

# Update on the aldehyde dehydrogenase gene (*ALDH*) superfamily

Brian Jackson,<sup>1</sup> Chad Brocker,<sup>1</sup> David C. Thompson,<sup>2</sup> William Black,<sup>1</sup> Konstandinos Vasiliou,<sup>1</sup> Daniel W. Nebert<sup>3</sup> and Vasilis Vasiliou<sup>1\*</sup>

<sup>1</sup>Molecular Toxicology and Environmental Health Sciences Program, Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Center, Aurora, CO 80045, USA

<sup>2</sup>Department of Clinical Pharmacy, University of Colorado Anschutz Medical Center, Aurora, CO 80045, USA

<sup>3</sup>Department of Environmental Health and Center for Environmental Genetics (CEG), University of Cincinnati Medical Center, Cincinnati, OH 45267, USA

\*Correspondence to: Tel: +1 303 724 3520; Fax: +1 303 724 7266; E-mail: vasilis.vasiliou@ucdenver.edu

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## Abstract

Members of the aldehyde dehydrogenase gene (*ALDH*) superfamily play an important role in the enzymic detoxification of endogenous and exogenous aldehydes and in the formation of molecules that are important in cellular processes, like retinoic acid, betaine and gamma-aminobutyric acid. *ALDH*s exhibit additional, non-enzymic functions, including the capacity to bind to some hormones and other small molecules and to diminish the effects of ultraviolet irradiation in the cornea. Mutations in *ALDH* genes leading to defective aldehyde metabolism are the molecular basis of several diseases, including gamma-hydroxybutyric aciduria, pyridoxine-dependent seizures, Sjögren–Larsson syndrome and type II hyperprolinaemia. Interestingly, several *ALDH* enzymes appear to be markers for normal and cancer stem cells. The superfamily is evolutionarily ancient and is represented within *Archaea*, *Eubacteria* and *Eukarya* taxa. Recent improvements in DNA and protein sequencing have led to the identification of many new *ALDH* family members. To date, the human genome contains 19 known *ALDH* genes, as well as many pseudogenes. Whole-genome sequencing allows for comparison of the entire complement of *ALDH* family members among organisms. This paper provides an update of *ALDH* genes in several recently sequenced vertebrates and aims to clarify the associated records found in the National Center for Biotechnology Information (NCBI) gene database. It also highlights where and when likely gene-duplication and gene-loss events have occurred. This information should be useful to future studies that might wish to compare the role of *ALDH* members among species and how the gene superfamily as a whole has changed throughout evolution.

**Keywords:** *ALDH*, aldehyde dehydrogenase, nomenclature, carbonyl metabolism, evolution, gene family

## Introduction

The aldehyde dehydrogenase gene (*ALDH*) superfamily is represented in all three taxonomic domains (*Archaea*, *Eubacteria* and *Eukarya*), suggesting a vital role throughout evolutionary history. Our understanding of the biological roles of this superfamily continues to expand in ways that are often unexpected and, perhaps, unprecedented for an enzyme family. As implied by their name,

members of this superfamily serve to metabolise both physiologically and pathophysiologically relevant aldehydes. This capacity prevents the accumulation of toxic aldehydes derived from endogenous production and/or exogenous exposures, which, if left unchecked, adversely affect cellular homeostasis and organismal functions.<sup>1</sup>

*ALDH* activity is also required for the synthesis of vital biomolecules through the metabolism of aldehyde intermediates, such as retinoic acid, folate

and betaine, to name a few.<sup>2–4</sup> Whereas the ability of the ALDH family members to metabolise reactive aldehydes represents a major underlying cytoprotective mechanism, it is important to recognise that ALDHs demonstrate functions that extend beyond detoxification. Accumulating evidence supports roles for ALDHs in the modulation of cell proliferation, differentiation and survival, especially through participation in retinoic acid synthesis.<sup>2</sup> Members of this superfamily also exhibit functions that appear to be independent of their enzyme activity, including absorption of ultraviolet (UV) irradiation in the cornea by acting as a crystallin and binding to hormones and other small molecules, including androgens, cholesterol, thyroid hormone and acetaminophen.<sup>2,5,6</sup>

Sequencing of the human genome and subsequent identification of mutations in *ALDH* genes associated with loss of ALDH enzyme activity have led to the identification of many disease associations, such as cataracts (ALDH1A1, ALDH3A1, ALDH18A1), seizures (ALDH7A1), hyperprolinaemia (ALDH4A1), heart disease (ALDH2), alcohol sensitivity (ALDH1A1, ALDH1B1, ALDH2), certain cancers (ALDH2) and a broad array of other metabolic and developmental abnormalities.<sup>2</sup> Recently, a role for ALDHs in normal and cancer stem cells has also been identified. For example, ALDH1A1 is differentially expressed in human haematopoietic stem cells (HSCs) and can be used as a stem cell marker for multiple cancers.<sup>2</sup> Similarly, ALDH1B1 is primarily expressed in stem cells in the normal colon and is strongly upregulated in human colonic adenocarcinomas.<sup>7,8</sup> As described by Nelson and colleagues,<sup>9</sup> genomic gene artefact identification becomes very important when using genotyping techniques to identify disease-causing alleles. Gene-duplication events, leading to multiple functional and/or non-functional genetic copies in the genome, can significantly complicate polymerase chain reaction (PCR)-based genotyping assays. Transgenic animal models have permitted the exploration of the functions of ALDHs under *in vivo* physiological and pathophysiological conditions.<sup>2</sup> These invaluable studies are heavily dependent upon our understanding of the mouse

and human genomes. In addition to mutations in *ALDH* genes within populations, there is a large variation in the number of *ALDH* genes between species.

During the past decade, the availability of gene and protein information has grown rapidly, primarily due to advances in gene-sequencing technologies. In the 2002 update of *ALDH* superfamily members,<sup>10</sup> 555 *ALDH* genes were listed, including 32 from *Archaea*, 351 from *Eubacteria* and 172 from *Eukarya*. Characteristic *ALDH* motifs were searched in 74 genomes: 16 in *Archaea*, 51 in *Eubacteria* and seven in *Eukarya*. A recent download from the current Pfam database (build version 24.0) includes 16,765 ALDH entries (listed as *aldehyd* in the Pfam database).<sup>11</sup> This update focuses on 11 representative vertebrate species in which the full genome has been sequenced: five primates, the cow, two rodents, two birds and one fish. Many of these genomes have been annotated automatically; generous algorithms list pseudogenes as protein-coding genes. This update attempts to describe the *ALDH* complement within these organisms and identify pseudogenes and gene-duplication events, when possible.

## Methods

Fully sequenced genomes from 11 representative species: primates (human, *Homo sapiens*; common chimpanzee, *Pan troglodytes*; common marmoset, *Callithrix jacchus*; Sumatran orangutan, *Pongo abelii*; Rhesus macaque, *Macaca mulatta*), the cow (*Bos taurus*), rodents (mouse, *Mus musculus*; rat, *Rattus norvegicus*), birds (zebra finch, *Taeniopygia guttata*; domestic chicken, *Gallus gallus*) and one fish (zebrafish, *Danio rerio*) were analysed.

*ALDH* genes were retrieved from Entrez Gene<sup>12</sup> using the terms 'ALDH' or 'aldehyde dehydrogenase'. Peptide sequences for each *ALDH* gene were retrieved from Entrez Protein<sup>12</sup> and aligned against a reference list of ALDH family members, including known human ALDHs and sequences from the NCBI's HomoloGene<sup>12</sup> using ClustalW.<sup>13</sup> To be included for description, a gene record was required to meet three criteria: 1) the protein product of the gene must be 'full-length' (ie

excludes known fragments and partial records); 2) the gene must have a known unique chromosomal location on the annotated genome; and 3) the gene must be listed as protein-coding (ie excludes known pseudogenes).

Parent genes were designated based on highest homology to the known human protein. Identified gene duplications were sequentially named according to nomenclature guidelines, based on decreasing sequence homology to the parent gene. Duplicated genes were further analysed to determine if they represented potentially new protein-coding genes or non-functional pseudogenes. Pseudogenes were identified according to criteria outlined previously<sup>9</sup> and assigned to the following categories: detritus pseudogenes (those which are fragments missing exons) and reverse-transcriptase events (those which resemble mRNA sequences and lack introns). If data suggested that a duplicated gene was protein coding, it was considered to be a new gene family member and named according to the previously established *ALDH* nomenclature system.<sup>14</sup> Zebrafish *aldh* genes were named according to the guidelines set out by the zebrafish nomenclature committee (<http://www.zfin.org>).<sup>15</sup> Pseudogenes in rodent (or fish) and non-rodent/non-fish genomes were appended with the suffix 'p' or 'P', respectively, and followed by a number designating multiple pseudogenes for a given gene family within each individual species.

It is, again, important to underscore that this initial analysis should be considered preliminary and subject to change as experimental evidence sheds light on actual protein function. Alignment and clustering of protein sequences were used as a basis for assigning homology. Sequences were aligned, and dendrograms based on neighbour-joining distances were created using a ClustalW webserver at <http://align.genome.jp>. Percentage amino acid (AA) identities were determined using the Needle webserver at (<http://www.ebi.ac.uk/Tools/emboss/align/>).<sup>16</sup>

To assess whether protein sequences were actively transcribed, we employed several methods. Numerous promoter-prediction programs were used, but none was sufficiently consistent across

species or discriminatory to be useful in the prediction of pseudogenes. The ratio of non-synonymous to synonymous ( $K_a/K_s$ ) nucleotide-substitution rates was used as a measure of selective pressure on each individual gene. Rates were calculated using homologous genes for all species in the current analysis, in order to determine ancestral states using the Bergen Center  $K_a/K_s$  Calculation Tool (<http://services.cbu.uib.no/tools/kaks/>) and default values, with the exception that the tree method was set to maximum likelihood.<sup>17</sup>

Copy number variants (CNV; defined here as gains and losses of DNA sequences >1 kilobase [kb]), insertions and deletions (InDels; gains and losses of DNA sequences of 100–999 base pairs [bp]), and inversions in human *ALDH* genes were retrieved from the Database of Genomic Variants.<sup>18</sup>

**Table 1.** List of all species examined in the current study, including the Latin name and common name and the number of unique *ALDH* genes found in each species. The data reflect the number of gene records found in the NCBI Gene Entrez database for each species, as of 13th March 2011

Latin name	Common name	# <i>ALDH</i> genes
<i>Homo sapiens</i>	Human	19
<i>Pan troglodytes</i>	Common chimpanzee	18
<i>Callithrix jacchus</i>	Common marmoset	16
<i>Pongo abelii</i>	Sumatran orangutan	18
<i>Macaca mulatta</i>	Rhesus macaque	20
<i>Bos taurus</i>	Cow	20
<i>Rattus norvegicus</i>	Norway rat	21
<i>Mus musculus</i>	House mouse	21
<i>Taeniopygia guttata</i>	Zebra finch	15
<i>Gallus gallus</i>	Chicken	14
<i>Danio rerio</i>	Zebrafish	25

## Results

Records for *ALDH* genes were retrieved and sorted for all 11 species analysed (Table 1). The number of records that met the above-mentioned criteria is provided (ie the number of genes excluding non-functional pseudogenes). The number of *ALDH* genes per species varied from 14 in chicken to 25 in zebrafish. There are currently 207 distinct genes present within the database for these 11 species; this is a greater than fourfold increase from 2002, when only 51 were annotated.<sup>10</sup> This allows for a much more comprehensive comparison of *ALDH* super-family members throughout vertebrate evolution during the past 450 million years. It is important to keep in mind that, for many species, some genes have yet to be identified. Further, many annotated genes may reflect gene-duplication events that represent non-functional pseudogenes. These situations will be explored in greater depth below.

The total number of human annotations has remained unchanged since 2005, with 19 functional protein-coding genes.<sup>19</sup> The chimpanzee and the orangutan genomes diverged from humans ~5 and ~14 million years ago (MYA), respectively.<sup>20,21</sup> Both the chimpanzee and orangutan genomes contain 18 *ALDH* genes, each corresponding to a known human orthologue. The macaque and common marmoset genomes are more distantly related. They diverged ~25 and 35–40 MYA<sup>22</sup> and contain 20 and 16 *ALDH* members, respectively. Orthologues for all 19 human genes were identified in mouse and rat. In addition, rodent genomes contain an *Aldh1a1* paralogue (*Aldh1a7*) and an *Aldh3b2* gene duplication, resulting in a total of 21 *Aldh* genes. The most recent common ancestor of humans and rodents lived 75–90 MYA.

The cow genome, which diverged from that of the human 80–100 MYA, has 20 annotated *ALDH* entries which, again, closely parallel human members. Variations include two gene duplications and one possible deletion. Both avian genomes currently lack orthologous entries for *ALDH1A1*, *ALDH1B1*, *ALDH1L1*, *ALDH3A1*, *ALDH3B2* and *ALDH16A1*. Moreover, the zebra finch genome is also missing annotated sequences for

*ALDH18A1* and includes two apparent gene duplications.

Table 2 summarises these *ALDH* orthologues, their chromosomal locations and the associated NCBI Entrez gene identification (ID) number for each of the 11 species. For zebrafish, Entrez gene ID 100334142 was listed as ‘aldehyde dehydrogenase 1A1-like [*D. rerio*]’. This gene record appears to be derived from an unplaced chromosomal fragment, however, because no genome location could be determined. In addition, alignment of the peptide sequence for this gene ID to other mammalian *ALDH1A1* protein sequences was poor. Specifically, sequence homology with human, mouse and rat *ALDH1A1* was only 26.2 per cent, 26.4 per cent and 26.8 per cent, respectively. NCBI BlastP analysis indicated that it most closely resembles bacterial *ALDH* proteins. Together, this evidence suggests that this record may represent bacterial contamination, rather than a true zebrafish gene; thus, we have not included this gene. This also makes the zebrafish the only species among the 11 analysed that lacks a record for *ALDH1A1*. Interestingly, a protein blast (blastp) search using human *ALDH1A1* and limiting results to fish species only (NCBI taxid: 7898) revealed *ALDH1A2* homologues in multiple species (including salmon, pufferfish, ricefish and bichir), but no records for *ALDH1A1* in any fish species. This is consistent with previous findings that indicate that *ALDH1A1* is not present in the teleost lineage.<sup>23</sup>

We found evidence for several gene duplications. Table 3 lists all genes that show duplications, compared with genes in the human genome. This table provides a summary of existing information available within the NCBI gene entries, as well as recommended gene names based on our analyses and current nomenclature guidelines.

Table 4 lists additional information related to peptide sequences and calculated sequence identities. Additional genes (increase in gene number, compared with humans) show peptide divergence of as little as 0.4 per cent (zebrafish *aldh2.2* and *aldh2.3*) and as much as 64.9 per cent (zebrafish *aldh3a2.1* and *aldh3a2.2*). In most cases, gene duplications have similar sizes, are often nearby on the same

**Table 2.** ALDH genes and duplicated genes across species with respective chromosome (Chr) locations. Numbers in parentheses indicate NCBI Entrez gene ID (GI). Records in bold text denote duplications compared with the human genome. Z, the sex Chr in birds (ZW system); cM, centiMorgans. Letter designations in mouse gene locations indicate chromosomal regions

Gene (by homology)	Primates		Rodents			Birds			Fish	
	Human	Orangutan	Cow	Rat	Mouse	Zebra finch	Chicken	Zebrafish*		
ALDH1A1	9q21.13 (216)	9 (100174688)	8 (281615)	1q51 (24188)	19 12.0 cM (11668)	Z (100223406)	Z (395264)			
ALDH1A2	15q21.3 (8854)	15 (100171834)	10 (535075)	8q24 (116676)	9 42.0 cM (19378)	10 (751771)	10 (395884)	7 (116713)		
ALDH1A3	15q26.3 (220)	15 (100452276)	21 (507093)	1q22 (266603)	7 (56847)	10 (100231202)	10 (395389)	7 (751785)		
			<b>28 (534200)</b>							
ALDH1A7				1q51 (29651)	19 20.0 cM (26358)					
ALDH1B1	9q11.1 (219)	9 (100174654)	8 (281618)	5q22 (298079)	4 B2 (72535)					
ALDH1L1	3q21.3 (10840)	3 (100172380)	3 (505677)	4 (64392)	6 (107747)		6 (798292)			
ALDH1L2	12q23.3 (160428)	12 (100459691)	5 (516864)	7q13 (299699)	10 (216188)	1A (100230131)	1 (418078)	4 (100333269)		
ALDH2	12q24.2 (217)	12 (100171596)	17 (508629)	12q16 (29651)	5 F-G1 (11669)	15 (100217978)	15 (416880)	5 (393462)		
								<b>5 (368239)</b>		
								<b>5 (100332355)</b>		
ALDH3A1	17p11.2 (218)	17 (100446485)	19 (281617)	10q22 (25375)	11 34.25 cM (11670)					
ALDH3A2	17p11.2 (224)	17 (100171557)	19 (513967)	10q22 (65183)	11 34.3 cM (11671)	19 (100230924)	19 (417615)	15 (323653)		
						<b>19 (100226132)</b>		<b>15 (10000026)</b>		
								<b>21 (100329417)</b>		
								<b>21 (447920)</b>		
ALDH3B1	11q13 (221)	11 (100450634)	29 (511469)	1q42 (309147)	19 (67689)	5 (100232483)	5 (428813)	5 (557008)		
			<b>29 (508879)</b>			<b>5 (100229547)</b>				
ALDH3B2	11q13 (222)			1q42 (688800)	19 (621603)					
				<b>1q42 (688778)</b>	<b>19 (73458)</b>					
ALDH3D1								3 (282559)		
ALDH4A1	1p36 (8659)	1 (10072770)	2 (100126042)	5q36 (641316)	4 66.1 cM (212647)	21 (100228902)	21 (419467)	11 (394133)		

Continued



Table 2. Continued

Gene (by homology)	Primates		Rodents			Birds			Fish	
	Human	Orangutan	Cow	Rat	Mouse	Zebra finch	Chicken	Zebrafish*	Zebrafish*	
<b>ALDH5A1</b> 6p22 (7915)	6 (100458767)	23 (532724)	17p11 (291133)	13 A3.1 (214579)	2 (100222151)	2 (420818)	16 (565235)	<b>16 (100330723)</b>		
<b>ALDH6A1</b> 14q24.3 (4329)	14 (100171652)	10 (327692)	6q31 (81708)	12 39.0 cM (104776)	5 (1002226750)	5 (423345)	17 (436647)			
<b>ALDH7A1</b> 5q31 (501)	5 (100461726)	7 (507477)	18q12.1 (291450)	18 29.0 cM (110695)	Z (100223716)	Z (426812)	10 (334197)			
<b>ALDH8A1</b> 6q23.2 (64577)	6 (100450228)	9 (513537)	1p12 (685750)	10 (237320)	3 (100222753)	3 (421695)	23 (447801)			
<b>ALDH9A1</b> 1q23.1 (223)	1 (100173126)	3 (537539)	13q24 (64040)	1 H2 (56752)	8 (100225645)	8 (424405)	8 (100005587)			
							<b>2 (399481)</b>			
							<b>8 (100006238)</b>			
<b>ALDH16A1</b> 19q13.33 (126133)	19 (100434496)	18 (506329)	1q22 (361571)	7 (69748)			3 (492710)			
<b>ALDH18A1</b> 10q24.3 (5832)	10 (100173488)	26 (514759)	1q54 (361755)	19 (56454)			6 (423976)	12 (557186)		
							<b>12 (100329417)</b>			

\*Zebrafish genes are named in accordance with nomenclature guidelines described at <http://www.zfin.org> and established by Mullins et al.<sup>15</sup>

**Table 3.** List of the Entrez Gene genes ID (GI), chromosome location, presence of introns, gene type and recommended gene name of all ALDH genes in this study that show evidence of gene duplication, compared with that in the human genome

Gene (by homology)	Species	NCBI Gene ID	NCBI Gene name	Chromosome	Chromosomal location		Introns	Gene type	Recommended gene name
					Ref Seq ID	Range			
<b>ALDH1A3</b>	Cow	507093	ALDH1A3	21	NC_007319.4	4,261,104–4,301,275	yes	Parent gene	ALDH1A3
		534200	LOC534200	28	NC_007329.4	11,750,749–11,762,637	yes	Pseudogene — detritus	ALDH1A3PI
<b>ALDH2</b>	Zebratfish*	393462	<i>aldh2a</i>	5	NC_007116.4	71,734,127–71,754,941	yes	Parent gene	<i>aldh2.1</i> *
		368239	<i>aldh2b</i>	5	NC_007116.4	71,708,861–71,732,452	yes	New gene	<i>aldh2.2</i> *
		100332355	LOC100332355	5	NC_007116.4	71,632,543–71,658,511	yes	New gene	<i>aldh2.3</i> *
<b>ALDH3A2</b>	Zebratfish*	323653	<i>adh3a2</i>	15	NC_007126.4	21,001,391–21,009,951	yes	Parent gene	<i>aldh3a2.1</i> *
		10000026	LOC10000026	15	NC_007126.4	20,970,670–20,976,922	yes	New gene	<i>aldh3a2.2</i> *
		100329417	LOC100329417	21	NC_007132.4	40,585,351–40,617,892	yes	New gene	<i>aldh3a2.3</i> *
		447920	<i>zgc:103715</i>	21	NC_007132.4	40,905,693–40,917,184	yes	Pseudogene — detritus	<i>aldh3a2p1</i>
	Zebra finch	100230924	LOC100230924	19	NC_011483.1	8,354,898–8,361,968	yes	Parent gene	ALDH3A2
		100226132	LOC100226132	19	NC_011483.1	8,364,080–8,368,708	yes	New gene	ALDH3A3
<b>ALDH3B1</b>	Cow	511469	ALDH3B1	29	NC_007330.4	47,708,146–47,722,523	yes	Parent gene	ALDH3B1
		508879	LOC508879	29	NC_007330.4	47,568,715–47,575,449	yes	New gene	ALDH3B4
	Zebra finch	100232483	LOC100232483	5	NC_011469.1	7,960,933–7,967,624	yes	Parent gene	ALDH3B1
		100229547	LOC100229547	5	NC_011469.1	7,968,165–7,973,465	yes	New gene	ALDH3B5
<b>ALDH3B2</b>	Rat	688800	ALDH3B2	1	NC_005100.2	206,549,529–206,553,424	yes	Parent gene	ALDH3B2
		688778	LOC688778	1	NC_005100.2	206,500,430–206,510,746	yes	New gene	ALDH3B3
	Mouse	621603	<i>Aldh3b2</i>	19	NC_000085.5	3,972,328–3,981,665	yes	Parent gene	<i>Aldh3b2</i>
		73458	1700055N04Rik	19	NC_000085.5	3,958,808–3,969,947	yes	New gene	<i>Aldh3b3</i>
<b>ALDH5A1</b>	Zebratfish*	565235	<i>aldh5a1</i>	16	NC_007127.4	35,584,243–35,592,745	yes	Parent gene	<i>aldh5a1.1</i> *

Continued

Table 3. Continued

Gene (by homology)	Species	NCBI Gene ID	NCBI Gene name	Chromosome	Chromosomal location		Introns	Gene type	Recommended gene name
					Ref Seq ID	Range			
		100330723	LOC100330723	16	NC_007127.4	35,723,717–35,735,263	yes	New gene	<i>aldh5a1.2*</i>
<b>ALDH7A1</b>	Macaque	702749	ALDH7A1	6	NC_007863.1	122,937,640–122,989,782	yes	Parent gene	ALDH7A1
		716090	LOC716090	14	NC_007871.1	68,342,919–68,344,780	no	Pseudogene — RT event	ALDH7A1P5
<b>ALDH9A1</b>	Zebrafish*	100005587	<i>aldh9a1a</i>	8	NC_007119.4	21,476,877–21,484,987	yes	Parent gene	<i>aldh9a1.1*</i>
		399481	<i>aldh9a1b</i>	2	NC_007113.4	4,838,438–4,863,128	yes	New gene	<i>aldh9a1.2*</i>
		100006238	LOC100006238	8	NC_007119.4	21,464,110–21,473,710	yes	New gene	<i>aldh9a1.3*</i>
<b>ALDH18A1</b>	Zebrafish*	557186	<i>aldh18a1</i>	12	NC_007123.4	29,670,615–29,686,508	yes	Parent gene	<i>aldh18a1.1*</i>
		100332705	LOC100332705	12	NC_007123.4	29,643,982–29,661,436	yes	New gene	<i>aldh18a1.2*</i>

\*Zebrafish genes are named in accordance with nomenclature guidelines described at <http://www.zfin.org> and established by Mullins et al.<sup>15</sup> RT, reverse transcription



**Table 4.** Tabulation of all ALDH genes in this study that show evidence of gene duplication, compared with that in the human genome. Included are protein lengths (in number of amino acids [AAs]),  $K_a/K_s$  values, RefSeq protein IDs and recommended protein names. '% AA identity' denotes the absolute number of identical AAs relative to the absolute number of AA locations. '% AA unaligned' indicates the percentage of AAs that are represented by either a gap in the alignment of either sequence or an overhang if one sequence is longer than the other. '% AA identity (unaligned excluded)' indicates the percentage of AA locations that are identical when unaligned AAs are excluded from the total number of AA locations. For example, a 127-AA fragment of a 537-AA protein, which is identical except for the truncation, would have  $127/537 = 23.6$  per cent identity, of which  $410/537 = 76.4$  per cent is represented by unaligned residues (AAs in the longer sequence that have no correlation with the shorter sequence) but, excluding those residues,  $127/127 = 100$  per cent paired AAs are identical. The final column indicates which sequences are being compared for percentage identity, percentage gaps and percentage identity (excluding gaps)

Species	Recommended gene name	RefSeq Protein ID	Protein length	Ka/Ks	Aligned sequences	% AA (unaligned)	% AA Identity (unaligned included)	% AA Identity (unaligned excluded)	Functional protein	Recommended protein name
Cow	ALDH1A3	XP_583647.3	537	0.234	–	–	–	–	Yes	ALDH1A3
	ALDH1A3P1	XP_001789867.1	127	0.260	(a)/(b)	76.4	23.6	100	No	Pseudogene
	Zebrafish <i>aldh2.1</i>	NP_956784.1	516	0.278	–	–	–	–	Yes	<i>Aldh2.1</i>
Zebrafish	<i>aldh2.2</i>	NP_998466.2	516	0.112	(a)/(b)	0	95.2	95.2	Yes	<i>Aldh2.2</i>
	<i>aldh2.3</i>	XP_002662252.1	516	0.041	(a)/(c)	0	95.2	95.2	Yes	<i>Aldh2.3</i>
					(b)/(c)	0	99.6	99.6	–	–
Zebrafish	<i>aldh3a2.1</i>	NP_997814.1	488	0.175	–	–	–	–	Yes	<i>Aldh3a2.1</i>
	<i>aldh3a2.2</i>	XP_001335979.2	489	0.402	(a)/(b)	1.8	63.1	64.9	Yes	<i>Aldh3a2.1</i>
	<i>aldh3a2.3</i>	XP_002666107.1	514	0.175	(a)/(c)	5.1	65.8	70.9	Yes	<i>Aldh3a2.3</i>
	<i>aldh3a2pl</i>	NP_001004658.1	169	0.190	(a)/(d)	65.6	23.5	89.1	No	Pseudogene
Zebra finch					(b)/(c)	7.5	57.4	64.9	–	–
					(b)/(d)	66	18.9	84.9	–	–
					(c)/(d)	67.1	31.3	98.4	–	–
Zebra finch	ALDH3A2	XP_002198810.1	510	0.396	–	–	–	–	Yes	ALDH3A2
Cow	ALDH3A3	XP_002196134.1	526	0.625	(a)/(b)	5.6	84.1	89.7	Yes	ALDH3A3
	ALDH3B1	NP_001068986.1	486	0.335	–	–	–	–	Yes	ALDH3B1
	ALDH3B4	XP_585724.2	486	0.550	(a)/(b)	4.5	80.9	85.4	Yes	ALDH3B4

Continued

Table 4. Continued

Species	Recommended gene name	RefSeq Protein ID	Protein length	Ka/Ks	Aligned sequences	% AA (unaligned)	% AA Identity (unaligned included)	% AA Identity (unaligned excluded)	Functional protein	Recommended protein name
<b>Zebra finch</b>	ALDH3B1	XP_002196917.1	450	0.308	–	–	–	–	Yes	ALDH3B1
	ALDH3B5	XP_002196928.1	341	0.434	(a)/(b)	39.9	53.2	93.1	Yes	ALDH3B5
<b>Rat</b>	Aldh3b2	XP_001068348.2	483	0.436	–	–	–	–	Yes	ALDH3B2
	Aldh3b3	XP_001068253.1	530	0.239	(a)/(b)	11	76.9	87.9	Yes	ALDH3B3
<b>Mouse</b>	Aldh3b2	NP_001170909.1	479	0.270	–	–	–	–	Yes	ALDH3B2
	Aldh3b3	XP_900106.1	479	0.229	(a)/(b)	0	86.4	86.4	Yes	ALDH3B3
<b>Zebrafish</b>	aldh5a1.1	NP_001103938.1	404	<0.001	–	–	–	–	Yes	Aldh5a1.1
	aldh5a1.2	XP_002664997.1	514	0.008	(a)/(b)	21.4	78.6	100	Yes	Aldh5a1.2
<b>Macaque</b>	ALDH7A1	XP_002804539.1	502	0.180	–	–	–	–	Yes	ALDH7A1
	ALDH7A1P5	XP_001111963.1	538	1.289	(a)/(b)	16.3	82.3	98.6	No	Pseudogene
<b>Zebrafish</b>	aldh9a1.1	NP_958879.1	508	0.126	–	–	–	–	Yes	Aldh9a1.1
	aldh9a1.2	NP_958916.1	518	0.154	(a)/(b)	1.9	71.2	73.1	Yes	Aldh9a1.2
	aldh9a1.3	NP_001119952.1	508	0.190	(a)/(c)	0	94.9	94.9	Yes	Aldh9a1.3
					(b)/(c)	1.9	70.3	72.2	–	–
<b>Zebrafish</b>	aldh18a1.1	NP_001077015.1	782	0.103	–	–	–	–	Yes	Aldh18a1.1
	aldh18a1.2	XP_002664020.1	782	0.826	(a)/(b)	0	100	100	Yes	Aldh18a1.2

chromosome (Chr) and show some degree of divergence (ie 70–95 per cent AA identity). Genes that have portions of the gene copied with no AA divergence include: cow *ALDH1A3P1* (127 of 537 AAs), zebrafish *aldh5a1.2* (404 of 514 AAs) and zebrafish *aldh18a1.2* (782 of 782 AAs). Zebrafish *aldh3a2.3* (169 of 514 AAs) represents a shortened copy which shows minor divergence (98.4 per cent identity).  $K_a/K_s$  ratios were calculated for all gene duplications. A value of  $<1.0$  indicates selective pressure to conserve the gene and suggests that it plays a functional role. All duplications were found to have a score of  $<1.0$ , except macaque *ALDH7A1P5* (to be discussed below).

### ALDH1

*ALDH1A1* is present in all species except zebrafish, confirming earlier studies.<sup>23</sup> In cow, there are two distinct records for *ALDH1A3*: the gene found on Chr 21 is full length (537 AAs) and represents the putatively functional parent gene (*ALDH1A3*), whereas the second is a detritus pseudogene on Chr 28 which appears to be the product of a partial gene-duplication event (*ALDH1A3P1*). The shorter genomic sequence would translate a peptide sharing 100 per cent sequence identity to only the 127 carboxy-terminal AAs of the full-length parent protein. Several gene duplications appear to have been conserved in rodents. One such gene is *ALDH1A7*, found in rats and mice. In both cases, the *ALDH1A7* gene is present on the same chromosome and in close proximity to *ALDH1A1*. Mouse *ALDH1A7* shares 92 per cent AA identity with mouse *ALDH1A1*, and studies have confirmed that the gene encodes inducible tissue-specific mRNA.<sup>24</sup> *ALDH1B1* is present in mammals but missing from birds and fish. *ALDH1L1* is missing from both bird species (zebra finch and chicken) but present in other species examined and thus may represent a deletion in the avian lineage.

### ALDH2

*ALDH2* appears to be one of many genes duplicated in zebrafish. It has been suggested that an

entire genome duplication event may have occurred after the divergence of teleosts and mammals;<sup>25</sup> this may explain the increased *ALDH* gene number in zebrafish. A second gene-duplication event appears to have occurred, giving rise to three zebrafish *aldh2* gene records (*aldh2.1-3*). The *aldh2.1* gene is believed to be the parent, based on homology with orthologous *ALDH2* protein sequences. Both *aldh2.2* and *aldh2.3* potentially encode full-length peptides. *Aldh2.2* is 95.2 per cent similar to *Aldh2.1* and *aldh2.3* may represent a more evolutionarily recent duplication of *aldh2.2*, as evidenced by 99.6 per cent AA identity between the *Aldh2.2* and *Aldh2.3* proteins.

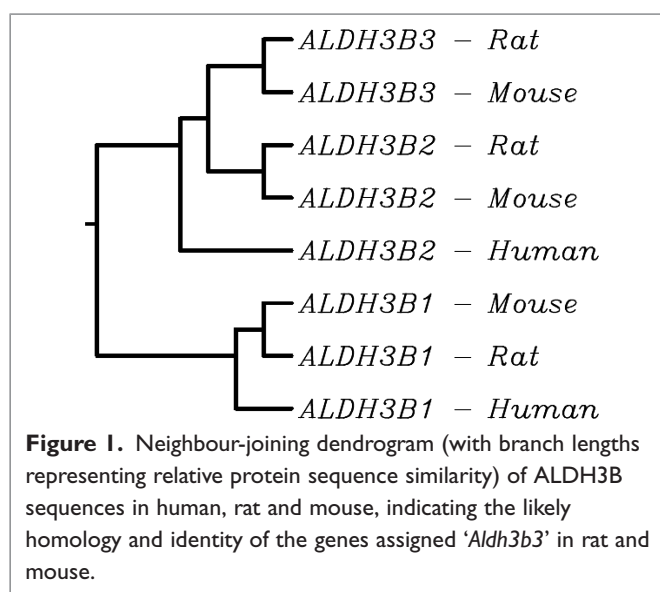
### ALDH3

The *ALDH3* genes show the most variation in gene number of any *ALDH* family among the organisms studied. *ALDH3A1* is missing from birds and fish but is present in every mammalian genome analysed in this study. The zebra finch has a duplicate *ALDH3A2* (*ALDH3A3*) entry which encodes a full-length peptide that shares 84.1 per cent identity with the parent protein. Four *ALDH3A2* homologues were identified within the zebrafish genome. The *aldh3a2.1* is considered the parent gene. The *aldh3a2.2* and *aldh3a2.3* full-length gene products, respectively, share 64.9 per cent and 70.9 per cent sequence identity with that of *Aldh3a2.1* and 64.9 per cent identity with each other. Zebrafish *aldh3a2p1* represents a partial gene duplication; the resulting 169-AA peptide would most likely undergo proteolytic degradation if translated.

*ALDH3B1* is duplicated in cow and zebra finch, as well as in zebrafish, on the proviso that *D. rerio* *aldh3d1* is also considered an *ALDH3B1* homologue. Zebrafish *Aldh3d1* shares 44 per cent AA identity with *Aldh3b1* and is listed in NCBI HomoloGene as a homologue of *ALDH3B1* (HomoloGene, data not shown).<sup>12</sup> Zebra finch *ALDH3B5* encodes a 341-AA peptide that shares 100 per cent sequence identity with the 228 amino-terminal AAs of the parent gene's protein. Cow and zebra finch *ALDH3B4* and *ALDH3B5* proteins share 80.9 per cent and 53.2 per cent

sequence identity with their respective parent genes, and 39.7 per cent with one another, indicating that none of the genes is an orthologue. Zebra finch *ALDH3B5* is shorter than *ALDH3B1* (341 versus 450 AAs) and, without this sequence gap, they share 93.1 per cent AA identity; it is unknown whether this smaller gene product is functional.

*ALDH3B2* is present as a single distinct gene in human, chimpanzee and macaque, whereas two copies occur in mouse and rat. *ALDH3B2* is absent from common marmoset, cow, zebra finch, chicken and zebrafish. Mouse and rat *ALDH3B3* share 86.4 per cent and 76.9 per cent AA identity, respectively, with the corresponding parent *ALDH3B2* proteins and 83.4 per cent identity with each other. The two *ALDH3B3* genes are found on corresponding syntenic chromosomes within their respective genomes. Presently, the protein product of Entrez Gene ID 688778 (*R. norvegicus*) is annotated as '*ALDH3B1* (predicted)'. Based on a phylogenetic clustering of *ALDH3B1* and *ALDH3B2* protein sequences (Figure 1), however, we believe it is better to name this protein *ALDH3B3*; this shows that both mouse and rat *ALDH3B3* proteins are in the *ALDH3B2* clade and are more similar to each other than to rodent or human *ALDH3B2* proteins. The alignment used for phylogenetic clustering can be seen in Supplementary Table S1.



#### ***ALDH4***

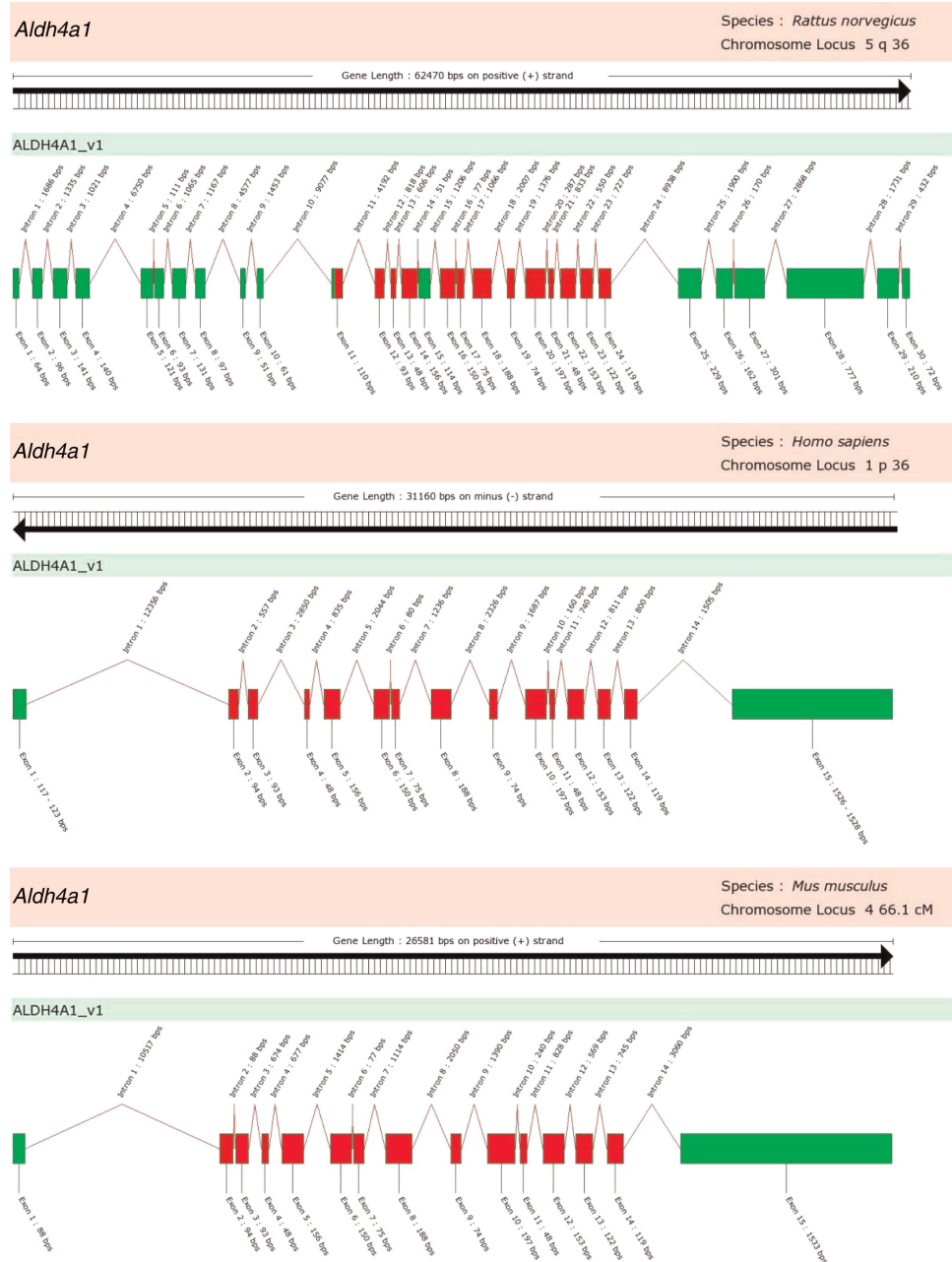
*ALDH4A1* is missing from chimpanzee and common marmoset but is present in all others. Previously, rat *ALDH4A1* had been conspicuously absent from the major databanks but it was recently added. During a BLAST search of the rat genome using various individual exon segments from mouse *Aldh4a1*, significant hits for *Aldh4a1* in the rat genome were identified on Chr 5q36 and it was determined to be a part of the fusion gene *LRRP Ba1-651*.<sup>26</sup> Figure 2 shows an assembled structure of this fusion gene with the *Aldh4a1* exons highlighted in red. Although it appears that these exons are transcribed and contain the conserved *ALDH* catalytic domain, it is not clear whether the gene product retains aldehyde dehydrogenase activity.

#### ***ALDH5* and beyond**

*ALDH5A1* is missing in marmoset and duplicated in zebrafish. The zebrafish duplication, *aldh5a1.2*, encodes a slightly truncated peptide (404 versus 514 AAs) which shares 100 per cent AA identity with the first 426 AAs and resides on the same Chr as *aldh5a1.1*.

*ALDH7A1* is duplicated in the macaque. The *ALDH7A1P5* duplication is located on Chr 14 and contains the complete *ALDH7A1* coding sequence; however, the sequence lacks any intronic regions, suggesting a reverse transcriptase-mediated duplication event. Furthermore, this gene has a  $K_a/K_s$  score of 1.289, indicating a lack of selective pressure to conserve this gene. This provides further evidence that *ALDH7A1P5* does not code for a functional protein.

In zebrafish, *aldh9a1* has three additional copies. The parent gene *aldh9a1.1* and *aldh9a1.3* reside on Chr 8; *aldh9a1.2* is found on Chr 2. Both *aldh9a1.2* and *aldh9a1.3* encode putative full-length proteins which respectively share 71.2 per cent and 94.9 per cent AA identity with *Aldh9a1.1* and 70.3 per cent sequence identity with each other. Zebrafish also contains a duplication of *aldh18a1*. The *aldh18a1.2* is found on the same chromosome and encodes a protein that is 100 per cent identical with that of the parent gene.



**Figure 2.** Comparison of ALDH4A1 from human and rat. Rat *Aldh4a1* is part of the larger fusion gene *LRRP Bal-651*.<sup>26</sup> The exons representing the *Aldh4a1* portion of this gene with homology to mouse and human are highlighted.

The naming of zebrafish genes required further genomic analyses in order to determine whether duplications originated from the ray-finned lineage whole-genome duplication event. Many of the duplicated genes reside within close proximity on the same chromosome, suggesting that they are

segmental duplications that resulted from misguided recombination processes during meiosis and not a product of the whole genome duplication that took place within the ray-fin lineage.<sup>27</sup> These include the *aldh2*, *aldh5a1* and *aldh18a1* paralogues, which are located in close proximity on Chr 5, 16 and 12,



respectively. It also includes *aldh3a2.1* and *aldh3a2.2*, located on Chr 15, as well as *aldh9a1.1* and *aldh9a1.3*, found on Chr 8. The gene architecture surrounding *aldh3a2.3* on Chr 21 does not support a duplicated chromosome, in that the region lacks other duplicated genes from Chr 15. Furthermore, studies looking at zebrafish gene duplications found that a high frequency of genes found on Chr 21 are duplicated on Chr 5 and none were identified on Chr 15, suggesting that Chr 5, rather than Chr 15, is the paralogous chromosome.<sup>28,29</sup> A similar situation was identified with respect to *aldh9a1.2* on Chr 2. Uridine–cytidine kinase-2 homologues (*uck2a* and *uck2b*) are found upstream of both *aldh9a1.1* and *aldh9a1.2*, supporting a tandem gene-duplication event; however, other genes in close proximity to this duplication do not show any homology between chromosomes 2 and 8.

#### Alternatively spliced transcriptional variants and CNVs of human *ALDH* genes

In addition to the increase in *ALDH* identification through genomic sequencing, other sources of complexity in the *ALDH* superfamily are being studied. Transcript sequencing has revealed that many *ALDH* genes encode multiple mRNA splice variants (for a review of human *ALDH* splice variants, see Black *et al.*<sup>30</sup>). Besides splice variants, CNVs have been reported for human *ALDH* genes. By querying the Database of Genomic Variants, 35 CNVs, 28 InDels and one inversion have been detected in the *ALDH* family, although these records are usually representative of one or several individuals (Supplementary Table S1). Of these 64 events, 33 were InDels entirely within intronic regions and may be silent. Others are likely to cause loss of function of the enzyme involved, including loss of the whole gene (11 events; occurred in *ALDHs* 1A3, 1B1, 3A1, 3B1, 5A1 and 16A1) or duplication, loss or inversion of exons within the coding sequence (16 events; occurred in *ALDHs* 1A3, 1L1, 1L2, 3A2, 3B2, 6A1 and 9A1). Finally, in a few cases, a region containing the entire gene and surrounding region was duplicated (four events; occurred in *ALDH3B1* and *ALDH3B2*).

## Discussion

The *ALDH* superfamily shows considerable diversity among vertebrate genomes, with species in the current study showing between 14 and 25 putatively protein-encoding genes. Many of the gene duplications discussed here probably encode functional proteins. There are also a number of duplication events that give rise to non-functional pseudogenes. Names were assigned to the 'new genes' and 'pseudogenes' (Table 3) according to the *ALDH* nomenclature system established in 1999.<sup>14</sup> The species-specific nomenclature system was used for zebrafish genes.<sup>15</sup> Pseudogenes were also named according to the standardised protocol.<sup>20</sup>

In the cow genome, *ALDH1A3P1* resembles the product of a partial gene duplication event. The coding region would translate a peptide sharing 100 per cent sequence identity to the 127 carboxy-terminal AAs of the full-length parent gene. Such a high degree of sequence identity is suggestive of a relatively recent evolutionary duplication. Even if the truncated gene encodes the 127-AA peptide; however, it lacks many highly conserved residues required for *ALDH* activity. Thus, the truncated peptide would probably be targeted for rapid degradation. As such, this gene represents a non-functional pseudogene and has been named accordingly.

*ALDH1B1* is present in mammals but missing from birds and fish. The high degree of AA sequence conservation between *ALDH2* and *ALDH1B1* suggests that the latter may be the product of a gene duplication event that occurred some time after the avian–land animal split around 310 MYA. Future analyses should consider other species, including amphibians and reptiles, in order to verify and more accurately pinpoint this evolutionary event.

Analysis of the *aldh2* gene duplications in zebrafish indicates that these represent protein-coding genes and not pseudogenes. As mentioned above, translation of either gene would result in a full-length peptide. The *aldh2.2* gene would encode a product 95.2 per cent identical to that of the parent gene *aldh2.1*. At 95.2 per cent AA identity, *aldh2.2*



represents a new gene. The *aldh2.3* homologue may represent a more evolutionarily recent duplication of *aldh2.2*, as evidenced by the ~99.6 per cent sequence identity noted. Therefore, *aldh2.3* is likely to be a gene-duplication event of *aldh2.2*. All three protein products include the conserved ALDH motifs and residues required for enzyme activity.

The *ALDH3* family showed the greatest variability among species. *ALDH3A1* facilitates cell cycle regulation and scavenging of reactive oxygen species, and acts as a corneal crystallin by filtering UV irradiation in the eye. *ALDH3A1* is missing from birds and fish but is present in every mammalian genome analysed in this study, suggesting that the gene evolved some time after 310 MYA. *ALDH3A1* is conserved among mammals and shows no apparent duplications. In some species, such as rabbit, it appears that *ALDH1A1* is expressed as a corneal crystallin instead of *ALDH3A1*.<sup>31</sup> Interestingly, zebrafish is the only species in this study that apparently lacks both *ALDH3A1* and *ALDH1A1*. Studies have suggested that zebrafish use scinla (cytosolic gelsolin) as a corneal crystallin instead.<sup>32–34</sup>

Zebra finch *ALDH3A3* encodes a full-length peptide that shares 84.1 per cent similarity with the *ALDH3A2* parent gene. Zebrafish has three *aldh3a2* duplications, which include two full-length genes (*aldh3a2.2* and *aldh3a2.3*) and a significantly truncated partial duplication (*aldh3a2p1*). The degree of sequence identity that *Aldh3a2.2* and *Aldh3a2.3* share with the parent peptide (64.9 per cent and 70.9 per cent, respectively) suggests that they diverged sufficiently long ago to be considered new *ALDH3A* family members. They also share 64.9 per cent identity with each other and less than 60 per cent identity with zebra finch *ALDH3A3*, suggesting that all three genes are paralogues rather than orthologues. Zebra finch *ALDH3A5* should also be considered a new functional *ALDH* family member. In addition, the zebrafish pseudogene *aldh3a2p1*, if translated, would share the highest degree of sequence identity with *aldh3a2.3*. Thus, the pseudogene most likely reflects a more recent partial duplication of this gene.

*ALDH3B1* is duplicated in both cow and zebra finch. The cow *ALDH3B4*-encoded protein would be full length and share 85.4 per cent identity to *ALDH3B1*, suggesting that it is a new *ALDH3B* family member. Zebra finch *ALDH3B5* shares an extremely high degree of homology with the amino-terminus of *ALDH3B1*. However, it lacks ~150 AAs that comprise the carboxy-terminus needed for enzyme oligomerisation. The truncated protein would still contain the conserved motifs required for ALDH activity. Until more experimental evidence becomes available, the *ALDH3B5* gene should be considered as putatively functional.

The mouse and rat *Aldh3b3* genes appear to represent new orthologous *ALDH* family members; the genes reside in syntenic chromosomal regions and share a high degree (83.4 per cent) of sequence identity with one another. The two proteins are more divergent than the rodent *ALDH3B2* orthologues, which share 89.9 per cent sequence identity.

*Aldh5a1* is another duplicated *ALDH* gene within the zebrafish genome. The duplication *aldh5a1.2* resides on the same chromosome as the *aldh5a1.1* parent gene, and the two share 100 per cent sequence identity. *Aldh5a1.2* encodes a peptide containing an additional 22 amino-terminal and 88 carboxy-terminal residues. It also shares greater sequence identity with the human *ALDH5A1* orthologue than *Aldh5a1.1* (65.5 per cent versus 51.4 per cent). This suggests that *aldh5a1.2* might actually be the parent gene and *aldh5a1.1* a slightly truncated version formed as the result of gene duplication.

As mentioned above, the macaque *ALDH7A1P5* genomic sequence lacks intronic regions, suggesting that a reverse transcriptase-mediated event gave rise to this pseudogene (ie having no adjacent promoter or other regulatory sequences). Four additional *ALDH7A1* pseudogenes have been identified on chromosomes 5q14 (*ALDH7A1P1*), 2q31 (*ALDH7A1P2*), 7q36 (*ALDH7A1P3*) and 10q21 (*ALDH7A1P4*).<sup>19</sup> Macaque *ALDH7A1P5* is located on Chr 14, which is not syntenic with human Chr 11 and does not share common origins with any of the human pseudogenes. Therefore, the event that gave rise to *ALDH7A1P5* must have taken place within the last 25 million years.

Three full-length *ALDH9A1* homologues were identified in zebrafish. The Aldh9a1.2 peptide shares 71.2 per cent and 70.3 per cent identity with Aldh9a1.1 and Aldh9a1.3, respectively. Aldh9a1.3 is 94.9 per cent identical to the parent Aldh9a1.1 peptide, suggesting that this duplication was a relatively recent event when compared with the duplication that gave rise to Aldh9a1.2. Hence, *aldh9a1.1*, *aldh9a1.2* and *aldh9a1.3* represent three distinct protein-coding *ALDH9* family members. The zebrafish genome also contains two copies of *aldh18a1*, which are found in very close proximity on Chr 12. Both genes are considered protein coding and would give rise to peptides of the same length which share 100 per cent sequence identity, suggesting a relatively recent duplication event.

*ALDH* gene-naming conventions dictate that (i) *ALDH* superfamily members sharing more than ~40 per cent AA identity belong to the same family (eg *ALDH1A*, *ALDH1B*, etc.), and (ii) *ALDH* family members that share greater than 60 per cent AA identity belong to the same subfamily (eg *ALDH1A1*, *ALDH1A2*, etc). This provides a convenient and systematic naming system for an entire superfamily. Interestingly, this does not always indicate homology properly; these rules in the cytochrome P450 (*CYP*) gene superfamily are known to break down when one includes evolutionarily distantly related animals.<sup>27</sup> For example, whereas zebrafish Aldh3d1 and Aldh3b1 share only 50 per cent AA identity, HomoloGene evidence and alignments suggest that *aldh3d1* is probably a duplication of *aldh3b1* (data not shown). Although *aldh3d1* has diverged considerably, it is likely to be more closely related to *aldh3b1* than the naming convention would suggest.

Many of these proteins have been defined based on genomic or dbEST data and have not been studied extensively. Many records remain in databases that are listed as 'protein-coding' but which instead may represent pseudogenes of various types. Furthermore, although the genes here do not have internal stop codons, without functional analysis, it is difficult to determine whether the genes might have other inactivating mutations or if they experience selective pressure. Although automated

prediction and naming of *ALDH* proteins from completely sequenced genomes have achieved a great deal of information in a short amount of time, the alignment, curation and naming of these genes remains an important task. The fact that no new human *ALDH* genes have been identified over the past six years and that most other vertebrates seem to have settled close to this number suggests that identification of *ALDH* superfamily members in vertebrates is nearing completion. Determining the function and biological importance of each family member still requires additional work, however. As more information becomes available, the web database resource at [www.aldh.org](http://www.aldh.org) (the aldehyde dehydrogenase gene superfamily resource center)<sup>35</sup> will be updated to reflect our current understanding of this diverse and essential gene superfamily.

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ALDH3B3 - Rat	MPMVLMPLMMPVLIILYNYRLVPCSVLRVLSTGGAVLKVSTGGAVLRVL	STGGTVLRRQITGGTDPFEEKLQRLKEAFNTGKTKMAKFRAEQLESLGQF
ALDH3B3 - Mouse	-----	-MSTKGGKPRADQGTDPFEEKLQRLKEAFNTGKTKAKFRAEQLSLGRF
ALDH3B2 - Rat	-----MYR	LEHKLISINTAPSCRAGPSEATLHTLREAFNAGRTRPAEFRTAQLQGLGRF
ALDH3B2 - Mouse	-----	-MSAAETGSEPSQAGPSEATLHSLREAFNAGRTRPTEFRTAQLRSLGRF
ALDH3B2 - Human	-----	-----
ALDH3B1 - Mouse	-----	-----MDSFEDKLQQLREAFKEGRTRSAEFRAAQLQGLSHF
ALDH3B1 - Rat	-----	-----MDSFEDKLQQLREAFNAGRTRSAEFRAAQLQGLSHF
ALDH3B1 - Human	-----	-----MDPLGDTLRLREAFHAGRTRPAEFRAAQLQGLGRF
ALDH3B3 - Rat	LQDNKQLHDLALDGLGKSAFESDMSEIILCQNEVDLALKNLQTMKDES	VSTNFLTKFSSAFIRKEPFGVLIIAPWNYPLNLMIMPLVGAIAAGNCVV
ALDH3B3 - Mouse	LQDNKQLHDLALDGLGKSGFESDMSEIILCENEVDLALKNLQTMKDEP	VSTNLLTKLSTAFIRKEPFGVLIIAPWNYPVNLMIIPLVGAIAAGNCVV
ALDH3B2 - Rat	LKDNKQLLDALAKDVGKSAFESDMSEIILCQNEVDLALKNLQTMKDES	VSTNFLTKFSSAFIRKEPFGVLIIAPWNYPLNLMIMPLVGAIAAGNCVV
ALDH3B2 - Mouse	LQENKELLQDALAKDVGKSGFESDMSEIILCENEVDLALKNLQTMKDEP	VSTNLLTKLSTAFIRKEPFGVLIIAPWNYPVNLMIIPLVGAIAAGNCVV
ALDH3B2 - Human	-----MKDEP	RSTNLFMKLDSVFIWKEPFGVLIIAPWNYPLNLTLLVLLVGAIAAGSCVV
ALDH3B1 - Mouse	LRDNKQQLQEAALQDLHKSFAFEAEVSEIAISQAEVDLALRNLRSWMKDEK	VSKNLATQLDSAFIRKEPFGVLIIAPWNYPLNLTLLVLLVGAIAAGNCVV
ALDH3B1 - Rat	LRDNKQQLQEAALQDLHKSFAFESEVSEIAISQAEVDLALRNLRSWMKDEK	VSKNLATQLDSAFIRKEPFGVLIIAPWNYPLNLTLLVLLVGAIAAGNCVV
ALDH3B1 - Human	LQENKQLLHDLALQDLHKSFAFESEVSEIVASQGEVTLALRNLRAWMKDER	VPKNLATQLDSAFIRKEPFGVLIIAPWNYPLNLTLLVLLVGAIAAGNCVV
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ALDH3B3 - Rat	LKPSEMSKNTEKVLAEELLQYLDQSCFAVVLGGPEETGQLLHKHFDYIFF	TGSPRVGKIVMAAAKHLTPITLELGGKNPCYVDDNCDPQTVANRVAWFR
ALDH3B3 - Mouse	LKPSEISKNTEKVLAEELLQYLDQSCFAVVLGGPEETGQLEHKKFDYIFF	TGSPRVGKIVMTAAAKHLTPITLELGGKNPCYVDDNCDPQTVANRVAWFR
ALDH3B2 - Rat	LKPSEMSKNTEKVLAEELLQYLDQSCFAVVLGGPEETGQLLHKHFDYIFF	TGSPRVGKIVMAAAKHLTPITLELGGKNPCYVDDNCDPQTVANRVAWFR
ALDH3B2 - Mouse	LKPSEISKNTEKVLAEELLQYLDQSCFAVVLGGPEETGQLEHKKFDYIFF	TGSPRVGKIVMTAAAKHLTPITLELGGKNPCYVDDNCDPQTVANRVAWFR
ALDH3B2 - Human	LKPSEISQGTQKVLAEVLPQYLDQSCFAVVLGGPEETGQLEHKKLDYIFF	TGSPRVGKIVMTAATKHLTPITLELGGKNPCYVDDNCDPQTVANRVAWFC
ALDH3B1 - Mouse	LKPSEISKATEKILAEVLPQYLDQSCFTVVLGGQETGQLEHKKFDYIFF	TGNAYVVGKIVMAAAKHLTPITLELGGKNPCYVDDNCDPQIVANRVAWFR
ALDH3B1 - Rat	LKPSEISKATEKILAEVLPQYLDQSCFAVVLGGQETGQLEHRRFDYIFF	TGNTYVVGKIVMAAAKHLTPITLELGGKNPCYVDDNCDPQTVANRVAWFR
ALDH3B1 - Human	LKPSEISKNVEKILAEVLPQYVQSCFAVVLGGPEETGQLEHRRFDYIFF	TGSPRVGKIVMTAAAKHLTPITLELGGKNPCYVDDNCDPQTVANRVAWFR
	*****.* : .**.*	*.*.*****.*
ALDH3B3 - Rat	YFNAGQTCVAPDYVLCSEMQRERLVPALQNAITRFYGDNPQTSPLNGRII	NQKHFERLQGLLGCGRVAIGGQSDGEGERYIAPTFLVDVQETEPMVQEEIF
ALDH3B3 - Mouse	YFNAGQTCVAPDYILCSQEMQQLVLPALQNAITRFYGDNPQTSPLNGRII	NQKHFKRLQGLLGCGRVAIGGQSDGEGERYIAPTFLVDVQETEPMVQEEIF
ALDH3B2 - Rat	YFNAGQTCVAPDYVLCSEMQRERLVPALQNAITRFYGDNPQTSPLNGRII	NQKHFERLQGLLGCGRVAIGGQSDGEGERYIAPTFLVDVQETEPMVQEEIF
ALDH3B2 - Mouse	YFNAGQTCVAPDYILCSQEMQRERLVPALQNSITRFYGDNPQTSPLNGRII	NQKHFKRLQGLLGCGRVAIGGQSDGEGERYIAPTFLVDVQETEPMVQEEIF
ALDH3B2 - Human	YFNAGQTCVAPDYVLCSEMQRERLVPALQSTITRFYGDNPQTSPLNGRII	NQKHFKRLQGLLGCGRVAIGGQSDGEGERYIAPTFLVDVQETEPMVQEEIF
ALDH3B1 - Mouse	YFNAGQTCVAPDYILCSQEMQRERLVPALQNAITRFYGDNPQTSPLNGRII	NQKHFKRLQGLLGCGRVAIGGQSDGEGERYIAPTFLVDVQETEPMVQEEIF
ALDH3B1 - Rat	YFNAGQTCVAPDYVLCSEMQRERLVPALQNAITRFYGDNPQTSPLNGRII	NQKHFERLQGLLGCGRVAIGGQSDGEGERYIAPTFLVDVQETEPMVQEEIF
ALDH3B1 - Human	YFNAGQTCVAPDYVLCSEMQRERLVPALQSTITRFYGDNPQTSPLNGRII	NQKHFKRLQGLLGCGRVAIGGQSDGEGERYIAPTFLVDVQETEPMVQEEIF
	*****.*	*.*
ALDH3B3 - Rat	GPILPLVTVRNLDEAIEFINRREKPLALYAYSNNVEVIKQVLARTSSSGF	CGNDGFMHMTLSSLPFGGVGSSGMGRYHGKFSFDTFSNQRACLLSFCGME
ALDH3B3 - Mouse	GPILPLVTVRSLDEAIEFMNREKPLALYAYSNNAEVIKQVLARTSSSGF	CGNDGFMYMTLSSLPFGGVGSSGMGRYHGKFSFDTFSNQRACLLSCPME
ALDH3B2 - Rat	GPILPLVTVRSLDEAVNFINRREKPLALYAFSNNQVVTQMLECTSSSGF	GGNDGFLYLTLPALPLGGVNSGMGRYHGKFSFDTFSHQRACLLSSPGME
ALDH3B2 - Mouse	GPILPLVTVRSLDEAIEFINRREKPLALYAFSNNQVNVQMLERTSSSGF	GGNDGFLYLTLPALPLGGVNSGMGRYHGKFSFDTFSHHRACLLRSPGME
ALDH3B2 - Human	GPILPIVNVQVDEAIKFINRREKPLALYAFSNNQVNVQMLERTSSSGF	GGNEGFTYISLLSVPFGGVGSGMGRYHGKFTFDTFSHRTCLLAPSGLE
ALDH3B1 - Mouse	GPILPLVTVRSLDEAIEFMNREKPLALYAFSKRSQVIKQVLARTSSSGF	CGNDGFMHMTLSSLPFGGVGSGMGRYHGKFSFDTFSNQRACLLRSPGME
ALDH3B1 - Rat	GPILPLVTVNLDEAIEFINRREKPLALYAFSKRSQVIKQVLARTSSSGF	CGNDGFMHMTLSSLPFGGVGSGMGRYHGKFSFDTFSNQRACLLRSPGME
ALDH3B1 - Human	GPILPIVNVQSLDEAIEFINRREKPLALYAFSNNQVVKRVLQTSSSGF	CGNDGFMHMTLASLPFGGVGSGMGRYHGKFSFDTFSHHRACLLRSPGME
	*****.*	*.*
ALDH3B3 - Rat	KLNDLRYPPYSPRRQQLRWAMGQSCTLL--	
ALDH3B3 - Mouse	KLNGLRYPPYSPRRQQLRWAIAGESCTLL--	
ALDH3B2 - Rat	KLNDLRYPPYGTWQQLISWAMGQSCTLL--	
ALDH3B2 - Mouse	KLNDLRYPPYGPWNQQLISWAI GSRCTLL--	
ALDH3B2 - Human	KLKEIHYPYTDWNQQLRWGMSQSCTLL--	
ALDH3B1 - Mouse	KINDLRYPPYSSRNLRLVLLVAMEERCCSCTLL	
ALDH3B1 - Rat	KINDLRYPPYTSRNLRLVLLVAMEKRCCSCTLL	
ALDH3B1 - Human	KLNALRYPPQSPRRLRLVLLVAMEAQCCSCTLL	
	*.*	

**Figure S1.** Alignment of *ALDH3B2* genes in human, rat and mouse created by ClustaW. Dashes (–) represent sequence gaps, asterisks (\*) represent identical amino acids (AAs), colons (:) represent very similar AAs, periods (.) represent less similar AAs, whereas spaces ( ) represent dissimilar AAs.

**Table S1.** Known copy number variations in humans. Included are the variation ID from the Database of Genomic Variants, *ALDH* family member, type (CNV – copy number variation with changes > 1 kb; InDel – insertions and deletions with changes 100–999 bp; inv — inversions with changes that invert the nucleotide sequence), whether the change was a loss or gain, site (intron — change only affects an intronic region; part — change affects one or more exons; whole — change affects the entire gene), sample size and chromosomal location

Variation ID	ALDH	Type	Gain/loss	Site	Sample size (variant/ controls)	Chr
26310	16A1	InDel	Gain	Intron	1/1	19q13.33
26311	16A1	InDel	Gain	Intron	1/1	19q13.33
26312	16A1	InDel	Gain	Intron	1/1	19q13.33
26313	16A1	InDel	Loss	Intron	1/1	19q13.33
109892	1A1	InDel	Gain	Intron	1/1	9q21.13
102109	1A2	CNV	Loss	Intron	1/1	15q22.1
25534	1A2	InDel	Loss	Intron	1/1	15q22.1
40101	1A2	InDel	Loss	Intron	1/1	15q22.1
41386	1A2	InDel	Loss	Intron	1/1	15q22.1
45349	1A2	InDel	Loss	Intron	1/1	15q22.1
45350	1A2	InDel	Loss	Intron	1/1	15q22.1
102186	1A3	CNV	Loss	Intron	1/1	15q26.3
11819	1A3	InDel	Loss	Intron	1/36	15q26.3
25599	1A3	InDel	Loss	Intron	1/1	15q26.3
25600	1A3	InDel	Loss	Intron	1/1	15q26.3
25601	1A3	InDel	Loss	Intron	1/1	15q26.3
40124	1A3	InDel	Loss	Intron	1/2	15q26.3
42429	1A3	InDel	Loss	Intron	1/1	15q26.3
42898	1A3	InDel	Loss	Intron	1/1	15q26.3
45395	1A3	InDel	Loss	Intron	1/1	15q26.3
61482	1A3	InDel	Loss	Intron	1/1	15q26.3
68446	1L1	InDel	Loss	Intron	1/39	3q21.2
106822	1L2	CNV	Gain	Intron	1/1	12q23.3
42760	3A2	InDel	Loss	Intron	1/1	17p11.2
24787	3B2	InDel	Loss	Intron	1/1	11q13.2
44926	3B2	InDel	Loss	Intron	1/1	11q13.2
81276	5A1	InDel	Gain	Intron	1/90	6p22.2

Continued



Table S1. Continued

Variation ID	ALDH	Type	Gain/loss	Site	Sample size (variant/ controls)	Chr
93550	5A1	CNV	Loss	Intron	2/90	6p22.2
99466	5A1	CNV	Loss	Intron	1/1	6p22.2
33982	7A1	InDel	Gain	Intron	1/1	5q23.2
97538	9A1	InDel	Gain	Intron	1/1	1q24.1
23991	9A1	InDel	Gain	Intron	1/1	1q24.1
11004	9A1	InDel	Loss	Intron	15/50	1q24.1
35661	16A1	CNV	Gain	Part	1/1	19q13.33
114045	1A3	CNV	Gain	Part	1/30	15q26.3
72379	1A3	CNV	Loss	Part	1/39	15q26.3
4352	1L1	CNV	2G 1L	Part	3/95	3q21.2
59786	1L1	Inv	Inversion	Part	1/1	3q21.2
68445	1L1	CNV	Loss	Part	1/39	3q21.2
107014	1L2	CNV	Loss	Part	1/1	12q23.3
88379	3A2	CNV	Loss	Part	1/90	17p11.2
88381	3A2	CNV	Loss	Part	1/90	17p11.2
3140	3A2	CNV	Loss	Part	4/270	17p11.2
65982	3B2	CNV	Gain	Part	2/450	11q13.2
85827	3B2	CNV	Loss	Part	2/90	11q13.2
53128	3B2	CNV	Loss	Part	2/1064	11q13.2
3055	6A1	CNV	Gain	Part	1/270	14q24.3
66668	6A1	CNV	Loss	Part	2/450	14q24.3
6793	9A1	CNV	Loss	Part	2/50	1q24.1
3856	3B1	CNV	Gain/loss	Whole	3/270	11q13.2
113072	3B1	CNV	Gain	Whole	1/30	11q13.2
30558	3B1	CNV	Gain	Whole	1/1	11q13.2
5275	3B2	CNV	Gain	Whole	1/272	11q13.1–11q13.2
5111	16A1	CNV	Loss	Whole	25/95	19q13.33
32261	16A1	CNV	Loss	Whole	18/30	19q13.32–19q13.33
5110	16A1	CNV	Loss	Whole	4/95	19q13.33
2201	1A3	CNV	Loss	Whole	3/269	15q26.3

Continued



**Table S1.** Continued

Variation ID	ALDH	Type	Gain/loss	Site	Sample size (variant/ controls)	Chr
47939	<i>1B1</i>	CNV	Loss	Whole	6/2906	9p13.1
30022	<i>3A1</i>	CNV	Loss	Whole	2/485	17p11.2
53160	<i>3B1</i>	CNV	Loss	Whole	2/1064	11q13.2
2931	<i>3B1</i>	CNV	Loss	Whole	8/270	11q13.2
29913	<i>3B1</i>	CNV	Loss	Whole	1/485	11q13.2
29914	<i>3B1</i>	CNV	Loss	Whole	1/485	11q13.2
47969	<i>5A1</i>	CNV	Loss	Whole	9/2906	6p22.2