44D	0.03
PPI	2	novel	T48T	0.01
PPI	2	rs1063385	V49L	NI
-	4	rs2276020	D172D	0.11
TPR 1	4	novel	L212L	0.01
TPR 1	4	rs3210041	M214L	NI
-	5	rs641081	Q228K	0.13
TPR 3	6	rs61741147	A276V	0.005
TPR 3	6	rs35665586	A297A	0.005
C-terminal α-helix	6	novel	V302V	0.005
C-terminal α-helix	6	rs4930199	Q307R	NI
C-terminal AHR binding	6	rs1049565	I327I	NI

NI, not identified; PPI, peptidyl-prolyl cis-trans isomerase FKBP-type; SNP, single nucleotide polymorphism; TPR, tetratricopeptide repeat; -, SNP between defined domains.

Peptidyl-prolyl cis-trans isomerase FKBP-type (PPI) domain (exon 2): Two SNPs were identified in exon 2 of the PPI domain, both of which were synonymous (**Table 3**). The polymorphism 132C>T (D44D) was the first SNP detected sequentially, and occurred at a low allelic frequency of 3% in the current population, similar to that reported in the NCBI dbSNP. The frequency in the current population was based on the heterozygous presence of this allele in six African-American individuals. The second SNP, 144C>T (T48T), was novel and rare (allelic frequency of 1%), having a heterogeneous presence in only two individuals, both of whom were Chinese. In addition, there have been three other SNPs identified in the PPI domain that were not detected in the current population: 36G>A (G12G), 68G>A (G23E), and 145G>T (V49L). However, this is not surprising considering that all are likely to have low allelic frequencies. In fact, of the three, only one (G12G) has been validated in a human population (allelic frequency of 1%); to date, there are no frequency data available on the two non-synonymous SNPs as reported in the NCBI dbSNP.

PPI and the first tetratricopeptide repeat (TPR 1) inter-domain (exon 4): The next SNP detected was 516C>T (D172D), located between the PPI and the first TPR domains. **Table 4** illustrates that this synonymous SNP had a moderate allelic frequency of 11% and was found

Table 3. Genotype and allele frequencies of SNPs in the PPI domain of the human *AIP* gene observed in 103 human samples

PPI domain

Exon 2: rs11822907: D44D

GCCACGTTCCACTACCGGACGCTGCACAGTGA **C/T**
ACGAGGGCACCGTGCTGGACGACAGCCGGGCTCGTGGCAAGCCCATG-
GAGCTCATCATTGGCAAGAAGTTCAAGCTGCCTGTGTGGGAGAC-
CATCGTGTGCACCATGCGAGAAGGGGAGATTGCCCAGT-
TCCTCTGTGACATCAAG

Group	People	C/C	C/T	T/T
Caucasian	29	1	-	-
African-American	26	0.769	0.231	-
Japanese	13	1	-	-
Mexican	10	1	-	-
Southeast Asian	7	1	-	-
Chinese	18	1	-	-
Totals	103	0.942	0.058	-

Exon 2: novel: T48T

GCCACGTTCCACTACCGGACGCTGCACAGTGACGACGAGGGCAC **C/T**
GTGCTGGACGACAGCCGGGCTCGTGGCAAGCCCATGGAGCTCATCAT-
TGGCAAGAAGTTCAAGCTGCCTGTGTGGGAGACCATCGTGTGCAC-
CATGCGAGAAGGGGAGATTGCCCAGTTCCTCTGTGACATCAAG

Group	People	C/C	C/T	T/T
Caucasian	29	1	-	-
African-American	26	1	-	-
Japanese	13	1	-	-
Mexican	10	1	-	-
Southeast Asian	7	1	-	-
Chinese	18	0.899	0.111	-
Totals	103	0.981	0.019	-

Table 4. Genotype and allele frequencies for rs2276020 SNP between the PPI and TRP 1 domains of the human *AIP* gene observed in 103 human samples

Between PPI and TPR 1 domains

Exon 4: rs2276020: D172D

GTGGAGAGCCCTGGCACGTACCAGCAGGACCCATGGGCCATGACAGA **C/T**
GAAGAGAAGGCAAAGGCAGTGCCACTTATCCACCAGGAGGGCAACCG-
GTTGTACCGCGAGGGGCATGTGAAGGAGGCTGCTGCCAAGTAC-
TACGATGCCATTGCCTGCCTCAAGAACCTGCAGATGAAG

Group	People	T/T	T/C	C/C
Caucasian	29	-	-	1
African-American	26	-	0.154	0.846
Japanese	13	-	0.385	0.615
Mexican	10	-	0.1	0.9
Southeast Asian	7	-	0.286	0.714
Chinese	18	-	0.556	0.444
Totals	103	-	0.214	0.786

Table 5. Genotype and allele frequencies for a novel SNP in the TRP 1 domain of the human *AIP* gene observed in 103 human samples

TRP 1 domain				
Exon 4: novel: L212L				
GTGGAGAGCCCTGGCACGTACCAGCAGGACCCATGGGCCATGACAGAC-GAAGAGAAGGCAAAGGCAGTGCCACTTATCCACCAGGAGGGCAACCG-GTTGTACCGCGAGGGGCATGTGAAGGAGGCTGCTGCCAAGTACT-ACGATGCCATTGCCTGCCTCAAGAAC **C/T** TGCAGATGAAG				
Group	People	C/C	C/T	T/T
Caucasian	29	1	-	-
African-American	26	1	-	-
Japanese	13	1	-	-
Mexican	10	1	-	-
Southeast Asian	7	1	-	-
Chinese	18	0.889	0.111	-
Totals	103	0.981	0.019	-

Table 6. Genotype and allele frequencies for rs641081 SNP between the TRP 1 and TRP 2 domains of the human *AIP* gene observed in 103 human samples

Between TPR 1 and TPR 2 domains				
Exon 5: rs641081: Q228K				
GAACAGCCTGGGTCCCCTGAATGGATCCAGCTGGAC **C/A** AGCAGATCACGCCGCTGCTGCTCAACTACTGCCAGTGCAAGCTGGTG-GTCGAGGAGTACTACGAGGTGCTGGACCACTGCTC-TTCCATCCTCAACAAGTACGACG				
Group	People	C/C	C/A	A/A
Caucasian	29	-	-	1
African-American	26	0.192	0.538	0.269
Japanese	13	-	-	1
Mexican	10	-	0.2	0.8
Southeast Asian	7	-	-	1
Chinese	18	-	-	1
Totals	103	0.049	0.155	0.796

in all ethnicities studied except for Caucasians. This allele was found in a heterogeneous state only, and was most prominent among the Chinese, Japanese, and Southeast Asian subpopulations. These data agree with prior reports for this SNP (rs2276020); in fact, there is a very large Japanese study of approximately 750 individuals registered in the NCBI dbSNP that reported an allelic frequency for this SNP that was very similar to that observed in the current Japanese subgroup.

TPR 1 domain (exon 4): The second novel SNP, 634C>T (L212L), was discovered within the 1st TRP domain (**Table 5**). This was another cytosine-to-thymine allele that had no impact on the leucine amino acid at this position. As with the first novel SNP, this also had a low allelic frequency of 1%; it also had a heterogeneous presence in two Chinese individuals, although these were not the same two individuals that harbored the novel T48T SNP. An additional non-synonymous SNP located in the TPR 1 domain, 640A>C (M214L), was reported previously in the NCBI dbSNP but was not detected in the current population.

TPR 1 and TPR 2 inter-domain (exon 5): A non-synonymous polymorphism was identified between the first and second TPR domains (**Table 6**). The 682C>A (Q228K) SNP was present at a moderate allelic frequency of 13%. This SNP was observed at a high genetic frequency in African-Americans and was also detected in two Mexican individuals in this study. The NCBI dbSNP indicated that this non-synonymous SNP (rs641081) is fairly common since it has been previously reported in a number of other population studies, including Asian subgroups.

TPR 3 domain (exon 6): Two SNPs were identified within the 3rd TPR domain of the *AIP* locus (**Table 7**). The first, 827C>T (A276V), was a very minor allele, being discovered in only one African-American individual and with an allelic frequency of 0.5%. Although this missense SNP sequence was just recently registered with the NCBI dbSNP (rs61741147), this is the first study to report an actual population frequency, providing evidence that the A276V SNP is in fact present in the human population, albeit at a very minor frequency. The second SNP identified in this domain was 891A>C (A297A), another rare polymorphism previously reported in the NCBI dbSNP (rs35665586) with a similarly low allelic frequency (only one African-American individual harboring the heterogeneous allele in both the current study and previous report). Though found within the same ethnic group, both SNPs were identified in different individuals, both of whom also carried the Q228K SNP.

C-terminal α-helix domain (exon 6): Only one SNP was identified in the C-terminal alpha helix (**Table 8**), a novel synonymous polymorphism (906G>A, V302V) found in a single sample from a Caucasian individual. Interestingly, this was the only SNP detected among the Caucasian subpopulation sampled in this study. Again, this heterozygous polymorphism was rare, having an allelic frequency of only 0.5%. Two other SNPs had been previously reported in NCBI dbSNP within the C-terminal domain—920A>G (Q307R) and 981C>T (I327I)—but neither were detected in the current study, nor have they been reported in any other human population to date. In addition, it is important to note that there has yet to be a non-synonymous SNP identified in the last five amino acids of the C-terminal domain, which is known to be the AHR-binding region of AIP.[1)]

In silico evaluation of the impact of novel SNPs on alternative splicing: The potential impact that the three novel SNPs might have on elements that regulate

Table 7. Genotype and allele frequencies of SNPs in the TRP 3 domain of the human *AIP* gene observed in 103 human samples

TRP 3 domain

Exon 6: rs61741147: A276V

ACAACGTCAAGGCCTACTTCAAGCGGGGCAAGGCCCACG **C/T**
GGCCGTGTGGAATGCCCAGGAGGCCCAGGCTGACTTTGCCAAAGTG-
CTGGAGCTGGACCCAGCCCTGGCGCCTGTGGTGAGCCGAGAGCTG-
CAGGCCCTGGAGGCACGGATCCGGCAGAAGGACGAAGAGGA-
CAAAGCCCGGTTCCGGGGGATCTTCTCCCATTGACAGGAG-
CACTTGGCCCTGCCTTACCTGCCAAGCCCACTGCTGCA-
GCTGCCAGCCCCCCTGCCCGTGCTGCGTCATGCTT-
CTGTGTATATAAAGGCCTTTATTTATCTCTC

Group	People	C/C	C/T	T/T
Caucasian	29	1	-	-
African-American	26	0.962	0.038	-
Japanese	13	1	-	-
Mexican	10	1	-	-
Southeast Asian	7	1	-	-
Chinese	18	1	-	-
Totals	103	0.99	0.01	-

Exon 6: rs35665586: A297A

ACAACGTCAAGGCCTACTTCAAGCGGGGCAAGGCCCACGCGGCCGT-
GTGGAATGCCCAGGAGGCCCAGGCTGACTTTGCCAA-
AGTGCTGGAGCTGGACCCAGC **C/A**
CTGGCGCCTGTGGTGAGCCGAGAGCTGCAGGCCCTGGAGGCACGGAT-
CCGGCAGAAGGACGAAGAGGACAAAGCCCGGTTCCGGGGGATCTT-
CTCCCATTGACAGGAGCACTTGGCCCTGCCTTACCTGCCAAGC-
CCACTGCTGCAGCTGCCAGCCCCCCTGCCCGTGCTGCGT-
CATGCTTCTGTGTATATAAAGGCCTTTATTTATCTCTC

Group	People	C/C	C/A	A/A
Caucasian	29	1	-	-
African-American	26	0.962	0.038	-
Japanese	13	1	-	-
Mexican	10	1	-	-
Southeast Asian	7	1	-	-
Chinese	18	1	-	-
Totals	103	0.99	0.01	-

alternative splicing was evaluated using the web-based FastSNP and HSF models. In general, the models predicted that the novel SNPs would have minimal impact on alternative splicing (**Table 9**). FastSNP did not attribute high risk scores to any of the three novel synonymous SNPs, although 144C>T (T48T) was predicted to have low to moderate effect on AIP as a result of diminished exonic enhancer motifs. As for affecting potential splicing sites, two of the three novel SNPs were situated within HSF-predicted splice sequence motifs: 634C>T (L212L) and 906G>A (V302V). Relative to the wildtype allele, however, neither of the nucleotide changes were predicted to have an effect on the potential splice sites—nor to generate new splice sites—as evidenced by the minute variation in consensus values.

Table 8. Genotype and allele frequencies for a novel SNP in the C-terminal α-helix domain of the human *AIP* gene observed in 103 human samples

C-Terminal alpha helix domain

Exon 6: novel: V302V

ACAACGTCAAGGCCTACTTCAAGCGGGGCAAGGCCCACGCGGCCGT-
GTGGAATGCCCAGGAGGCCCAGGCTGACTTTGCCAAAGTG-
CTGGAGCTGGACCCAGCCCTGGCGCCTGTGGT **G/A**
AGCCGAGAGCTGCAGGCCCTGGAGGCACGGATCCGGCAGAAGGACGAA-
GAGGACAAAGCCCGGTTCCGGGGGATCTTCTCCCATTGACAGGAG-
CACTTGGCCCTGCCTTACCTGCCAAGCCCACTGCTGCAGCT-
GCCAGCCCCCCTGCCCGTGCTGCGTCATGCTTCT-
GTGTATATAAAGGCCTTTATTTATCTCTC

Group	People	G/G	G/A	A/A
Caucasian	29	0.966	0.034	-
African-American	26	1	-	-
Japanese	13	1	-	-
Mexican	10	1	-	-
Southeast Asian	7	1	-	-
Chinese	18	1	-	-
Totals	103	0.99	0.01	-

Table 9. Summary of in silico analysis of novel AIP SNP impact on alternative splicing

SNP	AIP exon #	FastSNP		HSF potential splice sites					
		Level of risk†	Possible functional effect	Potential acceptor splice site(s)††	Relative nt position	CV variation (%)†††	Potential donor splice site(s)††	Relative nt position	CV variation (%)†††
144C>T (T48T)	2	Low–medium (2–3)	Splicing regulation	NI	-	-	NI	-	-
634C>T (L212L)	4	Very low (1)	Sense/synonymous	aagaac**t**tgcagAT	−6	−0.82	NI	-	-
906G>A (V302V)	6	Very low (1)	Sense/synonymous	gcctgtggt**a**agCC	−9	+1.33	GTGg**t**aagc	−5	+1.29
				tggt**a**agccgagAG	−4	−0.09			
				gt**a**agccgagagCT	−2	−0.17			

†Risk score determined by FastSNP decision tree analysis: 0 = no effect, 1 = very low effect, 2 = low effect, 3 = medium effect, 4 = high effect, 5 = very high effect.
††SNPs underscored and bolded in potential splice site sequences.
†††Consensus value variation from the wildtype.
NI, none identified: no potential splice sites were identified in this SNP region.

Discussion

The current genetic analysis identified eight exonic SNPs at the *AIP* locus in 103 human DNA samples, including three novel, but low-frequency polymorphisms. These novel SNPs are silent and therefore would not be expected to confer any direct functional effect on the protein product. A recent study predicted that two silent exonic AIP mutations identified in individuals diagnosed with familial isolated pituitary adenoma (FIPA) would result in splice variations, suggesting a possible mechanism whereby the silent polymorphisms identified in the general population might be responsible for functional variation indirectly.[24] None of the novel synonymous SNPs identified in the current study are located near the intron–exon–intron splice junctions, and therefore none would be expected to directly effect these important transcription sequences. However, an in silico evaluation was conducted to characterize the possible silent SNP effects on elements that regulate alternative splicing. The results indicate that these silent SNPs are unlikely to affect alternative splicing within the AIP transcript, although *in vitro* mRNA analysis will have to be performed to validate these predictions.

In the context of prior AIP SNP studies, there are now a total of 14 SNPs identified within the *AIP* locus. Of these, six result in amino acid substitutions and are therefore of potential functional importance, though only two of these (Q228K and A276V) were detected in the current study. The NCBI dbSNP lists four non-synonymous SNPs that were not detected in the current study: G23E, V49L, M214L, and Q307R. These amino acid substitutions are all located in functionally relevant domains within the AIP protein sequence and therefore suggest potential functional consequences (**Table 2**). Our understanding of the presence of these four SNPs in the population is uncertain, however, since no population frequency data have been reported for any of them to date.

AIP has been shown to play an important role in mammalian physiology and AHR-dependent pathways. Attempts by investigators to develop a viable AIP-null mouse line demonstrated that total loss of AIP at the whole animal level results in cardiovascular developmental issues that proved to be embryolethal.[11] Even an AIP hypomorphic mouse line, in which the AIP expression was severely depressed relative to wildtype, is characterized by a hepatocardiovascular deformity called ductus venosus.[12] More recently, however, investigators generated a mouse line wherein AIP expression was only depressed in hepatocytes.[25] It was observed in this mouse model that liver AHR expression was reduced by 60%. Most importantly, when these mice were administered dioxin, it was reported that loss of AIP protein either eliminated or reduced certain measures of hepatotoxicity (*e.g.*, increased serum ALT levels, severe hepatocellular hydropic degeneration, and hepatic focal inflammation).[25] These observations demonstrate the important role AIP plays in AHR dioxin-mediated toxicity. Incidentally, although no studies on the effect of low AIP expression on dioxin sensitivity have been conducted to date, reduction of AIP expression in humans was recently associated with non-germline somatotropinomas.[26]

The AIP mouse model results also suggest that polymorphisms at the *AIP* locus that have some impact on protein function would more likely confer a certain degree of dioxin resistance rather than enhance sensitivity. Though no AHR pathway functional assays have been conducted with the non-synonymous AIP SNPs, several studies of human AIP mutants have shown that polymorphisms either have no functional consequence or decrease function. In a yeast two-hybrid β-galactosidase system, Leontiou *et al.* illustrated that the C238Y (TPR 2 domain) and R271W (TPR 3 domain) point mutations, observed in individuals with familial or sporadic pituitary adenomas, resulted in a loss of PDE4A5 interaction when compared to wildtype.[15] More recently, Igreja *et al.* made a similar loss of function observation for two additional mutations (K103R and K241E) expressed in familial isolated pituitary ademonas.[24] None of these four mutations have been observed in the general population. Several other AIP point mutations have been associated with these pituitary adenomas, although their functional consequence has either been minimal or yet to be examined.[14,24,27]

In conclusion, this study identified three new *AIP* SNPs that are expressed at low frequencies in the human population, and although in silico analyses results suggest that they are not expected to affect transcriptional splicing or to have any other functional impact, these synonymous SNPs potentially can serve as useful genetic markers for future studies. Additionally, this study is the first to observe the non-synonymous SNP A276V in a human population, though it appears at a very low frequency. In contrast, the other non-synonymous AIP SNP identified in this study (Q228K) has been reported in a number of prior population genetic studies and occurs at a moderate allelic frequency in the human population. Both new non-synonymous SNPs may have potential functional relevance since they are located within a region of AIP that is known to be responsible for AIP-HSP90 protein-protein binding, an interaction important to the maintenance of AHR protein integrity and cellular localization. There have been no non-synonymous AIP SNPs reported in the last five amino acids at the tail end of the C-terminal α-helix (amino acids 326–330), the region responsible for AIP-AHR binding.[1] Given what is known about the influence that AIP has over AHR expression and mediation of dioxin toxicity, however, it does not seem plausible that any non-synonymous AIP SNP identified to date is apt to increase human sensitivity to dioxin-associated toxicities; if anything, it is more likely that these AIP SNPs would decrease dioxin sensitivity, given the evidence that single point mutations in this region disrupt the protein-protein interactions necessary to avoid AHR protein

degradation. Further investigation into the functional consequences of these AIP SNPs on AHR signaling is warranted.

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