AJHG, Volume *92*

SUPPLEMENTAL DATA

ALDH1A3 Mutations Cause Recessive Anophthalmia and Microphthalmia

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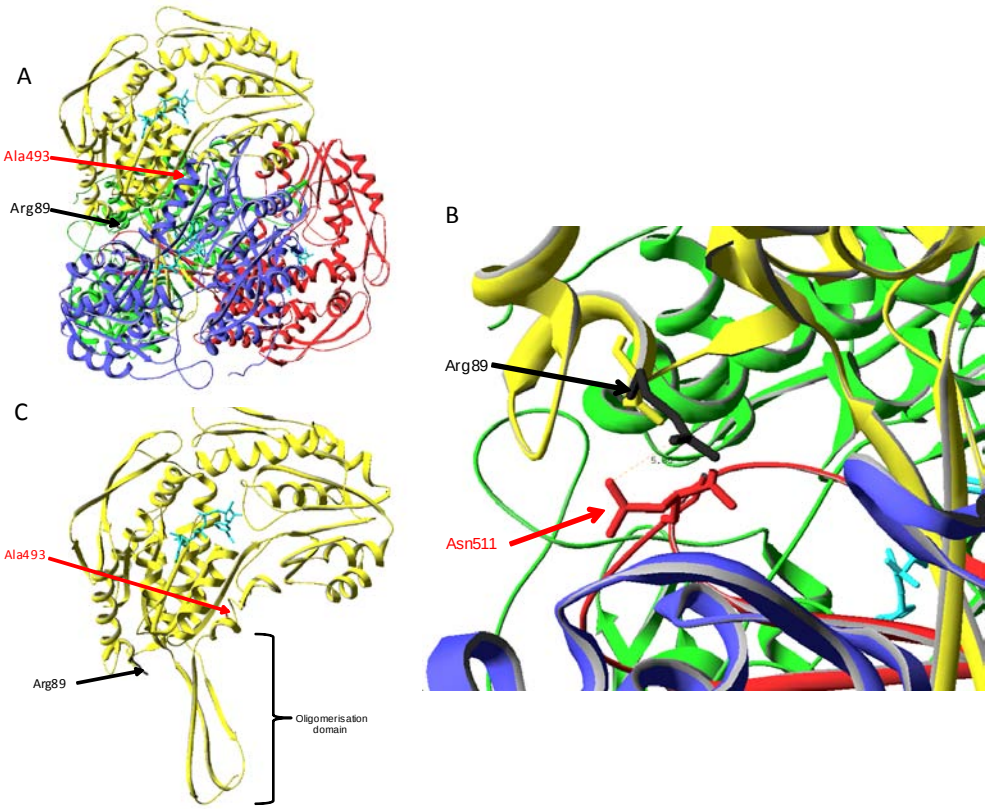

Figure S1. Ribbon Representation of the Human ALDH1A3 Tetramer

The 2.35 Å coordinate set for the tetrameric sheep liver class 1 aldehyde dehydrogenase with NAD bound (pdb code: 1BXS) was used as a template for modeling the human ALDH1A3 protein (71,3% sequence identity). (A) Each of the four monomers is shown in different colors (yellow, blue, green and red, respectively). . The four catalytic pockets are visualized with the four NAD co-crystallized with the sheep ALDH1 (1BXS) and are shown in light blue. (B) Magnification of the region around the Arg89 residue of a monomer. The interaction with the Asn511 residue at a distance of 5 Å in another monomer is shown. The replacement of the Arg89 by a cysteine is predicted to alter the interaction between the monomers. (C) Ribbon representation of a monomer (yellow) bound to NAD (light blue) showing the oligomerization domain and the position of the two residues affected by A/M mutations. The p.Ala493Pro mutation is predicted to alter severely the oligomerization domain of the enzyme.

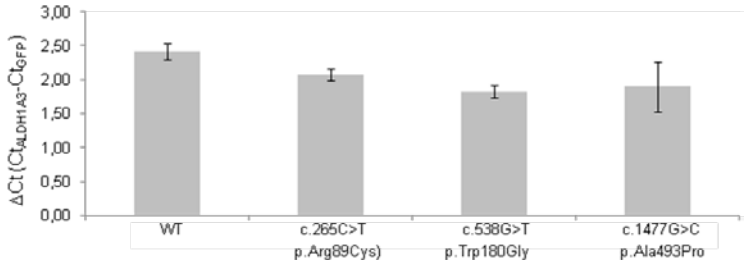

Figure S2. Relative Expression of GFP and ALDH1A3 mRNA in HEK293 Cells Measured by RT-qPCR

HEK293 cells were co-transfected with pCAG-GFP and pCMV6-Entry-ALDH1A3-WT-cMyc, pCMV6-Entry-ALDH1A3-Arg89Cys-cMyc, pCMV6-Entry-ALDH1A3-Trp180Gly-cMyc, pCMV6-Entry-ALDH1A3-Ala493Pro-cMyc plasmids, respectively. Total mRNA was extracted using the RNeasy Mini Kit (Qiagen, Courtaboeuf, France) according to manufacturer's protocol. cDNAs (5 µl of a 1:25 dilution in nuclease-free water) were subjected to real-time PCR amplification in a buffer (20 µl) containing MESA BLUE qPCR Master Mix Plus for Sybr Assay (Eurogentec, Angers, France) and 300 nmol/l of forward and reverse primers, on a Taqman 7900 HT Fast Real-Time PCR System (Applied Biosystems, Courtaboeuf, France). For each cDNA sample, the mean of cycle threshold (Ct) values was calculated from triplicates (SD <0.5 Ct). ALDH1A3 expression levels were normalized by using the relation ΔCt ($Ct_{ALDH1A3}$ - Ct_{GFP}). Amplification efficiency was calculated for each primer-pair using fourfold serial dilution curves (1:5, 1:25, 1:125, 1:625). No reverse transcriptase and no template control reaction were used as negative controls in each run. The quantitative data are the means ± SEM of three independent experiments. The graph shows that the expression of the wild-type and mutant *ALDH1A3* mRNAs are in the same range.

A

Filter	sub	del	ins	hom	het	Genes
None	47 594	197	199	20 268	27 722	16 634
Heterozygote variants excluded	20 268	0	0			9897
Variants with frequency ≥ 1% in dbSNP, EVS, 1000Genome excluded.	834	0	0			689
Variants in UTR, non-coding, intergenic and deep intronic regions and synonymous variants excluded	223	0	0			174
Polyphen and sift prediction begnin excluded	28	0	0			27

B

Gene	chr	Start	End	All	sub	ins	del	Coding	Silent	Splicing	Stop
NBPF8	1	144593863	144622156	1	1	0	0	0	0	0	0
OR2T35	1	248802088	248803059	1	1	0	0	1	0	0	0
SLC25A22	11	790975	798816	1	1	0	0	1	0	0	0
SORL1	11	121323412	121504887	1	1	0	0	1	0	0	0
OR10G7	11	123909273	123910217	1	1	0	0	1	0	0	0
MSANTD2	11	124636894	124671069	1	1	0	0	1	0	0	0
ZFHX2	14	23990569	24025901	1	1	0	0	1	0	0	0
AHNAK2	14	105404081	105445194	1	1	0	0	1	0	0	0
ITGA11	15	68594550	68725002	1	1	0	0	1	0	0	0
ALDH1A3	**15**	**101402629**	**101457331**	**1**	**1**	**0**	**0**	**1**	**0**	**0**	**0**
CTB-134H23.2	16	29050423	29064547	1	1	0	0	1	0	0	0
CLEC18B	16	74443029	74456149	1	1	0	0	1	0	0	0
FASN	17	80036715	80056606	1	1	0	0	1	0	0	0
TCEB3C	18	44555073	44556949	1	1	0	0	1	0	0	0
HIF3A	19	46800803	46847190	1	1	0	0	1	0	0	0
FUT2	19	49199728	49209707	1	1	0	0	1	0	0	0
RGPD4	2	108443888	108507797	1	1	0	0	1	0	0	0
PLEKHB2	2	131862920	132111782	1	1	0	0	1	0	0	0
KRTAP10-6	21	46011649	46012886	1	1	0	0	1	0	0	0
EIF4G1	3	184032783	184053646	1	1	0	0	1	0	0	0
LEAP2	5	132208514	132211238	1	1	0	0	1	0	0	0
PCDHB7	5	140552743	140556457	1	1	0	0	1	0	0	0
CTAGE9	6	132030081	132032706	1	1	0	0	1	0	0	0
TRGV9	7	38357118	38358962	1	1	0	0	1	0	0	0
ZAN	7	100331749	100395919	1	1	0	0	1	0	0	0
CPSF1	8	145618944	145635253	1	1	0	0	1	0	0	0
PCSK5	9	78506060	78977755	1	1	0	0	1	0	0	0

Table S1. Whole Exome Resequencing Summary. (A) Filtering strategy of the variants resulting in selection of 28 substitutions in 27 genes. sub: substitution, del: deletion, ins: insertion, hom: homozygous, het: heterozygous, UTR: untranslated region. (B) Genes harboring nonsynonymous

homozygote variants predicted to be deleterious or with unknown effect using the Polyphen and SIFT programs. *ALDH1A3* is the only gene located in the 15q26.3 region. Chr: chromosome.

Exon number	Forward sequence (5'-3')	Reverse sequence (5'-3')	Size of amplified product (bp)
1	GAGCGGGCTGCGCAGTGT	CCGAGACGTCCCGCGAAA	354
2	GGTGGACAAGATGGATAAGA	GCCAGTTCTGTCTTATAGCT	299
3	CCAAACTGCAGTCACCTCAA	CACGACCACACAAAACCAG	368
4-5	GGTGCATCTGACTGTGAG	GCTTGTTCAACGCTGGTG	745
6	CCTCCACAAAGGCATCGTTG	GCCACTGTCCCATCTCGT	392
7	GGATGAGAAGCCCAGGTC	GCCTGTCAAAGGAAAAGCTC	376
8	GAGAGCCAGGTGGTGGCA	GCACACATCTTACTCTCAGT	323
9	GCAGCTGTCACCAGTCCT	GGGACCCTGTAGGCGGTT	348
10	GGCTTGACAAGAACATGCAG	AAGGATTTCTGGGATCCCTG	371
11-12	GCTGAAGCAATGTTTGGACG	GCAGATTGGAGCCTGTGTC	1611
13	CTCCAACGGCCTGATGGA	CAGTAGATGTAAAGCCTCCAG	298

Table S2. Primers Used for MutationScreening of *ALDH1A3*.

Oligonucleotides	Sequence (5'-3')
ALDH1A3-c.265T_forward	GCCTTCCAGAGGGGCTCGCCATGG**T**GCCGGCTGGATGCCCTGAGTCGT
ALDH1A3-c.265A_reverse	CCCACGACTCAGGGCATCCAGCCGGC**A**CCATGGCGAGCCCCTCTGGAA
ALDH1A3-c.1477C_forward	GAAATGGCAGAGAACTAGGTGAATAC**C**CTTTGGCCGAATACACAGAAGTG
ALDH1A3-c.1477G_reverse	CACTTCTGTGTATTCGGCCAAAG**G**GTATTCACCTAGTTCTCTGCCATTTC
ALDH1A3-c.620C_forward	CATGGTCCTGAAGCCTGCGG**C**GCAGACACCTCTCACCGCCCTTT
ALDH1A3-c.620G_reverse	AGGGCGGTGAGAGGTGTCTGC**G**CCGCAGGCTTCAGGACCATGGT

Table S3. Primers Used for Site-Directed Mutagenesis of pCMV6-Entry-*ALDH1A3* Vector

The mutant nucleotides appear in bold underlined.

mRNA	Forward primer (5'-3')	Reverse primer (5'-3')
GFP	GFP-F_TCCAGCAGGACCATGTGATC	(GFP-R)_GTCCGCCCTGAGCAAAGA
ALDH1A3	ALDH1A3-ex12.13F_TGGCAGAGAACTAGGTGA	(c-Myc-R)_GCCAGATCCTCTTCTGAGAT

Table S4. RT-qPCR Primers Used to Assess *ALDH1A3* and *GFP* Expression in Transiently Transfected HEK293 Cells

The use of a primer located in the c-Myc tag with the ALDH1A3-ex12.13F allowed specific amplification of the *ALDH1A3* mRNA encoded by the plasmids.