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Gender influences monoallelic expression of *ATP10A* in human brain

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Abstract

Human chromosome 15q11-13 and the syntenic region of mouse chromosome 7 contain multiple imprinted genes necessary for proper neurodevelopment. Due to imprinting, paternal 15q11-13 deficiencies lead to Prader-Willi syndrome (PWS) while maternal 15q11-13 deficiencies cause Angelman syndrome (AS). The mechanisms involved in parental imprinting of this locus are conserved between human and mouse, yet inconsistencies exist in reports of imprinting of the maternally expressed gene *Atp10a/ATP10A*. Excess maternal 15q11-13 dosage often leads to autism-spectrum disorder therefore further investigation to characterize the true imprinting status of *ATP10A* in humans was warranted. In this study, we examined allelic expression of *ATP10A* transcript in 16 control brain samples, and found that 10/16 exhibited biallelic expression while only 6/16 showed monoallelic expression. Contrary to the expectation for a maternally expressed imprinted gene, quantitative RT-PCR revealed significantly reduced *ATP10A* transcript in Prader-Willi syndrome brains with two maternal chromosomes due to uniparental disomy (PWS UPD). Furthermore, a PWS UPD brain sample with monoallelic *ATP10A* expression demonstrated that monoallelic expression can be independent of imprinting. Investigation of factors that may influence allelic *ATP10A* expression status revealed that gender has a major affect, as females were significantly more likely to have monoallelic *ATP10A* expression than males. Regulatory sequences were also examined, and a promoter polymorphism that disrupts binding of the transcription factor Sp1 also potentially contributes to allelic expression differences in females. Our results show that monoallelic expression of human *ATP10A* is variable in the population and is influenced by both gender and common genetic variation.

Introduction

Human chromosome 15q11-13 contains a cluster of imprinted genes implicated in multiple neurodevelopmental disorders. Due to the parent-of-origin specific gene expression of this locus, paternal deficiencies of 15q11-13 cause Prader-Willi syndrome (PWS), while maternal deficiencies cause Angelman syndrome (AS) (Knoll et al. 1989). PWS is characterized by hypotonia at birth followed by hyperphagia induced obesity later in life, and mild cognitive impairments with obsessive compulsive behaviors (Bittel and Butler 2005). Contrastingly, AS is characterized by severe neurologic impairments including profound mental retardation, lack of speech, seizures, ataxia, and unprovoked laughter (Cassidy et al. 2000). Duplications of 15q11-13 often lead to the diagnosis of autism-spectrum disorder (ASD), and are also subject to effects of imprinting, as maternally derived 15q11-13 duplications specifically cause the ASD phenotype (Cook et al. 1997). Rett syndrome (RTT), an autism-spectrum disorder caused by mutations in *MECP2* (Methyl CpG binding protein 2) (Amir et al. 1999) also has been

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linked to 15q11-13, as MeCP2 binds throughout the locus (Yasui et al. 2007) and expression of some 15q11-13 genes is altered in RTT brain (Hogart et al. 2007; Samaco et al. 2005).

The cluster of imprinted genes present on human chromosome 15q11-13 and the syntenic region of mouse chromosome 7C have conserved genomic structure and regulation (Nicholls and Knepper 2001). Rare microdeletions mapped in patients with PWS and AS revealed that imprinting throughout the locus is regulated by a bipartite imprinting center consisting of a 4 kb region in the 5' end of *SNRPN* (PWS imprinting control region) and an 880 bp region (AS imprinting control region) 35 kb upstream of *SNRPN* (Nicholls and Knepper 2001). Differential DNA methylation is associated with maintenance of the maternal imprint in paternally expressed genes. While imprinting and differential methylation of the paternally expressed genes appears to be ubiquitous, imprinting of the maternally expressed gene *UBE3A* is restricted to brain in both human and mouse (Albrecht et al. 1997; Rougeulle et al. 1997; Vu and Hoffman 1997). The mechanism involved in paternal *UBE3A* silencing is enigmatic, however, some evidence has pointed to a role for a neuron-specific *UBE3A* antisense transcript that is regulated by promoter sequences at the imprinting center (Landers et al. 2004; Runte et al. 2004; Yamasaki et al. 2003).

In addition to *UBE3A*, a second maternally expressed imprinted gene, *ATP10A*, has been identified within 15q11-13. While one report found that *ATP10A* exhibits imprinted expression similar to *UBE3A*, with biallelic expression in non-neuronal tissues (Herzing et al. 2001b), another study reported imprinted expression of *ATP10A* in lymphoblasts as well as brain (Meguro et al. 2001). Imprinting status of the mouse ortholog of *ATP10A* has been disputed—with one report that *Atp10a* is paternally imprinted in hippocampus and olfactory bulb (Kashiwagi et al. 2003), and a different study finding that *Atp10a* is biallelically expressed in all tissues (Kayashima et al. 2003). Given the conflicting evidence for the imprinting status of mouse *Atp10a*, and the apparent discrepancy in human *ATP10A* tissue-specific imprinting, we sought to further characterize the imprinting of *ATP10A* in human brain to better understand the potential role of this gene in multiple neurodevelopmental disorders.

Materials and methods

Genotyping and allelic expression

Post-mortem cerebral cortex samples (Brodmann Area 9) were obtained from the NICHD Brain and Tissue Bank for Neurodevelopmental Disorders. DNA and RNA isolation were performed as previously described (Hogart et al. 2007). Single nucleotide polymorphisms (SNPs) were identified within exons of *ATP10A* using the UC Santa Cruz Genome Browser (<http://genome.ucsc.edu>) and the NCBI SNP database (<http://www.ncbi.nlm.nih.gov/SNP/>). Polymorphisms were screened by NEBcutter (www.neb.com) to find SNPs that disrupted restriction enzyme recognition sequences. Three SNPs within *ATP10A* exon 18, rs2076741, rs2078743, and rs2076744, are differentially cleaved by *AclI*, *Hpy99I*, and *AclI*, respectively. Approximately 10 ng of brain gDNA was PCR amplified using standard PCR conditions and primers flanking *ATP10A* exon 18 (Primers listed in Supplementary Table 1). PCR products were digested using an excess of restriction enzyme for 3 hours. RT-PCR amplification of *ATP10A* cDNA in heterozygous individuals followed previously described methods (Hogart et al. 2007). Restriction enzyme digestion of cDNA products was used to assess allelic expression. Band intensities were quantitatively measured with AlphaEaseFC™ version 4.0 software and only individuals with a single expressed allele were scored as monoallelic. Sequencing of *ATP10A* cDNA was used to verify allelic expression results. Quantitative RT-PCR of PWS, AS, and control *ATP10A* brain cDNA was performed according to previously described methods (Hogart et al. 2007). Briefly, crossing point values for *ATP10A* transcript were normalized to the housekeeping gene *GAPDH* using the comparative CT method (Applied

Biosystems). Significant differences in *ATP10A* expression in PWS and AS samples compared to control values were evaluated using the Student's t test.

Analysis of regulatory sequences

Overlapping primers were designed to amplify 2.1kb of the 5' end of *ATP10A* with the strongest degree of conservation according to the UCSC Genome Browser. Genomic brain DNA from five individuals with monoallelic *ATP10A* expression (510, 1027, 1136, 1275, 1321) and five with biallelic *ATP10A* expression (22, 103, 285, 1029, 4192) was PCR amplified. DNA sequencing was performed by the UC Davis Division of Biological Sciences Sequencing Facility. Sequencing chromatograms were compared to the human genome reference sequence (March 2006 assembly) to identify rare or novel sequence variants. All characterized SNPs in the dbSNP build 128 (<http://www.ncbi.nlm.nih.gov/SNP/>) within the 2.1 kb region were analyzed. Allele frequencies were calculated within our population by dividing the number of occurrences of each SNP by the total number of alleles for each polymorphism. Transcription factor binding sites were analyzed with the Transcription Element Search System (TESS) TRANSFAC v6.0 database available online (<http://www.cbil.upenn.edu/cgi-bin/tess/tess>). Additional sequencing was performed to genotype two intronic SNPs (rs4906777 and rs4566129) that potentially disrupt characterized MeCP2 binding sites (Yasui et al. 2007). Fisher's exact test was used to determine if regulatory and coding SNPs were significantly overrepresented in samples with monoallelic or biallelic *ATP10A* expression.

Bisulfite Sequencing

Bisulfite sequencing was used to analyze DNA methylation of *ATP10A* in human brain. First, 1 µg of genomic DNA was converted with the EZ DNA Methylation-Gold kit (Zymo) according to the manufacturer's protocol. Primers amplifying the 5' CpG island of *ATP10A* and the intronic MeCP2 binding site (Figure 3a) were designed using MethPrimer (www.urogene.org/methprimer/index1.htm) (Primers listed in Supplementary Table 1). PCR amplification was performed for 38-40 cycles with standard reaction conditions and approximately 25 ng of bisulfite converted genomic DNA. PCR products were cloned and sequenced according to previously described methods (Hogart et al. 2007).

Results

As *ATP10A* is proposed to exhibit neuron-specific imprinted expression similar to *UBE3A*, we performed allelic expression analysis of *ATP10A* in a panel of control cerebral cortex samples. Individuals analyzed in our study included a wide range of ages (1 day to 46 years old), various ethnic backgrounds, and a relatively equal proportion of males and females (Table 1). Genomic DNA from control brain samples was genotyped for polymorphisms in *ATP10A* exons. cDNA was synthesized from heterozygous individuals, and quality was verified by RT-PCR amplification of the housekeeping gene *GAPDH*. Restriction enzyme digestion and sequencing performed on *ATP10A* cDNA revealed that 37.5% (6/16) of control brain samples exhibited monoallelic expression of *ATP10A* while 62.5% (10/16) of control samples showed biallelic expression (Table 1 and Figure 1a and 1b). Allele frequencies of SNPs used to detect allelic expression were similar between individuals with monoallelic *ATP10A* expression and individuals with biallelic *ATP10A* expression (Table 2). Additionally, exon haplotypes that were transcriptionally silent in some individuals were expressed in other individuals, suggesting that choice of allelic expression is independent of tightly linked genetic factors (Table 2).

Quantitative RT-PCR analysis of AS and PWS cerebral cortex was used to investigate *ATP10A* expression from maternal versus paternal chromosomes independently. AS samples with a maternal 15q11-13 deletion (AS del) had significantly reduced *ATP10A* transcript

compared to controls, as expected. Prader-Willi deletion (PWS del) brain samples had reduced transcript but were not significantly different than controls (Figure 1b), similar to observed expression patterns for *UBE3A* (Hogart et al. 2007). Surprisingly, Prader-Willi syndrome maternal UPD (PWS UPD) samples had significantly reduced *ATP10A* transcript (Figure 1b) unlike the significantly elevated *UBE3A* levels observed in the previous report (Hogart et al. 2007). Rett syndrome (RTT) brain samples showed a trend towards reduced *ATP10A* transcript that was not significantly different from controls, again differing from previously published results for *UBE3A* (Hogart et al. 2007; Samaco et al. 2005).

To investigate potential explanations for the unexpected *ATP10A* expression deficiencies in PWS UPD samples, we performed allelic expression analysis of *ATP10A* cDNA in both PWS samples. Consistent with reports that the majority of PWS maternal UPD cases are heterodisomic due to meiosis I nondisjunction (Fridman and Koiffmann 2000), both PWS UPD samples were heterozygous for coding SNPs within *ATP10A* (Figure 2). Sequencing chromatograms of *ATP10A* cDNA revealed that both maternal alleles were equally expressed in PWS 1447. In striking contrast, PWS 1290 expressed only one maternal *ATP10A* allele (Figure 2). Monoallelic expression of *ATP10A* in an individual with maternal disomy together with biallelic *ATP10A* expression in the majority of controls implicate factors other than parental origin in determining monoallelic expression of *ATP10A*.

To address the question of whether *ATP10A* monoallelic expression is due to polymorphisms in regulatory regions, we sequenced 2.1 kb of the promoter in control individuals to look for promoter variants that may correlate with monoallelic expression of *ATP10A*. While no novel variants were identified through sequencing, three characterized SNPs were observed. Allele frequencies for promoter SNPs rs2076748, rs4906796, and rs2076749 are shown in Table 3. Allele frequencies for rs4906796 and rs2076749 were similar between individuals with monoallelic and biallelic *ATP10A* expression, however the G allele of rs2076748 was absent from samples with monoallelic expression (Table 3). Interestingly, the A allele disrupts an Sp1 binding site while the ancestral G allele preserves the consensus sequence for this ubiquitous transcription factor. This single promoter polymorphism does not completely explain the variability in monoallelic expression, as some individuals with biallelic *ATP10A* expression were also homozygous for the A allele. Interestingly, all four females homozygous for the A allele exhibited monoallelic expression.

In addition to the proximal promoter, we investigated polymorphisms in an intronic *ATP10A* sequence that was shown by chromatin immunoprecipitation in human cells to be a strong binding site for the neuronal epigenetic factor MeCP2 (Yasui et al. 2007). MeCP2 binds to methylated CpG dinucleotides (Meehan et al. 1992) and functions as a modulator of gene expression (Chahrour et al. 2008; Yasui et al. 2007), therefore we hypothesized that SNPs that disrupt CpG sites may affect allelic expression. Genotyping of polymorphisms that abolish CpG dinucleotides (rs4906777 and rs4566129) revealed that individuals with monoallelic expression showed a trend towards higher frequencies of SNPs that disrupt CpG sites (A allele for rs4906777 and T allele for rs4566129). In addition, all four males homozygous for the A allele of rs4906777 were biallelic for *ATP10A* transcript, while all three females homozygous for the A allele were monoallelic. While the allele distributions for SNPs rs4906777 and rs4566129 were not significantly different between individuals with monoallelic and biallelic *ATP10A* expression, the female PWS UPD sample with monoallelic expression lacked both polymorphic CpG sites (Figure 3c). Interestingly, ethnicity did not correlate with monoallelic expression (Table 1), however we found a significant correlation based on gender ($p = 0.0128$), with female samples being significantly overrepresented in the monoallelic expression category (Table 3). While we included the PWS UPD samples in our gender analysis to increase sample size, analysis of gender exclusively in control samples also yielded a significant

association ($p = 0.035$), with females significantly overrepresented in the monoallelic *ATP10A* category.

The correlation between gender and monoallelic expression led us to hypothesize that gender-specific epigenetic differences may contribute to allelic expression of *ATP10A*. Bisulfite sequencing was used to analyze DNA methylation of the *ATP10A* promoter CpG island in each brain sample (Figure 3). While overall *ATP10A* promoter methylation was low, the female PWS UPD sample (1290) with monoallelic expression exhibited one clone with elevated promoter methylation compared to the male PWS UPD sample (1447) (Figure 3b). While this evidence does not strongly support an epigenetic basis for the allelic expression differences, the presence of a highly methylated clone in the female PWS UPD sample does suggest that DNA methylation may contribute to the silencing of one maternal allele. Bisulfite sequencing confirmed that the MeCP2-bound sequence of the *ATP10A* intron including the polymorphic CpG sites was normally heavily methylated, and normal methylation levels were present in both PWS UPD brains (Figure 3c). Bisulfite sequencing was also used to analyze promoter methylation in control brain samples (Figure 4). All samples showed variable low levels of promoter methylation, ranging from 1-12% but individuals with monoallelic *ATP10A* expression (light grey bars) did not have increased promoter methylation compared to individuals with biallelic *ATP10A* expression (dark grey bars). Furthermore, no significant differences were observed between males and females for *ATP10A* promoter methylation. These results suggest that promoter methylation is not a general mechanism that accounts for variable monoallelic expression of *ATP10A*.

Discussion

Discrepancies in the imprinting status of the mouse *Atp10a* gene led us to question if human *ATP10A* imprinting was similarly variable. In our survey of *ATP10A* allelic expression in human control brain only a minority of individuals exhibited monoallelic *ATP10A* expression and allelic expression status appeared to be influenced by gender. Due to the anonymous nature of tissue donation we were unable to determine if the preferentially expressed allele in controls was always of maternal origin. Interestingly, monoallelic *ATP10A* expression in PWS UPD brain demonstrated that maternal alleles can be silenced and raises the possibility that *ATP10A* monoallelic expression is independent of parental chromosome origin.

Quantitative RT-PCR analysis of *ATP10A* transcript in PWS and AS brain samples also did not support a strict maternal basis for *ATP10A* expression. AS deletion samples with a single paternal 15q11-13 allele had significantly reduced *ATP10A* expression compared to controls, while PWS deletion samples were not significantly different from controls. This maternal chromosome bias in expression is in agreement with the original reports that concluded that *ATP10A* is a maternally expressed imprinted gene (Herzing et al. 2001a; Meguro et al. 2001). A parental chromosome bias in gene expression in PWS and AS brain was also observed for the neighboring gene *GABRB3*, although this gene is not imprinted (Hogart et al. 2007) demonstrating that this evidence alone is insufficient proof of imprinting. Contrasting to the elevated *ATP10A* in PWS UPD lymphoblasts reported by Bittel et al (Bittel et al. 2003), we observed significantly reduced *ATP10A* in PWS UPD brain compared to control brain. Tissue specific differences in *ATP10A* regulation, biological differences between different PWS UPD individuals, and technical differences between microarray expression profiling and qRT-PCR analysis may all contribute to the different observations between our reports. Consistent with Bittel et al (Bittel et al. 2003) we previously reported elevated *UBE3A* expression in the same PWS UPD brain samples (Hogart et al. 2007). Opposite directional changes in the two maternally expressed genes *ATP10A* and *UBE3A* in PWS UPD brain samples (Figure 1b and (Hogart et al. 2007) suggests that regulation of *ATP10A* is distinct from *UBE3A* imprinting in brain.

Unexpectedly, we found a significant association between gender and allelic expression status, with females being more likely than males to exhibit monoallelic *ATP10A* expression. Additionally, the A allele of the *ATP10A* promoter polymorphism rs2076748 disrupts a consensus binding site for the ubiquitous transcription factor Sp1, and may contribute to allelic expression differences between individuals. Interestingly the single male control sample with monoallelic *ATP10A* expression (1027) was homozygous for the A allele of the promoter variant rs2076748, and a female with biallelic expression was homozygous for the G allele of this variant, suggesting that gender combined with differences in genetic background influences monoallelic expression in the population. As *ATP10A* is physically located between the strictly biallelically expressed gene *GABRB3* and an actively imprinted gene *UBE3A*, we hypothesize that this gene is influenced by neighboring imprints, but unlike *GABRB3* (Hogart et al. 2007), *ATP10A* does not appear to have a strong overriding mechanism to maintain biallelic expression over monoallelic expression.

Recently a genome-wide study of allelic expression revealed that 5% of autosomal genes are subject to random monoallelic expression (Gimelbrant et al. 2007). Interestingly, although *ATP10A* was not mentioned in the study, a closely related gene, *ATP11A*, was found to be subject to random monoallelic expression. Consistent with our variable allelic expression results in control brain, analysis of *ATP10A* allelic expression in human embryonic stem cell lines found that 8 of 12 informative cell lines had biallelic *ATP10A* expression while only 4 of 12 had monoallelic expression (Kim et al. 2007). Strict monoallelic expression of other 15q11-13 imprinted genes, *SNRPN*, *NDN*, and *MAGEL2* in the stem cell lines and our observation of frequent biallelic expression of *ATP10A* in brain, favors the interpretation that the variability in *ATP10A* allelic expression in stem cells is due to normal differences between individuals.

ATP10A is a member of the P-type ATPase family of proteins that are involved in active transport of molecules across the phospholipid membrane (Flamant et al. 2003). Mice heterozygous for a deletion spanning *Atp10a* exhibit increased body fat and obesity specifically when the deletion is maternally inherited (Dhar et al. 2000). Wildtype, maternal deletion, and paternal deletion mice showed similar levels of *Atp10a* transcript, leading to the conclusion that the maternal-specific inheritance of the obesity phenotype was most likely due to maternal environment effects instead of imprinting (Dhar et al. 2000). Further characterization of the *Atp10a* maternal-deletion mice revealed gender differences in severity of the obesity-related phenotypes with females being more significantly affected than males (Dhar et al. 2004). In light of our observation of gender influencing monoallelic expression of *ATP10A* in humans, it is interesting to speculate that similar effects may be influencing *Atp10a* allelic expression in mice.

Multiple human disorders are caused by abnormal dosage of 15q11-13, therefore understanding how genes within this locus are expressed is critical to understanding the molecular pathogenesis related to abnormal 15q11-13 karyotypes. Genotype-phenotype analyses of PWS and AS patients have revealed that many genes throughout 15q11-13 contribute to the clinical manifestations of patients (Lossie et al. 2001; Torrado et al. 2007). Additionally, as maternally derived 15q11-13 duplications often lead to autism-spectrum disorder, the two maternally expressed imprinted genes *ATP10A* and *UBE3A* are prime candidate genes for the 15q11-13 associated autism phenotype. Further studies are required to determine if any behavioral consequences result from abnormal *ATP10A* expression. Our analysis of *ATP10A* imprinting reveals that this gene is subject to complex regulation that is influenced by gender as well as parental-origin and genetic factors. Given this result, dysregulation of *ATP10A* may occur in previously unexpected ways, and potentially contributes to some of the obesity-related complications in PWS and some individuals with 15q11-13 duplications (Dennis et al. 2006; Ungaro et al. 2001).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Analysis of *ATP10A* allelic expression in cerebral cortex. (a) Genomic DNA (upper panel) and cDNA (lower panel) from heterozygous control brain samples was digested with *AcII* to examine allelic expression. Upper band corresponds to uncut C allele and lower band corresponds to digested G allele. cDNA band quantification shown below the image reflects the percent that each allele contributes to the total densitometry. (b) Sequencing chromatograms of *ATP10A* cDNA confirm the allelic expression shown in (a). Arrows indicate the SNP differentially cleaved by *AcII*. (c) qRT-PCR analysis of *ATP10A* transcript in controls (n = 8), Angelman syndrome deletions (n = 2), Prader-Willi syndrome deletions (n = 2), Prader-Willi syndrome maternal uniparental disomy (n = 2), and Rett syndrome (n = 5). Error bars represent the S.E.M. for each category with significance values, * p < 0.02 and ** p < 0.001.

Figure 2.

Allelic expression analysis of *ATP10A* in PWS UPD brain. Sequencing chromatograms of *ATP10A* brain gDNA (upper panels) show that both PWS UPD individuals are heterozygous for polymorphisms within *ATP10A*. Sequencing of *ATP10A* cDNA (lower panels) reveals that PWS 1447 showed biallelic expression while PWS 1290 exhibited monoallelic expression of *ATP10A*.

Figure 3.

DNA methylation analysis of *ATP10A* regulatory sequences. (a) Schematic of *ATP10A* gene organization with vertical lines representing coding exons. Gray boxes indicate the relative locations of the 5' CpG island and the intronic MeCP2 binding site. (b) Bisulfite sequencing of the 5' CpG island in PWS 1290 and PWS 1447. Each horizontal line represents an individual clone, with filled circles representing methylated CpG sites and unfilled circles representing unmethylated CpG sites. (c) Bisulfite sequencing results from the MeCP2 binding site with polymorphic CpG sites indicated with stars (site 3 and site 7). A control sample heterozygous for polymorphisms in site 3 and site 7 shows that both sites are normally methylated. PWS 1290 lacks both polymorphic CpG sites while PWS 1447 lacks only site 3.

Figure 4.

ATP10A CpG island methylation in control brain. Bisulfite sequencing was used to assess methylation of the *ATP10A* 5' CpG island in control brain samples with monoallelic (light grey) and biallelic (dark grey) *ATP10A* expression. Percent methylation represents the number of methylated CpG sites divided by the total number of possible CpG sites for all clones analyzed (10-12 clones per individual).

Table 1
Summary of brain samples information and allelic expression status of *ATP10A*

Sample	Case #	Age	Ethnicity ^a	Gender ^b	<i>ATP10A</i> Expression
Control	1321	62 d	AA	F	monoallelic
Control	510	2 y	CA	F	monoallelic
Control	1275	2 y	AA	F	monoallelic
Control	1711	27 y	CA	F	monoallelic
Control	1027	22 y	CA	M	monoallelic
Control	1136	33 y	CA	F	monoallelic
Control	35	1 d	AA	M	biallelic
Control	1055	96 d	CA	M	biallelic
Control	22	113 d	CA	M	biallelic
Control	390	125 d	CA	F	biallelic
Control	103	2 y	AA	F	biallelic
Control	629	7 y	AA	M	biallelic
Control	1065	15 y	CA	M	biallelic
Control	1029	29 y	HP	M	biallelic
Control	285	30 y	AA	M	biallelic
Control	4192	46 y	CA	M	biallelic
PWS	1290	44y	CA	F	monoallelic
PWS	1447	45y	CA	M	biallelic

^a ethnicity identified by donor information, AA, African American, CA, Caucasian, HP, Hispanic

^b gender, M, male, F, female

Table 2

Allele frequencies of *ATP10A* exonic SNPs

	rs2076741 (G/C)	rs2078743 (T/C)	rs2076744 (G/A)	Haplotypes Expressed	Haplotypes Not Expressed
Monoallelic	G 0.92 (6) C 0.08	T 0.67 (6) C 0.33	G 0.8 (5) A 0.2	GCG, CTG, GTA, GTG	GTA, GTG, GCG
Biallelic	G 0.77 (11) C 0.23	T 0.73 (11) C 0.27	G 0.78 (9) A 0.22		
P value	0.389	0.714	1		

Numbers in parentheses indicate the number of individuals genotyped for each polymorphism
P values represent comparison of each SNP between monoallelic and biallelic *ATP10A* expression categories using Fisher's exact test.

Table 3

Allele frequencies of *ATP10A* regulatory polymorphisms

	Promoter SNPs		Intron SNPs			Gender (M/F)
	rs2076748 (A/G)	rs4906796 (C/G)	rs2076749 (T/A)	rs4906777 (A/G)	rs4566129 (T/C)	
Monoallelic	A 1 (5) G 0	C 0.8 (5) G 0.2	T 0.5 (3) A 0.5	A 0.83 (6) G 0.17	T 0.67 (6) C 0.33	M 0.14 (7) F 0.86
Biallelic	A 0.6 (5) G 0.4	C 0.7 (5) G 0.3	T 0.67 (4) A 0.33	A 0.59 (11) G 0.41	T 0.41(11) C 0.59	M 0.82 (11) F 0.18
P value	0.087	1	0.58	0.252	0.282	0.013

Numbers in parentheses indicate the number of individuals genotyped for each polymorphism
P values represent comparison of each SNP or gender between monoallelic and biallelic *ATP10A* expression categories using Fisher's exact test.