

Report

The Human Aminophospholipid-Transporting ATPase Gene *ATP10C* Maps Adjacent to *UBE3A* and Exhibits Similar Imprinted Expression

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Maternal duplications of the imprinted 15q11-13 domain result in an estimated 1%–2% of autism-spectrum disorders, and linkage to autism has been identified within 15q12-13. *UBE3A*, the Angelman syndrome gene, has, to date, been the only maternally expressed, imprinted gene identified within this region, but mutations have not been found in autistic patients. Here we describe the characterization of *ATP10C*, a new human imprinted gene, which encodes a putative protein homologous to the mouse aminophospholipid-transporting ATPase *Atp10c*. *ATP10C* maps within 200 kb distal to *UBE3A* and, like *UBE3A*, also demonstrates imprinted, preferential maternal expression in human brain. The location and imprinted expression of *ATP10C* thus make it a candidate for chromosome 15-associated autism and suggest that it may contribute to the Angelman syndrome phenotype.

Most of the imprinted transcripts that, to date, have been identified within 15q11-13 (fig. 1a) exhibit exclusive paternal expression, loss of which results in the contiguous gene-deletion syndrome Prader-Willi syndrome (PWS). Mutation of *UBE3A*, the only known maternally expressed gene in this region, is sufficient to cause the classic Angelman syndrome (AS) phenotype (MIM 105830) (Kishino et al. 1997; Matsuura et al. 1997), although the phenotype of patients with AS and a 15q11-13 deletion is more severe (Cassidy et al. 2000). Paternal duplication of 15q11-13 has no obvious phenotype, but maternal duplication results in a spectrum of phenotypes, ranging from language delay to autism (MIM 209850) (Browne et al. 1997; Cook et al. 1997). The phenotype is more severe in proportion to the number of intrachromosomal maternal copies of this segment, and patients carrying a marker chromosome containing two additional copies of maternal 15q11-13 have AS-like features (Wolpert et al. 2000). Autism may also be a component of AS (Steffenburg et al. 1996). The association of these autism-spectrum phenotypes with *UBE3A* is unclear, since mutations in *UBE3A* have not been found in karyotypically normal autistic individuals

(Veenstra-VanderWeele et al. 1999), and linkage analyses suggest the existence of candidate loci between *UBE3A* and D15S156 (Cook et al. 1998; Bass et al. 2000). Together, these results support a hypothesis that another gene in this region may play a role in some forms of autism and in modification of the AS phenotype.

In the course of mapping the 15q11-13 region (Christian et al. 1998), our laboratory had localized STS-N35112, contained within the expressed-sequence tag (EST) KIAA0566 (AB011138) (Ishikawa et al. 1998), to the ~1-Mb interval between *SNRPN* and D15S986, by minimal-tile YAC mapping. The sequence of KIAA0566 is contained within AC023449.3 and AC020639.16, which were used to identify 3' exon-intron boundaries of the KIAA0566 transcript. Sequence-homology database searches have identified a further six bacterial artificial chromosomes (BACs), which both hybridize, by FISH, to chromosome 15 and are positive, by PCR, for KIAA0566 exons (not shown). Comparisons between Unigene Resources ESTs (accession number Hs.44697) identified a single 3' splice variant (fig. 1b).

KIAA0566 corresponds to the putative 3' end of the human *ATP10C* gene (LocusLink accession number 10080; also, numerous GenBank accession numbers, which are available at the GenBank Overview web site and are listed below in the "Electronic Database Information" section). The 5' portion of the complete *ATP10C* transcript was reconstructed by the sequencing of Image clone 784559, which partially overlaps the

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0002-9297/2001/6806-0022\$02.00

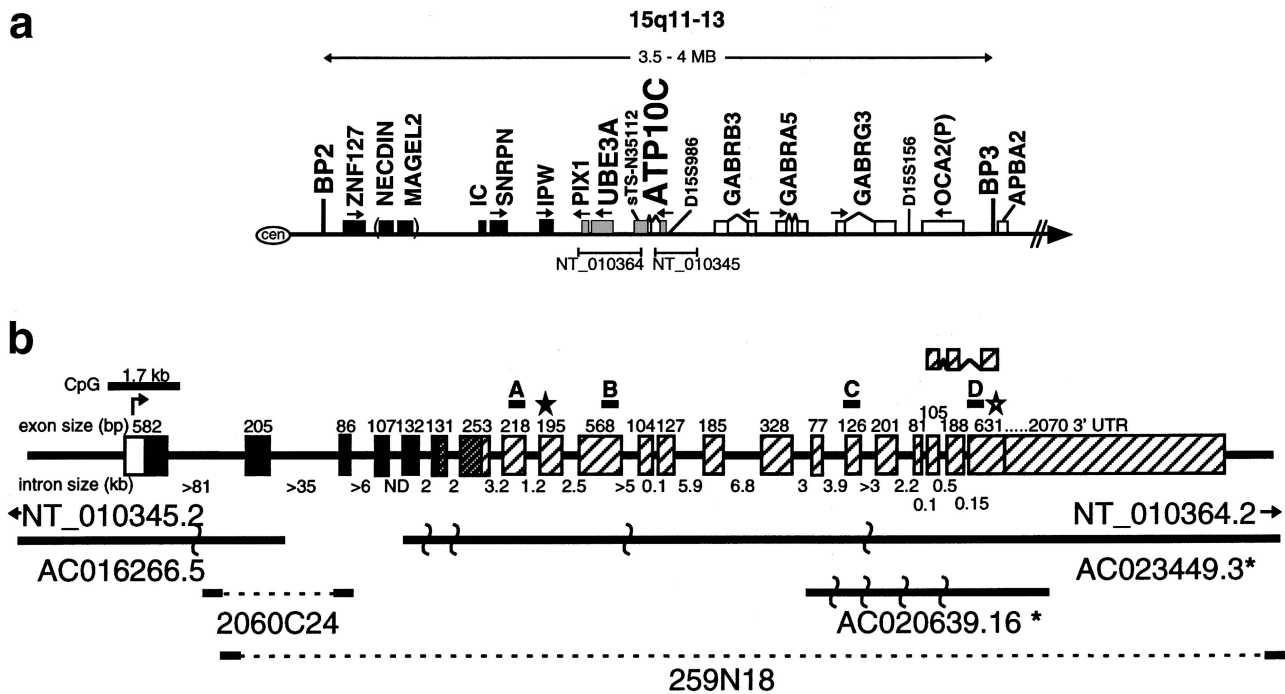


Figure 1 Genomic and physical map of *ATP10C*. *a*, Diagram showing that *ATP10C* maps immediately distal to *UBE3A* and is transcribed in the same orientation. Imprinted genes are represented by shaded (in the case of maternal expression) and blackened boxes (in the case of paternal expression). Genomic-sequence contigs overlapping *ATP10C* are shown below the map (where they are denoted by the prefix “NT_”). *b*, Diagram showing that *ATP10C* comprises 21 exons spanning >160 kb. Exon sizes (in bp) are shown above the gene, and intron sizes (in kb) are shown below the gene. Hatched exons represent sequence present in the KIAA0566 EST AB011138; shaded exons represent sequence present in Image clone 784559. Alternate splicing of exons 19–21 is indicated above the gene; the translation start site in exon 1 is indicated by an arrow. Contig sequence/RPCI-11 BACs spanning *ATP10C* are shown below the gene. Genomic sequences from BAC clones are indicated by solid black lines, which are broken by a slash at sites of sequence gaps. Sequence contigs (denoted by the prefix “NT_”) and sequence accession numbers (AC) also are shown; an asterisk (*) indicates sequence not corresponding to its assigned BAC. A–D are primers used in RT-PCR of an imprinted-expression panel (fig. 2a). The solid-black star indicates exon 9 polymorphism 1728 C→T (T→M); the star containing a white center indicates exon 21 polymorphism 4582 A→G.

5' end of KIAA0566, revealing a further 615 bp of upstream open reading frame (ORF). This sequence in turn overlaps RPCI-11 BAC 2C7 (AC016266.5), and analysis of the 2C7 flanking sequence suggested a further extension of the ORF, including a methionine, resulting in a gene of 21 exons encoding a putative protein of 1,499 amino acids. Expression of RNA corresponding to the complete ORF from fibroblasts and various brain regions has been confirmed by reverse-transcriptase PCR (RT-PCR). Comparison of the human *ATP10C* sequence with that of the mouse P-ATPase class V homologue (AF156549) (Halleck et al. 1999), recently mapped to the syntenic mouse chromosome 7 (Dhar et al. 2000), yields a total similarity, between the full-length mouse and human genes, of 84% at the nucleotide level and 80% at the protein level.

Genomic sequence (AC023449.3) overlapping exons 5–21 of *ATP10C* is contained within the chromosome 15 contig NT_010364, which contains *UBE3A* and thus indicates that *ATP10C* transcription is in the same ori-

entation as that of *UBE3A* (fig. 1a). The sequence of 5' *ATP10C*, including exon 2, is contained within the chromosome 15 contig NT_010345. To complete the contig across *ATP10C*, we identified BACs RPCI-11 259N18 and CIT-D 2060C24, by BAC end-sequence homology. These BACs both hybridize, by FISH, to chromosome 15 and are positive, by PCR, for exons 2–3 and exons 2–21, respectively, and complete the chromosome 15 genomic contig from *PIX1* through *GABRB3* (fig. 1b).

To determine the imprint status of *ATP10C*, we tested intron-spanning primer pairs (e.g., C and D or A and B; see fig. 1b) on a chromosome 15–expression panel, comprising cDNA from samples of normal brain, PWS brain, and AS brain, which express, respectively, both the maternal and paternal 15q11-13 gene alleles, the maternal allele only, or the paternal allele only. The expression panel demonstrated that there was a paucity of expression from AS brain samples (fig. 2a), suggesting preferential maternal expression and highlighting *ATP10C* as a second imprinted, maternally expressed

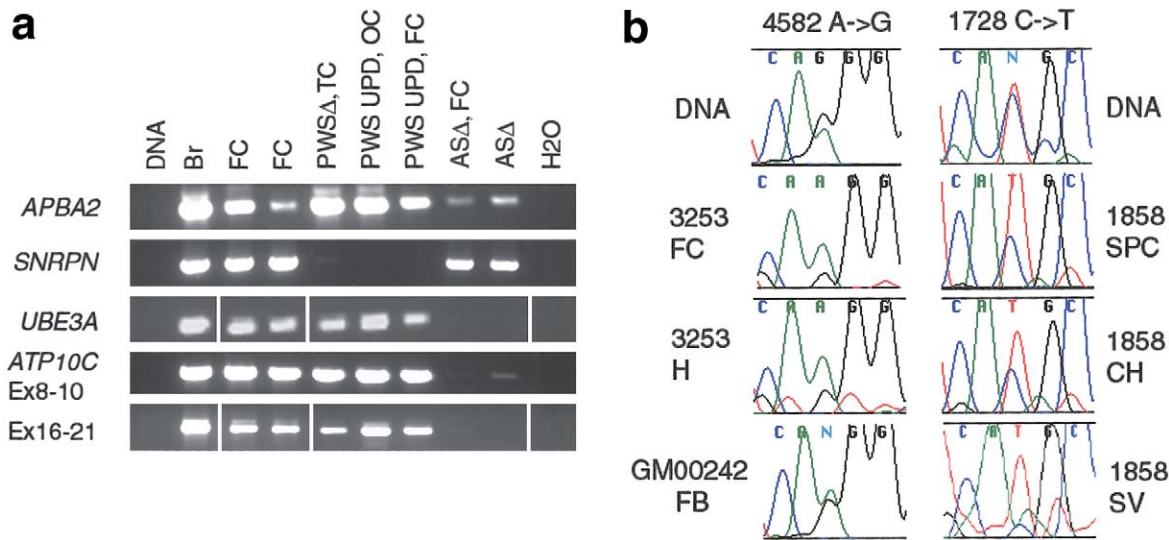


Figure 2 Imprinted expression of *ATP10C* in brain. *a*, RT-PCR of samples from normal brain, PWS brain, and AS brain, demonstrating minimal expression of *ATP10C* in samples from AS brain but normal levels of expression in samples from PWS brain, suggesting preferential maternal expression of *ATP10C*. *ATP10C* primers, as indicated in figure 1 as follows: A, 5'-TGGTGCACAGAACCCAGAGC; B, 5'-AATCGAAA-CCCAGTGTGTGC (755 bp); C, 5'-ATTCTTCACGGGCATTGGTGC; and D, 5'-TTCCTGGTCAACTGACGTGC (715 bp). Other primers are as follows: *APBA2*, 5'-TGGACAAACCACCAATAGGC and 5'-ATCTTCTCTCTGGTCATGGGC (696 bp); *SNRPN*, 5'-GCTCCATCTACTCTTTGAAGC and 5'-CTTTTCTTCACGCTCTGGTTGC (338 bp); *UBE3A*, 5'-CTCTTCTTGCAGTTTACAACG and 5'-CTTGAGTATTCGGGAAGTAAAAGC (152 bp). Δ = 15q11-13 deletion (in fig. 1a, the deletion including BP2-BP3); UPD = chromosome 15 uniparental disomy; OC = occipital cortex; TC = temporal cortex; Br = total brain. *b*, Sequence analyses of RT-PCR products for *ATP10C* exon 21 (left-side traces) and exon 9 (right-side traces) polymorphisms, demonstrating preferential expression of a single allele in all brain regions tested. As shown in the top row, both nucleotides are detected in genomic DNA of heterozygous, normal individuals, with the trace for the 4582 polymorphism typically overrepresenting the "G" allele and with the trace for the 1728 polymorphism detecting both nucleotides at roughly equal levels ("N"). Sequence traces from RT-PCR products from brain samples of normal individuals 3253 and 1858 show preferential expression of a single allele in all regions tested. The normal fibroblast line GM00242 demonstrates slight but consistent preferential expression of the maternal "A" allele; parental origin of alleles for individuals 3253 and 1858 could not be determined. Duplicate RNA isolates for each individual/brain region were reverse-transcribed, PCR was performed with several primer combinations, and the various PCR products were sequenced (not shown); preferential expression of the same allele (4582 "A" in 3253 and 1728 "T" in 1858) was observed in almost all products sequenced. FC = frontal cortex; H = hippocampus; SPC = superior parietal cortex; CH = cerebellar hemisphere; SV = superior vermis; FB = fibroblast.

gene in 15q11-13. No difference in *ATP10C* transcription level is apparent in a fibroblast expression panel (not shown). Similarly, by this assay, *UBE3A* also exhibits only exclusively maternal expression in adult brain (Rougeulle et al. 1997).

Ongoing sequence analysis of *ATP10C* in a cohort of autistic and normal patients has identified two polymorphisms, one a silent change in exon 21 and the other resulting in a threonine-to-methionine amino acid change in exon 9 (figs. 1*b* and 2*b*). Sequencing of the RT-PCR products from five heterozygous, normal individuals demonstrated consistent preferential expression of one allele in each case. Discrepancy in peak heights for each allele was most marked in the samples from various brain regions from the two individuals from whom such tissue was available, although it was not possible to determine the parental origin of the preferentially expressed allele. The allele-signal differences between genomic and cDNA samples from fibroblasts were less marked but also consistent and, in the line

(GM00242; Coriell Cell Repository) where the allele origin was known, it was the maternal allele that was preferentially expressed (fig. 2*b*). This suggests that regulation of the degree of differential *ATP10C* imprinted expression is tissue specific, whereas the imprint itself may be more ubiquitous. Imprinting of *ATP10C* expression is further supported by the observation of an obesity phenotype in mouse, occurring only with maternal p-locus deletions that span the region containing the mouse homologue, *pfatp* (Dhar et al. 2000). Taken together with the minimal expression in AS brain, these results indicate that *ATP10C* represents the second in a cluster of maternally expressed imprinted genes in 15q11-13. Work is currently ongoing to determine whether imprinted *ATP10C* expression is regulated by the *SNRPN* Imprinting Center (IC) in a manner similar to the regulation of *UBE3A* expression.

ATP10C is an interesting candidate for the autism-spectrum phenotypes associated with chromosome 15 and maternal rearrangements thereof, as well as for con-

tributing to the severity of deletion-associated AS. In addition to its map location and imprint status, the putative function of ATP10C as an aminophospholipid-transporting ATPase would involve it in maintenance of cell membrane integrity (Halleck et al. 1998), and it may therefore be critical for cell signaling in the CNS. Indeed, loss of members of another subfamily of P-type ATPases is responsible for Menkes disease and Wilson disease (DiDonato and Sarkar 1997). Specific localization of *Atp10c* transcript in mouse brain to cerebellar granule cells, the hippocampus, and cells surrounding the corpus callosum (Halleck et al. 1999) is especially intriguing, since all these areas (a) have been suggested as being preferentially involved in autism (Lord et al. 2000) and (b) overlap regions of imprinted *Ube3a* expression in mouse (Albrecht et al. 1997). Identification of imprinted *ATP10C* expression therefore paves the way both for more-extensive analysis of chromosome 15 imprinting mechanisms and for further understanding of the etiology of neurologic defects in AS and autism.

Note added in proof.—As this report was in press, Meguro et al. (2001) reported imprinted expression of *ATP10C* in lymphoblasts from patients with AS and deletion of either 15q11-13 or IC.

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Electronic Database Information

GenBank Overview, <http://www.ncbi.nlm.nih.gov/Genbank/GenbankOverview.html> (for *ATP10C* 5'UTR–3'UTR exonic/flanking sequence [accession numbers AY029487–AY029504] and complete *ATP10C* cDNA sequence [accession number AH010630])
 LocusLink, <http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi> (for *ATP10C* [accession number 10080])
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for autism [MIM 209850] and AS [MIM 105830])
 Unigene Resources, <http://www.ncbi.nlm.nih.gov/UniGene> (for ESTs [accession number Hs.44697])

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