SHORT REPORT

Exon organisation of the mouse gene encoding the Adrenoleukodystrophy related protein (ALDRP)

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ALDR is one of the four genes encoding an ATP Binding Cassette (ABC) hemi-transporter of the peroxisomal membrane so far identified in mammalian cells. The best known of these is X-ALD, whose dysfunction has been causally associated with X-linked adrenoleukodystrophy. ALDR and X-ALD protein product are closely related and we show here that this striking conservation is maintained at the genomic level. Although extending to a larger genomic region, the organisation of the mouse ALDR gene mirrors exactly that of X-ALD. This supports further the hypothesis that among the four known peroxisomal ABC hemi-transporters ALDRP is the most likely candidate as a modifier contributing to the phenotypic variability of X-linked adrenoleukodystrophy.

Keywords: adrenoleukodystrophy related gene; adrenoleukodystrophy; gene structure; ATP binding Cassette transporter

X-linked Adrenoleukodystrophy is a severe demyelinating disorder associated with impaired catabolism of very long chain fatty-acids (VLCFA)¹ and characterised by a high degree of phenotypic variability, even within a family. The molecular basis of the disease is the loss of function of a peroxisomal ABC (ATP Binding Cassette) hemi-transporter encoded by the *ALD* gene.^{2,3} The comparison with the inactivation of homologous genes in yeast suggests that the ALD protein (ALDP) may be involved in peroxisomal transport of activated VLCFA.⁴ Attempts to generate a mouse

model for adrenoleukodystrophy by the knock out of the *ALD* gene were partially disappointing since no neurological abnormalities were detected. These mice express a biochemical phenotype somewhat milder than observed in human patients.^{5,6} This suggests that related hemi-transporters, potentially able to dimerise with ALDP in a functional complex, may play a modifier role in the final phenotypic outcome of the disease. In other biochemical pathways, heterodimerisation of hemi-transporters is known to have a key functional role.^{7,8} At least three other ABC hemi-transporters are located in the membrane of the peroxisome, but their combinatorial assembly in one or more functional transporters is yet unknown.⁹ They are encoded by the *ALDR*, *PMP70* and *PMP70*-related genes.^{10–13} The ALDR protein (ALDRP) is the closest to ALDP with 66% amino acid identity, whereas

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Received 14 January 1998; revised 22 April 1998; accepted 19 May 1998

PMP70 and PMP70R score at 35% and 25% identity with ALDP respectively. Hence, it seems reasonable to consider the *ALDR* gene a likely candidate as a modifier of the phenotype in mouse or in human patients carrying ALD null mutations. The *ALDR* gene (*ALDL1* in the official GDB nomenclature) maps to human chromosome 12 and mouse chromosome 15.¹⁴ In mouse, it is predominantly expressed in brain and adrenals^{10,15} in contrast to the rather ubiquitous expression of the *ALD* gene. We describe here the structural

organisation of the *ALDR* gene and extend the analysis of conservation between ALD and ALDR at the genomic level.

A mouse 129SV genomic library (Stratagene, La Jolla, CA, USA, in lambda FIXII vector) was screened with the full length ALDR cDNA. Labelling of the probe by random-primed method, highly stringent hybridisation conditions and phage plate purification¹⁶ led to the isolation of eight individual phages ordered in two non-overlapping contigs. Phage overlaps were

A

B

ALDP	M-PVLSTP--RPSRV-TTLKRTAVVLALTAYGVHKIYPLVRQCLT-PA-RGPQVPAGEPTQ-----EASGATATK--AGMNRVFLQRLALLRLFFPRVL	87
ALDRP	MIHMLNAAAYRVKWRSGAAKRAACLVAAAYALKTLYPITGKRLKQPGHRKAKAEAYSPAENREILHCTETICKKPAPGLNAAFFKQLLELRKILFFPKLV	100
	_____ TM1 _____ TM2 _____	
ALDP	CRETGLLALHSAALVSRFTLSVYVARLDGRLARCIVRKDPRAFSWQLLQWLLIALPATFINSAIRYLEGQLALSFRSRLVAHAYGLYFSQQTYYRVSNMD	187
ALDRP	TTETGWLCLHSVALISRTFLSIYVAGLDGKIVKSIVEKKPRTFIIKLIKWLMIAPATFVNSAIRYLECKLALAFRTRVLVDHAYETYFANQTYKVINMD	200
	_____ TM3 _____ TM4 _____	
ALDP	GRLRNPDSLQSLTEDVVAFAASVAHLYSNLTKPLLDVAVTSYTLRAARSRGAGTAWPSAIALGLVVFLTANVLAFAFPKFGELVAEEARRKGELRYMHSRVV	287
ALDRP	GRLANPDQSLTEDIMMFSQSVVAHLYSNLTKPILDVILTSYTLIRTATSRGASPIGPTLLAGLVVYATAKVLKACSPKFGSLVAEEAHRKGYLRVHSRII	300
	_____ 1 _____ TM5 _____ TM6 _____ 2 _____	
ALDP	ANSEETAFYGGHEVELALLQHSYQDLASQINLILLERLWYVMLEQFLMKYVMSASGLLMVAVPIITATGYAESDSEAMKKALEMKEEELVSRTEAFTI	387
ALDRP	ANVEETAFYRGHKVEMKQLQKCYKALAYQNNLILSKRLWYIMIEQFLMKYVWSGCLIMVAIPIITATGFADGDLEDGPK-----QAMVSDRTEAFTT	393
	_____ 3 _____ 4 _____	
ALDP	ARNLLTAAADATERIMSSYKEVTELAGYTARVYEMFQVFEDVKHCRFKRTGDLEEAQAGPGVMVQSGVHVEGPLKIQGQVVDVEQGIICENIPIITPTGE	487
ALDRP	ARNLLASGADATERIMSSYKEITELAGYTARVYNMFVWFDEVKRGYIKRTVT-QEPENHSKRGGNLELPLSDTLAIGTVIDVDHGIICENVPIITPAGE	492
	_____ 5 _____ 6 _____ ABC _____	
ALDP	VVASLNIRVEEGMHLLITGPNCGCKSSLFRILGGLWPTYSGVLYKPPQRMFYIPQRPYMSVGLSLRDQVIYPDSAEDMRKGCSEQQLEAILGIVHLRH	587
ALDRP	VVASRLNFKVEEGMHLLITGPNCGCKSSLFRILSGLWPFYEGVLYKPPQHMFIYIPQRPYMSLGLSLRDQVIYPDSADDREKGYTDQDLERILHSHVLYH	592
	_____ 7 _____ 8 _____ 9 _____	
ALDP	ILQREGGWAEVCDWVDVLSGGGEKQIRIGMARMFYHRPKYALLDECTSAVSIDVEGKIFQAAKDAGIALLSITHRPSLWKYHHTLLQFDGEGGWKFEKLDSA	687
ALDRP	IVQREGGWDAVMDWVDVLSGGGEKQRMGMARMFYHKPKYALLDECTSAVSIDVEGKIFQAAIGAGISLLSITHRPSLWKYHHTLLQFDGEGGWRFELQDTA	692
ALDP	ARLSLTEEKQRLQQLAGIPKMQGRLQELRQILGEAAAPVQPLVPGVPT	736
ALDRP	IRLTLSSEKQKLESQLAGIPKMQGRLNELCKILGEDSVLTKTIQTPEKTS	741

Figure 1 **a** Genomic map of the 50 kb region encompassing the mouse ALDR gene. *EcoRI*, *SacI*, *XbaI* and *BamHI* restriction sites are indicated. Exons and introns are numbered. **b** Alignment of murine ALD and ALDR protein showing the perfect conservation of splicing sites. The location of the transmembrane spanners (TM) and the ATP Binding Cassette (ABC) are shown above the line. The last and first residues for each exon are underlined and hatched. Intron numbers are indicated

identified by restriction mapping with four enzymes (EcoRI, Xba I, Sac I, BamHI) and hybridisation of phages with partial cDNA probes or specific oligonucleotides spaced along the coding sequence. Shotgun subcloning of the relevant phage fragments in pBSKS vector (Stratagene, La Jolla, CA, USA) was carried out, allowing sequencing of the exon-introns boundaries in both directions (Pharmacia, Biotech, Uppsala, Sweden, T7 Polymerase sequencing kit on double-stranded templates). The size of the introns was estimated by PCR either on whole phages or their subclones and confirmed when possible on genomic DNA from 129SV mice. Oligonucleotides used as primers were derived from the cDNA sequence (17bp sequences starting from the following positions on the GB AC Z48670: 918, 1220, 1331, 1510, 1664, 1708, 1911, 2004, 2129 for the sense and 1314, 1423, 1596, 1640, 1773, 1950, 2077, 2149, 2312 for the antisense primers) and chosen in order to allow the amplification reaction to be performed in standard conditions. The gap between the two sets of overlapping phages, estimated as smaller than 2 kb on the basis of genomic Southern analysis (not shown) with probes derived from the 3' end of the cDNA could in fact be filled by PCR amplification on genomic DNA with a pair of primers targeted at the last two exons.

The resulting overall genomic map spans more than 50 kb (Figure 1a), and was confirmed by Southern blotting on genomic DNA (not shown). The *ALDR* gene is composed of 10 exons and 9 introns of highly

variable size. This structure mirrors exactly that of the human and mouse *ALD* genes^{17,18} with a perfect conservation of positions of splice sites (Figure 1b). The size of the coding sequence in exons 2–10 is quite homogeneous, whilst the first exon encodes 313 amino acids (42% of the whole protein) and contains four out of the six predicted transmembrane segments (Figure 1B). The structure of the *ALD* and *ALDR* genes is very different from that of the *PMP70* gene, a more distant relative, with 23 exons¹⁹ (GB AC #X83467 to #X83489). The mouse *ALDR* gene is larger than the mouse and human *ALD* genes (around 22 kb each); this is due to larger introns in particular the last four^{6–9} which span in *ALDR* more than 20 kb as compared with less than 4 kb in *ALD*. All the exon-intron junctions in the *ALDR* gene conform to the known rules of splicing site (Figure 2).

The knowledge of the structure of the mouse *ALDR* gene will allow the engineering, by homologous recombination, of an *ALDR* knock-out mouse. This will be important since the recently constructed *ALD* knock-out mice do not show neurologic abnormalities.^{5,6} The mouse *ALD* and *ALDR* genes show a very different pattern of expression, somewhat complementary in several organs.¹⁵ Moreover, the mouse *ALDR* is much more highly expressed than its human counterpart,^{20,10} in particular in adrenals and might thus play a more important role in VLCFA catabolism. Although the association of two homologous hemi-transporters into a functional complex has been assessed in mammals⁷ and in yeast,⁹ the facultative dimerisation with alternative partners has so far been reported only in the case of white, brown, and scarlet in *D. melanogaster*.⁸ The presence of several ABC transporters on eukaryote peroxisomal membranes allows the hypothesis that tissue specific complexes, assembled from different subunits, exist. In this context, however, the role of *ALDR* as a modifier or facultative dimerisation partner remains still to be ascertained. These questions will hopefully be answered by comparing and crossing *ALDR* knock-out mice with *ALD* knock-out mice.

	1143	AG	gtaagacaggcattaaagca	
ttttttttcccccttttttag	1144 GT	EXON 2	1324 TG	gtaataacatccatgttta
ttctgtgatttttctcatag	1325 AT	EXON 3	1446 AG	gtatttctaatttcggccgta
tctttatatgttgatttttag	1447 AT	EXON 4	1612 AG	gtaattaacttcaggccttg
aaactgttccttgattatag	1613 GA	EXON 5	1707 AA	gtaagatgatattcaagaaa
aattttattttctgaccttag	1708 GT	EXON 6	1853 AG	gtaagaaactttttatatca
gtgtgcttggtgttttctag	1854 GC	EXON 7	1999 AG	gtagactgtgccattacttt
tttttttattttattttctag	2000 GA	EXON 8	2084 AA	gtaagtacgctacgttcagg
gtttatttgtttgttttctag	2085 AC	EXON 9	2210 TG	gtacgtgctcacagcatcct
actttgttttaacacacag	2211 GA	EXON 10		

Figure 2 Exon-introns junctions for *ALDR* conform to consensus splice sites. Intron sequences flanking each exons are shown as well as the first and last two bases of the exons (in capitals). The position of each exon limit with respect to the cDNA sequence is indicated

Acknowledgements

CB and NTC were supported respectively by an ARC and AFM grant. This work was supported by institutional grants from INSERM and CNRS, specific grants from LLNC and from EEC concerted action on peroxisomal leukodystrophy (JLM).

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