Comparative studies of vertebrate aldehyde dehydrogenase 3: Sequences, structures, phylogeny and evolution. Evidence for a mammalian origin for the *ALDH3A1* gene

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ABSTRACT

Mammalian ALDH3 genes (*ALDH3A1*, *ALDH3A2*, *ALDH3B1* and *ALDH3B2*) encode enzymes of peroxidic and fatty aldehyde metabolism. *ALDH3A1* also plays a major role in anterior eye tissue UV-filtration. BLAT and BLAST analyses were undertaken of several vertebrate genomes using rat, chicken and zebrafish ALDH3-like amino acid sequences. Predicted vertebrate ALDH3 sequences and structures were highly conserved, including residues involved in catalysis, coenzyme binding and enzyme structure as reported by Liu and coworkers (1997) for rat *ALDH3A1*. Phylogeny studies of human, rat, opossum, platypus, chicken, xenopus and zebrafish ALDH3-like sequences supported three hypotheses: (1) the mammalian *ALDH3A1* gene was generated by a tandem duplication event of an ancestral vertebrate *ALDH3A2* gene; (2) multiple mammalian and chicken *ALDH3B*-like genes were generated by tandem duplication events within genomes of related species; and (3) vertebrate *ALDH3A* and *ALDH3B* genes were generated prior to the appearance of bony fish more than 500 million years ago.

Key words: aldehyde dehydrogenase; ALDH3; enzyme structure; gene duplication; evolution; aldehyde metabolism

1. Introduction

The aldehyde dehydrogenase (*ALDH*; EC 1.2.1.3) gene super-family (<http://aldh.org/superfamily.php>) encodes many ALDHs which perform diverse roles in aldehyde metabolic pathways in the body, including retinoid, lipid, alcohol, amino acid, neurotransmitter, xenobiotic and drug metabolism [1,2]. The mammalian *ALDH3* gene family has been investigated predominantly because of the roles of ALDH3 isozymes in fatty and peroxidic aldehyde metabolism, and the high ALDH3 activity

levels in cornea, stomach, hepatomas and liver microsomes [3-8]. Four major mammalian *ALDH3*-like genes and enzymes have been reported including *ALDH3A1*, which encodes the major corneal, stomach, and hepatoma isozyme [4-6] and acts in corneal UV-filtration [7-8]; *ALDH3A2*, which encodes the liver microsomal fatty aldehyde dehydrogenase [9,10], which has been implicated in the Sjögren-Larsson syndrome [11]; *ALDH3B1*, encoding a human lung, prostate and kidney cytosolic ALDH [12,13]; and *ALDH3B2*, encoding a human salivary gland and placental ALDH [14].

This study describes the predicted sequences, structures and phylogeny of vertebrate *ALDH3* genes and enzymes for platypus (*Ornithorhynchus anatinus*), chicken (*Gallus gallus*), frog (*Xenopus tropicalis*) and zebrafish (*Danio rerio*), and compares these results **to** those previously reported for human (*Homo sapiens*), rat (*Rattus norvegicus*) and other mammalian *ALDH3* isozymes. Structures for vertebrate *ALDH3* isozymes and gene locations for *ALDH3-like* genes were obtained using data from the respective genome sequences. This paper extends previous studies which examined the structures and properties of opossum (*Monodelphis domestica*) *ALDH3-like* genes and proteins [15,16]. Phylogenetic analyses also describe the relationships and potential origins of *ALDH3A-like* and *ALDH3B-like* genes during mammalian and vertebrate evolution.

2. Materials and methods

2.1 Vertebrate ALDH3 Gene and Enzyme Identification

BLAT (BLAST-Like Alignment Tool) *in silico* studies were undertaken using the UC Santa Cruz genome browser [<http://genome.ucsc.edu/cgi-bin/hgBlat>] [17,18] with the default settings. GenBank [<http://www.ncbi.nlm.nih.gov/Genbank/>] and UniProtKB/Swiss-Prot Database [<http://au.expasy.org>] sequences for rat *ALDH3A1*, *ALDH3A2*, *ALDH3B1* and *ALDH3B2* (Table 1) were used to interrogate platypus

(*Ornithorhynchus anatinus*), chicken (*Gallus gallus*), frog (*Xenopus tropicalis*) and zebrafish (*Danio rerio*) genome sequences [19-22]. Gene locations, predicted gene structures and ALDH3 protein subunit sequences were observed for each ALDH examined for those regions showing identity with the respective *ALDH* gene products (Table 1). Unless otherwise stated, all residue numbering is as in [27], according to the rat ALDH3A1 sequence.

2.2 Alignment of Vertebrate ALDH3 Residues

Alignments of human ALDH3A1 [23], ALDH3A2 [11], ALDH3B1 [12] and ALDH3B2 [14], rat ALDH3A1 [4] and of other predicted vertebrate ALDH3A1, ALDH3A2, ALDH3B1 and ALDH3B2 sequences (see Tables 1 & 2) were undertaken using a ClustalW-technique [24] (<http://www.ebi.ac.uk/clustalw/>).

2.3 Predicted Secondary and Tertiary Structures for Human ALDH3A1, ALDH3A2, ALDH3B1 and ALDH3B2 Subunits

Predicted secondary and tertiary structures for human ALDH3A1, ALDH3A2, ALDH3B1 and ALDH3B2 subunits were obtained using web tools [25,26] (<http://swissmodel.expasy.org/workspace>). The tertiary structure for rat ALDH3A1 [27] served as a reference for obtaining these tertiary structures: human ALDH3A1 (residues 4-448); ALDH3A2 (residues 5-443), ALDH3B1 (residues 5-446) and ALDH3B2 (residues 4-366) using the rat ALDH3A1 template at 2.6 Å resolution (PDB ID 1ad3B).

2.4 Prediction of Transmembrane Regions for Vertebrate ALDH3

Predictions of transmembrane helices for human, rat, platypus, chicken, frog and zebrafish ALDH3 sequences were conducted using the web resources of the Center for Biological Sequence Analysis, Technical University of Denmark TMHMM Server v. 2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>).

2.5 Phylogenetic Studies and Sequence Divergence

Alignments of vertebrate ALDH3 sequences with the nematode (*C. elegans*) ALDH3 sequence as the 'outgroup' were assembled using BioEdit v.5.0.1 and the default settings [28]. Alignment of ambiguous regions, including the amino and carboxyl termini, were excluded prior to phylogenetic analysis yielding alignments of 261 residues for comparisons of vertebrate ALDH3A1, ALDH3A2, ALDH3B1 and ALDH3B2 sequences (Tables 1 & 2). Evolutionary distances were calculated using the Kimura option [29] in TREECON [30]. Phylogenetic trees were constructed from evolutionary distances using the neighbor-joining method [31] and rooted with the *C. elegans* ALDH3 sequence (Reference Sequence NM_071144). Tree topology was reexamined by the boot-strap method (100 bootstraps were applied) of resampling and only values that were highly significant (≥ 90) are shown [32]. Percentage sequence identities for the ALDH3 family subunits examined were determined using the SIM-Alignment tool for Protein Sequences [26] (<http://au.expasy.org/tools/sim-prot.html>).

3. Results and Discussion

3.1 Alignments of rat and human ALDH3A1, human ALDH3A2, ALDH3B1 and ALDH3B2 and vertebrate ALDH3B2 amino acid sequences

Amino acid sequence alignments for rat ALDH3A1 [4] and human ALDH3A1 [23], ALDH3A2 [11], ALDH3B1 [12] and ALDH3B2 [14] are shown in Figure 1. Comparisons of the latter four ALDH sequences with the rat ALDH3A1 sequence (tertiary structure previously reported) [27] enabled identification of key residues which may contribute to catalysis, structure and function. Table 3 identifies some of these key residues for vertebrate ALDH3A1, ALDH3A2, ALDH3B1 and ALDH3B2 sequences and the nematode (*C. elegans*) ALDH3-like sequence. Active site residues which are considered essential for catalysis (Asn114; Glu209; Cys243; Glu 333) [27, 33, 34] were conserved for all of these ALDH3 sequences. Fifteen glycine residues were also retained for all of these ALDH3 sequences examined

(Table 3) which is consistent with the roles proposed by Hempel and coworkers [33] in contributing to the structure of rat ALDH3A1. Two of these conserved residues (Gly187 and Gly211) are located within the stem of the active site funnel. Three proline residues were also retained (Pro103, 317 and 340) which **appear to** contribute significantly to rat ALDH3A1 tertiary structure [27]. In addition, a novel dinucleotide-binding motif and NAD(P) binding domain near the N-terminal end of the α D helix (188Gly-Ser-Thr-Ala-Val-193Gly) [27] was predominantly retained for the human ALDH3A1, ALDH3A2, ALDH3B1 and ALDH3B2 sequences (Figure 1).

Amino acid sequence alignments for human ALDH3B1 [12] with mammalian and chicken ALDH3B2-like amino acid sequences (Table 2) are shown in Figure S1 (see supplementary data). Essential ALDH3 active site, nucleotide phosphate binding and structural glycine and proline residues were predominantly retained for each of the vertebrate ALDH3B2 sequences examined, although major differences in sequence length were observed, particularly for the primate ALDH3B2 sequences, which lacked 81 residues of the N-terminal sequence. In addition, the horse ALDH3B2 sequence lacked a hydrophobic residue near the active site Asn114 (Phe118 in rat ALDH3A1) and four residues near the active site Cys residue (Cys243 in rat ALDH3A1). Also, chicken ALDH3B2 revealed two major deleted segments in comparison with the human ALDH3B1 sequence but retained other key ALDH3A1 residues.

Sequence identities for vertebrate ALDH3-like sequences were examined (see Table S1 in supplementary data) with high levels of sequence identities observed for the human, rhesus monkey, rat, opossum and platypus ALDH3A1 sequences (80-98%). Comparisons of sequence identities between **human**, rhesus monkey, rat, opossum, platypus, chicken, frog and zebrafish ALDH3A2 sequences were also examined, with 66-96% identities being observed as well as high levels of identity for more closely related species (eg. human and rhesus monkey ALDH3A2

were 96% identical). Lower pairwise identities were observed between ALDH3A1 and ALDH3A2 sequences from the same mammalian species (69-73%), and in comparing ALDH3A1 and ALDH3A2 sequences with the human ALDH3B1 and ALDH3B2 sequences (50-57%). This supports a proposal that the identified vertebrate ALDH sequences are members of the ALDH3 family but are products of the same *ALDH3* gene sub-class in each case (Table S1).

3.2 Predicted Structures for Human ALDH3A1, ALDH3A2, ALDH3B1 and ALDH3B2

Predicted secondary structures for human ALDH3A2, ALDH3B1 and ALDH3B2 subunits were compared in Figure 1 with the previously reported structure for rat ALDH3A1 [27]. Similar α -helix and β -strand locations were observed for each of the ALDH3 subunits examined. The most significant difference related to the extra α -helix at the carboxy-terminus for the human ALDH3A2 sequence, which showed a high probability as a transmembrane sequence which was also observed for the chicken ALDH3A2 sequence (Figure 2). Given the reported location of human and mouse ALDH3A2 within the liver endoplasmic reticulum [3,9], and the fact that the C-termini occur on the same face of the dimer [27, 35], it is likely that this sequence is responsible for the membrane-bound location for this enzyme. In contrast, human ALDH3A1, ALDH3B1 and ALDH3B2 sequences showed only low probability regions for transmembrane sequences, which supports their location within the cytoplasm, as previously reported for rat ALDH3A1 [4,33]. Moreover, ALDH3A1 serves as a major soluble or 'crystallin-like' corneal protein for several mammals, and acts as a UV-filtering agent, protecting anterior eye tissues from UV-induced damage [7,8,36].

Predicted tertiary structures for human ALDH3A1, ALDH3A2, ALDH3B1 and ALDH3B2 were compared with rat ALDH3A1 [27] (Figure 3). Similar structures were observed for each enzyme, including the 3 major domains: the catalytic domain and the NAD binding domain, which are separated by the active site cleft, and an oligomerisation domain, which is involved in forming dimers of the active enzyme

[27]. The predicted human ALDH3B2 tertiary structure, however, showed a more open conformation, apparently resulting from the loss of three helices at the N-terminus ($\alpha 1$ - $\alpha 3$), in comparison with the human ALDH3A1 structure (Figure 3).

3.3 Predicted gene locations and exonic structures for vertebrate ALDH3-like genes

Tables 1 and 2 summarize the predicted locations, sizes and number of coding exons for vertebrate *ALDH3A*-like and *ALDH3B*-like genes examined, respectively. These were based on BLAST interrogations of *ALDH3*-like transcript databases (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) using the reported sequences for rat and mouse *ALDH3A1* [4,37], *ALDH3A2* [9], *ALDH3B1* and *ALDH3B2* [38], chicken *ALDH3A2* [39] and zebrafish *ALDH3A2* [22] and BLAT analyses of vertebrate and nematode (*Caenorhabditis elegans*) genomes using the UC Santa Cruz Genome Browser [18] (<http://genome.ucsc.edu/cgi-bin/hgBlat>).

Mammalian *ALDH3A1* and *ALDH3A2* genes were located between 26 (mouse) and 141 (opossum) kilobases apart on the same chromosome (or shotgun contig for guinea pig, cat and platypus genomes) but were usually encoded on opposite strands and contained 9 or 10 coding exons (Table 1). *ALDH3A2* genes were typically 3-4 times larger than *ALDH3A1* genes and identified in all vertebrate genomes examined, whereas *ALDH3A1* genes **are** apparently restricted to mammalian genomes, including marsupial [opossum (*Monodelphis domestica*)] and monotreme [platypus (*Ornithorynchus anatinus*)] species.

Mammalian *ALDH3B1* and *ALDH3B2* genes were also located on the same chromosome (but different from the *ALDH3A* genes), and were 43 (guinea pig) to 349 (human) kilobases apart and also encoded on opposite strands with 7-10 coding exons (Table 2). Mammalian *ALDH3B1* genes were typically 3-5 times larger than for the *ALDH3B2* genes. Single *ALDH3B*-like genes were identified in lower vertebrate genomes, including frog (*Xenopus tropicalis*) and several fish genomes

(eg *Danio rerio*), which were designated as *ALDH3B1* genes for consistency with the chicken and mammalian gene nomenclature

Predicted start sites for 10 coding exons were identified for the rat and human *ALDH3A1* and human *ALDH3A2* genes, which were located in similar or aligned positions in each case, with the exception of the exon 9/10 boundary, for which an extended *ALDH3A2* exon 9 sequence was observed (Figure 1). Comparisons of coding exons for the human *ALDH3B1* and *ALDH3B2* sequences revealed similarities, with the exception of coding exons 1 and 2 and the last exon boundary for the human *ALDH3B1* sequence, which were absent from the human *ALDH3B2* sequence.

3.4 Phylogeny and evolution of vertebrate *ALDH3A* and *ALDH3B* sequences

A phylogram (Figure 4) was calculated by the progressive alignment of vertebrate *ALDH3* amino acid sequences with a nematode (*C. elegans*) *ALDH3*-like sequence used to 'root' the tree, which clustered into three main branches, corresponding to the *ALDH3A1*-like, *ALDH3A2*-like and *ALDH3B*-like proteins and genes. The observed *ALDH3A1* sequences were restricted to mammals, including marsupial (opossum) [16] and monotreme (platypus) species, whereas *ALDH3A2*-like sequences were observed for all vertebrates examined, including several bony fish species. The tandem locations of mammalian *ALDH3A*-like genes (Table 1), the higher levels of identities for *ALDH3A1* and *ALDH3A2* sequences (>69%) (Table S1) and the phylogram generated from calculating genetic distances for vertebrate *ALDH3A*-like sequences (Figure 4) support an hypothesis that mammalian *ALDH3A1* genes were generated by a tandem duplication of a vertebrate ancestral *ALDH3A* gene, prior to the emergence of mammals during vertebrate evolution.

In contrast, mammalian and chicken *ALDH3B1* and *ALDH3B2* sequences grouped together on single branches of the phylogram, indicating that these genes are products of recent species (or related species) gene duplication events of an ancestral vertebrate *ALDH3B* gene. This is further supported by the tandem locations for these genes on mammalian and chicken chromosomes and the high

level (88%) of amino acid sequence identity for the human ALDH3B1 and ALDH3B2 sequences (Table S1). In addition, given that fish genomes contained both *ALDH3A*- and *ALDH3B*-like genes, these results also support an hypothesis that the common ancestor for *ALDH3* genes predated the evolutionary appearance of vertebrates, which has been estimated at >500 million years ago [40].

3.5 Functions of Vertebrate ALDH3 Isozymes

Several metabolic roles have been previously described for mammalian ALDH3A1 (Table 4). These include serving as a detoxifying agent for peroxidic aldehydes in the body [41,42]; a multifunctional protein in the eye, including roles as a corneal and lens 'crystallin' in several mammalian species [36]; a protective agent against oxidative damage in the lens and cornea [43]; a UV-radiation filter in mammalian anterior eye tissues [6-8,44-46]; in cyclophosphamide and related cancer drug metabolism [47]; as well as participating in the oxidative stress response and cell homeostasis [48].

Human ALDH3A2 (also called fatty aldehyde dehydrogenase) plays a major role in the body in the metabolism of long chain fatty aldehydes and several genetic mutations have been reported in human populations which lead to the recessive Sjögren-Larsson syndrome (SLS) [10,11]. One of the numerous mutations which gives rise to the SLS involves Asp-247 [33]. Asp-247 is conserved in all ALDH3s considered here, with the exception of a conservative Glu substitution in the chicken 3B1 enzyme. Mammalian ALDH3A2 is predominantly located within liver microsomes [3], via a hydrophobic C-terminal transmembrane helix [9], a property which is also shared with chicken ALDH3A2 (Figure 2).

The metabolic role(s) for the *ALDH3B*-like genes and proteins have not been described in mammals, however it is likely that they perform

detoxification roles for endogenous and exogenous aldehydes in the body, particularly in the adrenals and lung (*ALDH3B1*) and the epidermis (*ALDH3B2*), where these genes exhibit high levels of expression [12-14]. However, deletions in various *ALDH3B2* sequences (eg human, chimpanzee, rhesus, horse and chicken *ALDH3B2*) raise questions concerning their functional viability.

4. Conclusions

BLAST and BLAT analyses of several vertebrate genome databases were undertaken using amino acid sequences reported for rat *ALDH3A1*, *ALDH3A2* and *ALDH3B1*, and for chicken and zebrafish *ALDH3A2* enzymes, for interrogation of vertebrate genomes. Evidence is presented for at least four *ALDH3-like* genes in mammalian genomes, including two pairs of closely localized genes on separate chromosomes: [*ALDH3A1-ALDH3A2*] and [*ALDH3B1-ALDH3B2*]. Predicted amino acid sequences and secondary/tertiary structures for the vertebrate *ALDH3-like* subunits showed a high degree of similarity with rat *ALDH3A1*, which served as a reference structure for these *ALDH* isozymes [27]. Phylogenetic analyses supported three hypotheses concerning the molecular evolution of vertebrate *ALDH3-like* genes: (1) the mammalian *ALDH3A1* gene was generated by a tandem duplication event of an ancestral vertebrate *ALDH3A2* gene; (2) multiple mammalian and chicken *ALDH3B-like* genes were generated by tandem duplication events within genomes of related species; and (3) vertebrate *ALDH3A* and *ALDH3B* genes were generated within an ancestral genome, prior to the appearance of bony fish, more than 500 million years ago [40].

Conflict of interest statement

None

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Table 1: Aldehyde dehydrogenase (ALDH3A) genes and enzymes examined

GenBank mRNA (or cDNA) IDs identify previously reported sequences or predicted sequences (see <http://www.ncbi.nlm.nih.gov/Genbank/>); UNIPROT refer to UniprotKB/Swiss-Prot IDs for individual ALDHs (see <http://kr.expasy.org>). ALDH sequences are provided by the above sources. Vertebrate ALDH3A (ALDH3A1 and ALDH3A2) protein sequences were obtained from blast analyses of vertebrate genome sequences National Center for Biotechnology Information web tools (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and rat ALDH3A1 [4] and ALDH3A2 [9] sequences. Predicted exon/intron locations and gene sizes were obtained by BLAT interrogations of vertebrate genomes using the predicted ALDH sequences and UC Santa Cruz web tools (<http://genome.ucsc.edu>).

Animal	Species	ALDH Gene	RefSeq mRNA /Predicted [^]	Amino acids	Chromosomal coordinates	Gene Size (strand)	Coding exons	Uniprot ID
Human	<i>Homo sapiens</i>	3A1	NP_000682.3	453	17:19,582,228-19,589,034	6,807 (-ve)	10	P30838
Orangutan	<i>Pongo pygmaeus abelii</i>	3A1	XP_002834247 [^]	453	17:7,479,940-7,486,748	6,809 (-ve)	10	na
Rhesus monkey	<i>Macaca mulatta</i>	3A1	XP_001102220 [^]	453	16:19,254,826-19,261,848	7,023 (-ve)	10	na
Marmoset	<i>Callithrix jacchus</i>	3A1	XP_002748120 [^]	453	5:77,143,814-77,150,337	6,524 (-ve)	10	na
Mouse	<i>Mus musculus</i>	3A1	BC150677 ¹	453	11:61,022,244-61,031,916	9,673 (+ve)	10	P47739
Rat	<i>Rattus norvegicus</i>	3A1	NP_114178.1	453	10:47,368,564-47,374,585	6,022 (+ve)	10	P11883
Guinea Pig	<i>Cavia porcellus</i>	3A1	ENSCPOT00000015825 [^]	453	Contig71:688,016-693,637	5,622 (-ve)	10	na
Dog	<i>Canis familiaris</i>	3A1	NM_001082420	453	5:43,466,510-43,474,545	8,036 (+ve)	10	A3RF36
Horse	<i>Equus caballus</i>	3A1	XP_001918234 [^]	452	11:58,772,116-58,778,729	6,614 (+ve)	10	na
Cow	<i>Bos taurus</i>	3A1	NP_001159985	453	19:34,796,256-34,802,049	5,794 (+ve)	10	na
Cat	<i>Felix catus</i>	3A1	Contig151381.2_1 [^]	453	Contig151381:66,101-72,175	6,075 (-ve)	10	na
Opossum	<i>Monodelphis domestica</i>	3A1	XP_001372749 [^]	462	2:518,942,352-518,961,160	18,809 (-ve)	9	na
Platypus	<i>Ornitharynchus anatinus</i>	3A1	XP_001508369 [^]	453	Ultra69:171,876-181,973	10,221 (-ve)	9	na
Human	<i>Homo sapiens</i>	3A2	NP_000373.1	485	17:19,492,877-19,515,861	22,985 (+ve)	10	P51648
Orangutan	<i>Pongo pygmaeus abelii</i>	3A2	NM_001131237	485	17:7,384,858-7,412,594	27,737 (+ve)	10	na
Rhesus monkey	<i>Macaca mulatta</i>	3A2	XP_001101304 [^]	485	16:19,163,302-19,185,793	22,492 (+ve)	10	Q60HH8
Marmoset	<i>Callithrix jacchus</i>	3A2	XP_002748108 [^]	485	5:77,054,299-77,082,410	28,112 (+ve)	10	na
Mouse	<i>Mus musculus</i>	3A2	NM_007437	484	11:61,058,257-61,080,629	22,373 (-ve)	10	P47740
Rat	<i>Rattus norvegicus</i>	3A2	NP_113119	484	10:47,404,346-47,420,947	16,602 (-ve)	10	P30839
Guinea pig	<i>Cavia porcellus</i>	3A2	ENSCPOT00000013925 [^]	481	Contig71:634,338-658,061	23,724 (+ve)	9	na
Dog	<i>Canis familiaris</i>	3A2	XP_536656 [^]	484	5:43,521,358-43,539,078	17,721 (-ve)	10	na
Horse	<i>Equus caballus</i>	3A2	XP_001503562 [^]	594	11:57,639,505-57,662,302	28,958 (-ve)	11	na
Cow	<i>Bos taurus</i>	3A2	NP_001095454	485	19:34,839,902-34,851,760	11,850 (-ve)	10	na
Cat	<i>Felix catus</i>	3A2	ENSFCAT00000005181 [^]	450 ²	Contig151381:1,268-8,073 ²	13,688 (+ve) ²	9 ²	na
Opossum	<i>Monodelphis domestica</i>	3A2	XP_001365258 [^]	507	2:519,102,420-519,137,640	35,221 (-ve)	10	na
Platypus	<i>Ornitharynchus anatinus</i>	3A2	ENSOANT00000020116 [^]	470	Ultra69:122,029-136,432	14,404 (+ve)	10	na
Chicken	<i>Gallus gallus</i>	3A2	NP_001006223	490	19:6,791,454-6,794,935	3,482 (+ve)	9	Q5ZLE2
Frog	<i>Xenopus tropicalis</i>	3A2	NP_001016537	486	Scaffold72:1,185,178-1,196,603	11,426 (-ve)	10	na
Zebrafish	<i>Danio rerio</i>	3A2.1	NP_997814 ³	488	21:40,884,680-40,915,474	30,795 (-ve)	10	A7YFF4 ³
	<i>Danio rerio</i>	3A2.2	NP_997814 ³	488	21:40,587,096-40,617,889	30,794 (+ve)	10	A7YFF4 ³
Tetraodon	<i>Tetraodon nigroviridis</i>	3A2	GSTENT10018876001 [^]	529	7:8,245,367-8,252,752	7,386 (-ve)	10	na
Fugu	<i>Takifugu rubripes</i>	3A2	ENSTRU00000031834 [^]	472 ⁴	Un ⁵ :142,311,988-142,315,606	3,619 (+ve)	9 ⁴	na
Stickleback	<i>Gasterosteus aculeatus</i>	3A2	ENSGACT00000027123 [^]	478	VIII:19,058,006-19,064,550	6,545 (-ve)	10	na
Medaka	<i>Oryzias latipes</i>	3A2	ENSORILT00000003139 [^]	493	14:8,460,321-8,469,158	8,838 (-ve)	10	na
Nematode	<i>Caenorhabditis elegans</i>	3	NM_071144	437	V:1,996,109-1,999,581	3,405 (-ve)	6	na

Table 2: Aldehyde dehydrogenase (ALDH3B) genes and enzymes examined

GenBank mRNA (or cDNA) IDs identify previously reported sequences or predicted sequences (see <http://www.ncbi.nlm.nih.gov/Genbank/>); UNIPROT refer to UniprotKB/Swiss-Prot IDs for individual ALDHs (see <http://kr.expasy.org>). ALDH sequences are provided by the above sources. Vertebrate ALDH3B (ALDH3B1 and ALDH3B) protein sequences were obtained from blast analyses of vertebrate genome sequences National Center for Biotechnology Information web tools (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and rat ALDH3B1 [12] and ALDH3B2 [14] sequences. Predicted exon/intron locations and gene sizes were obtained by BLAT interrogations of vertebrate genomes using the predicted ALDH sequences and UC Santa Cruz web tools (<http://genome.ucsc.edu>).

Animal	Species	ALDH	RefSeq mRNA /Predicted ^A	Amino acids	Chromosomal coordinates	Gene Size	Coding	Uniprot ID
		Gene				(strand)	exons	
Human	<i>Homo sapiens</i>	381	BC013584 ¹	468	11:67,539,344-67,551,956	12,613 (+ve)	10	P43353
		382	NP_000686.2	385	11:67,187,265-67,190,741	3,477 (-ve)	7	P48448
Chimpanzee	<i>Pan troglodytes</i>	381	ENSPTRT00000007417 ^{A2}	468	11:66,232,082-66,245,136	13,055 (+ve)	9	na
		382	ENSPTRT00000045259 ^{A2}	384	11:66,158,638-66,162,590	3,953 (-ve)	8	na
Orangutan	<i>Pongo pygmaeus abelii</i>	381	ENSPPYT00000003575 ^{A2}	415 ²	11:8,105,491-8,114,867 ²	9,377 ² (-ve)	8 ²	na
		382	chr11.117.1 ^{A2}	375 ²	11:8,253,496-8,256,960 ²	3,465 ² (+ve)	7 ²	na
Rhesus monkey	<i>Macaca mulatta</i>	381	XP_001103842 ^A	468	14:6,693,135-6,705,696	12,562 (-ve)	9	na
		382	ENSMUT000000026056 ^A	385	14:6,790,986-6,794,458 ³	3,473 ³ (+ve)	7 ²	na
Marmoset	<i>Callithrix jacchus</i>	381	XP_002755684 ³	286 ³	11:123,522,170-123,536,620 ³	14,451 (+ve)	7 ³	na
		382	chr11.13.129 ^A	225 ^A	11:123,450,401-123,451,610 ^A	1,210 (-ve) ^A	4 ^A	na
Mouse	<i>Mus musculus</i>	381	BC046597 ¹	468	19:3,913,897-3,923,900	10,004 (-ve)	9	Q80VQ0
		382	BC100370 ¹	479	19:3,972,576-3,981,189	8,614 (+ve)	10	na
Rat	<i>Rattus norvegicus</i>	381	BC083850 ¹	468	1:206,438,748-206,449,646	10,899 (-ve)	9	Q5X142
		382	ENSRNOT00000024064 ^A	406	1:206,550,307-206,553,421	3,115 (+ve)	8	na
Guinea Pig	<i>Cavia porcellus</i>	381	ENSCPOT00000023180 ^A	468	Contig42:8,154,009-8,163,573	9,565 (+ve)	9	na
		382	ENSCPOT00000002557 ^A	460	Contig42:8,107,562-8,111,470	3,909 (-ve)	9	na
Dog	<i>Canis familiaris</i>	381	XP_533211 ^A	468	18:52,828,366-52,839,937	11,572 (-ve)	9	na
		382	ENSFAST00000017888 ^A	233 ³	18:52,883,871-52,885,936 ³	2,066 ³ (+ve)	5 ³	na
Horse	<i>Equus caballus</i>	381	XP_001498441 ^A	468	12:27,399,098-27,412,262	13,165 (+ve)	9	na
		382	ENSECAT00000004191 ^A	461	12:27,344,081-27,348,172	4,092 (-ve)	10	na
Cow	<i>Bos taurus</i>	381	BC147958 ¹	468	29:47,597,154-47,610,539	13,386 (-ve)	9	Q1JPA0
		382	ABG67109 ¹	319	29:47,459,617-47,463,844	4,228 (-ve)	7	na
Cat	<i>Felis catus</i>	381	scaffold_101138.001.1 ^A	433	scaffold101138:41,262-54,588	13,327 (+ve)	9	na
		382	scaffold_135347_2.1 ^A	142 ⁷	scaffold135347:28,737-33,205 ⁷	4,469 ⁷ (+ve)	3 ⁷	na
Opossum	<i>Monodelphis domestica</i>	381	XP_001362651 ^A	465	Un ⁶ :7,615,103-7,631,311	5,708 (+ve)	8	na
Chicken	<i>Gallus gallus</i>	381	XP_426371 ^A	496	5:79,353-85,060	5708 (+ve)	10	na
		382	chr5.006.1 ^A	440	5:70,087-78,443	8,357 (+ve)	10	na
Frog	<i>Xenopus tropicalis</i>	381	NP_001120622 ^A	469	Scaffold1331:29,673-49,585	19,913 (+ve)	9	na
Zebrafish	<i>Danio rerio</i>	381	NP_775328 ^A	473	3:31,432,266-31,439,644	7,379 (+ve)	9	na
Tetraodon	<i>Tetraodon nigroviridis</i>	381	CAG05951 ¹	537	Un ⁶ :5,227,801-5,231,123	3,323 (-ve)	7	na
Fugu	<i>Takifugu rubripes</i>	381	NP_001153657 ^A	511	Un ⁶ :336,405,772-336,414,383	8,612 (-ve)	13	na
Stickleback	<i>Gasterosteus aculeatus</i>	381	ENSACT00000007269 ^A	494	Xl:1,354,585-1,360,263	5,679 (+ve)	10	na
Medaka	<i>Oryzias latipes</i>	381	ENSORLT000000021685 ^A	471	8:25,485,641-25,491,169	5,529 (-ve)	10	na

Table 3: Key aldehyde dehydrogenase (ALDH) amino acid residues for mammalian ALDH3 sequences.

Sequences examined include: human ALDH3A1 (human 3A1); rat ALDH3A1 (rat 3A1); cow ALDH3A1 (cow 3A1); opossum ALDH3A1 (opossum 3A1); human ALDH3A2 (human 3A2); rat ALDH3A2 (rat 3A2); opossum ALDH3A2 (opossum 3A2); chicken ALDH3A2 (chicken 3A2); zebrafish ALDH3A2 (zebrafish 3A2); human ALDH3B1 (human 3B1); human ALDH3B2 (human 3B2); and *C.elegans* ALDH3-like sequence (elegans 3). Identification of predicted key catalytic and structural amino acid residues is based on 3D structural studies for rat ALDH3A1 [27]. S refers to substrate. See Figure 1 for the complete amino acid sequences for human and rat ALDHs.

Predicted	human	rat	cow	opossum	human	rat	cow	opossum	chicken	zebrafish	human	human	elegans
Function	3A1	3A1	3A1	3A1	3A2	3A2	3A2	3A2	3A2	3A2	3B1	3B2	3
s binding	Glu210 Cys244	Glu210 Cys244	Glu210 Cys244	Glu210 Cys244	Glu207 Cys241	Glu207 Cys241	Glu207 Cys241	Glu207 Cys241	Glu210 Cys244	Glu208 Cys241	Glu210 Cys244	Glu129 Cys263	Glu210 Cys244
catalysis	Asn115	Asn115	Asn115	Asn115	Asn112	Asn112	Asn112	Asn112	Asn115	Asn116	Asn112	Asn34	Asn116
glycine residues	Gly106 Gly132	Gly106 Gly132	Gly106 Gly132	Gly106 Gly132	Gly103 Gly129	Gly103 Gly129	Gly103 Gly129	Gly103 Gly129	Gly106 Gly132	Gly104 Gly130	Gly106 Gly132	Gly25 Gly51	Gly106 Gly132
chain folding	Gly168-9 Gly188	Gly168-9 Gly188	Gly168-9 Gly188	Gly168-9 Gly188	Gly165-6 Gly185	Gly165-6 Gly185	Gly165-6 Gly185	Gly165-6 Gly185	Gly168-9 Gly188	Gly166-7 Gly186	Gly168-9 Gly188	Gly87-8 Gly107	Gly168-9 Gly188
roles	Gly212-3 Gly241 Gly307 Gly337 Gly384-5 Gly403-4 Gly409	Gly212-3 Gly241 Gly307 Gly337 Gly384-5 Gly403-4 Gly409	Gly212-3 Gly241 Gly307 Gly337 Gly384-5 Gly403-4 Gly409	Gly212-3 Gly241 Gly307 Gly337 Gly384-5 Gly403-4 Gly409	Gly209-10 Gly238 Gly304 Gly334 Gly381-2 Gly400-1 Gly406	Gly209-10 Gly238 Gly304 Gly334 Gly381-2 Gly400-1 Gly406	Gly209-10 Gly238 Gly304 Gly334 Gly381-2 Gly400-1 Gly406	Gly209-10 Gly238 Gly304 Gly334 Gly381-2 Gly400-1 Gly406	Gly212-3 Gly241 Gly307 Gly337 Gly384-5 Gly403-4 Gly409	Gly210-1 Gly239 Gly305 Gly335 Gly382-3 Gly401-2 Gly407	Gly212-3 Gly241 Gly307 Gly337 Gly384-5 Gly403-4 Gly409	Gly131-2 Gly160 Gly226 Gly251 Gly263 Gly322-3 Gly328	Gly212-3 Gly241 Gly308 Gly340 Gly387-8 Gly406-7 Gly412
conserved proline residues	Pro104 Pro318 Pro341	Pro104 Pro318 Pro341	Pro104 Pro318 Pro341	Pro104 Pro318 Pro341	Pro101 Pro315 Pro338	Pro101 Pro315 Pro338	Pro101 Pro315 Pro338	Pro101 Pro315 Pro338	Pro104 Pro318 Pro341	Pro102 Pro316 Pro339	Pro104 Pro318 Pro341	Pro23 Pro237 Pro260	Pro104 Pro322 Pro344
stem of active site	Gly188 Gly212 Glu334 Phe336	Gly188 Gly212 Glu334 Phe336	Gly188 Gly212 Glu334 Phe336	Gly188 Gly212 Glu334 Phe336	Gly185 Gly209 Glu331 Phe333	Gly185 Gly209 Glu331 Phe333	Gly185 Gly209 Glu331 Phe333	Gly185 Gly209 Glu331 Phe333	Gly188 Gly212 Glu334 Phe336	Gly186 Gly210 Glu332 Phe334	Gly188 Gly212 Glu334 Phe336	Gly107 Gly131 Glu253 Phe255	Gly188 Gly212 Glu337 Phe339

Table 4: Major tissue distribution, substrates, functions and gene 'knock-out impacts for mammalian aldehyde dehydrogenases (ALDH)

3A1-ALDH3A1; 3A2-ALDH3A2; 3B1-ALDH3B1; 3B2-ALDH3B2 Reference sources are identified. See ALDH function review in [53].

ALDH	Major tissue and cellular distribution	Substrate/Function	Gene Knock-out Impacts
3A1	corneal epithelial cells [36,37,44] lens epithelial cells [44] stomach, trachea, lung, tongue [49] hepatoma [4]	peroxidic aldehyde oxidation [4,7,42,44] generation of NAD(P)H for UV-B absorption [7,36,37] C6+ aliphatic and aromatic aldehyde oxidation [4,7,42] cyclophosphamide and related cancer drug metabolism [47] oxidative stress regulation and cell homeostasis [48]	UV-B sensitivity [36,37,52] corneal dystrophy [36,37,52]
3A2	liver, kidney, adrenals [9,49]	fatty aldehyde oxidation [9,10]	Sjogren-Larsson syndrome [10]
3B1	kidney, lung [12,49]	aldehyde oxidation [12]	
3B2	salivary gland, adrenals [14,49]	aldehyde oxidation [13,14]	

Figure 1: Amino acid sequence alignments for human and rat aldehyde dehydrogenases ALDH3 sequences (color version)

See Table 1 for sources of ALDH sequences; 3A1 Rat-rat ALDH3A1; 3A1 Human-human ALDH3A1; 3A2 Human-human ALDH3A2; 3B1 Human-human ALDH3B1; 3B2 Human-human ALDH3B2; * shows identical residues; : shows 2 conservative substitutions; . shows 3 conservative substitutions; **bold font** shows known or predicted exon junctions; predicted or known [27] β -sheet (grey shading) and α -helix (yellow shading) secondary structures are shown; key residue identification is based on previous 3D studies of rat ALDH3A1 and likely predicted roles for amino acid residues [27]: **AS**-active site residues: **N**, **Asn114**; **E**, **Glu209** and **Glu 333**; and **C**, **Cys243**: **initiation** methionines are removed; α -helices and β -sheets are identified as α A, α B or α 10 etc or β 0, β 1 etc, respectively, as identified by Liu and coworkers [27]; **NP binding** refers

to coenzyme binding site; note the C-terminal **helix** predicted for the human ALDH3A2 sequence.

Figure 2: Predicted transmembrane helices for human and chicken aldehyde dehydrogenases ALDH3-like sequences (color version)

See Table 1 for sources of ALDH sequences. The TMHMM web tools of the Center for Biological Sequence Analysis, Technical University of Denmark TMHMM Server plots the probability of the ALDH sequence forming a **transmembrane helix** (0-1) (shown in **red** for the relevant amino acid sequences) (<http://www.cbs.dtu.dk/services/TMHMM/>). The predicted C-terminal transmembrane helices observed for human and chicken AL3A2 sequences are identified. Regions of the ALDH sequence predicted to be located inside or outside the membrane are shown in **blue** and **pink**, respectively.

Figure 3: Predicted tertiary structures for human ALDH3A1, ALDH3A2, ALDH3B1 and ALDH3B2 sequences (color version)

The predicted tertiary structures were obtained using the SWISS MODEL web site (<http://swissmodel.expasy.org/workspace>) and the predicted or known amino acid sequences for human ALDH3A1, ALDH3A2, ALDH3B1 and ALDH3B2 (see Table 1); the rainbow color code describes the predicted 3-D structures from the N- (blue) to C-termini (red); the designation of the three domains is based on the rat ALDH3A1 structure [27].

Figure 4: Proposed gene duplication events during the evolution of vertebrate aldehyde dehydrogenase ALDH3 (A) and phylogram of vertebrate and nematode aldehyde dehydrogenase ALDH3 sequences (B)

Each branch of the phylogram is labeled with the species name followed by an abbreviation of the gene name (3A1-ALDH3A1; 3A2-ALDH3A2; 3B1-ALDH3B1; 3B2-ALDH3B2; and 3-*C.elegans* ALDH3-like) sequences. Note the clustering into three ALDH groups (ALDH3A1, ALDH3A2 and ALDH3) of sequences. A genetic distance scale is shown. The number of times a clade (sequences common to a node or branch) occurred in the bootstrap replicates are shown. Only replicate values of 90 or more which are highly significant are shown with 100 bootstrap replicates performed in each case. Note the significant separation of clades for the mammalian ALDH3A1, and vertebrate ALDH3A2 and ALDH3B sequences. The *C.elegans* sequence was used to 'root' the tree.

Supplementary Table S1: Percentage identities for vertebrate and nematode aldehyde dehydrogenase (ALDH3-like) amino acid sequences

ALDH Gene	hu3A1	rh3A1	ra3A1	op3A1	pl3A1	hu3A2	rh3A2	ra3A2	op3A2	pl3A2	ch3A2	zf3A2	hu3B1	hu3B2	el3
hu3A1	100	98	86	80	83	71	70	70	73	74	69	63	57	52	51
rh3A1	98	100	86	80	83	71	70	70	74	75	70	63	56	52	51
ra3A1	86	86	100	75	80	68	68	69	69	72	68	62	57	52	52
op3A1	80	80	75	100	79	70	69	68	71	72	67	62	54	52	50
pl3A1	83	83	80	79	100	75	74	73	73	83	70	65	57	53	52
hu3A2	71	71	68	70	75	100	96	87	82	82	76	66	56	52	51
rh3A2	70	70	68	69	74	96	100	86	82	80	77	66	55	51	51
ra3A2	70	70	69	68	73	87	86	100	81	79	78	67	56	51	50
op3A2	73	74	69	71	73	82	82	81	100	79	78	64	55	50	49
pl3A2	74	75	72	72	83	82	80	79	79	100	74	66	57	54	51
ch3A2	69	70	68	67	70	76	77	78	78	74	100	68	56	51	50
zf3A2	63	63	62	62	65	66	66	67	64	66	68	100	55	53	46
hu3B1	57	56	57	54	57	56	55	56	55	57	56	55	100	88	46
hu3B2	52	52	52	52	53	52	51	51	50	54	51	53	88	100	40
el3	51	51	52	50	52	51	51	50	49	51	50	46	46	40	100

Numbers show the percentage of amino acid sequence identities; hu3A1-human ALDH3A1; rh3A1-rhesus ALDH3A1; ra3A1-rat ALDH3A1; op3A1A-opossum ALDH3A1; pl3A1-platypus ALDH3A1; hu3A2-human ALDH3A2; rh3A2-rhesus ALDH3A2; ra3A2-rat ALDH3A2; op3A2-opossum ALDH3A2; pl3A2-platypus ALDH3A2; ch3A2-chick ALDH3A2; zf3A2-zebrafish ALDH3A2; hu3B1-human ALDH3B1; hu3B2-human ALDH3B2; el3-*C.elegans* ALDH3-like sequence. The numbers in **bold** show higher sequence identities for ALDHs from within the same sub-family eg 3A1, 3A2 and 3B.

Figure S1: Amino acid sequence alignments for human ALDH3B1 and vertebrate ALDH3B2 sequences

See Table 2 for sources of ALDH3B sequences; 3B1 hu-human ALDH3B1; 3B2 hu-human ALDH3B2; 3B2 ch-chimpanzee ALDH3B2; 3B2 rh-rhesus ALDH3B2; 3B2 ho-horse ALDH3B2; 3B2 ra-rat ALDH3B2; 3B2 mo-mouse ALDH3B2; 3B2 gp-guinea pig ALDH3B2; 3B2 ck-chicken ALDH3B2; * shows identical residues; : shows 2 conservative substitutions; . shows 3 conservative substitutions; **bold font** shows known or predicted exon junctions; predicted or known [27] β -sheet (grey shading) and α -helix (yellow shading) secondary structures are shown; key residue identification is based on previous 3D studies of rat ALDH3A1 and likely predicted roles for amino acid residues [27]: **AS**-active site residues: **N**, Asn114; **E**, Glu209 and **Glu 333**; and **C**, Cys243: initiation methionines are removed; α -helices and β -sheets are identified as αA , αB or $\alpha 10$ etc or $\beta 0$, $\beta 1$ etc, respectively, as identified by Liu and coworkers [27]; **NP binding** refers to coenzyme binding site; note the C-terminal **helix** predicted for the human ALDH3A2 sequence.