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Brief Communication

X-linked adrenoleukodystrophy in a chimpanzee due to an *ABCD1* mutation reported in multiple unrelated humans

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A B S T R A C T

Background: X-linked adrenoleukodystrophy (X-ALD) is a genetic disorder leading to the accumulation of very long chain fatty acids (VLCFA) due to a mutation in the *ABCD1* gene. *ABCD1* mutations lead to a variety of phenotypes, including cerebral X-ALD and adrenomyeloneuropathy (AMN) in affected males and 80% of carrier females. There is no definite genotype-phenotype correlation with intrafamilial variability. Cerebral X-ALD typically presents in childhood, but can also present in juveniles and adults. The most affected tissues are the white matter of the brain and adrenal cortex. MRI demonstrates a characteristic imaging appearance in cerebral X-ALD that is used as a diagnostic tool.

Objectives: We aim to correlate a mutation in the *ABCD1* gene in a chimpanzee to the human disease X-ALD based on MRI features, neurologic symptoms, and plasma levels of VLCFA.

Methods: Diagnosis of X-ALD made using MRI, blood lipid profiling, and DNA sequencing.

Results: An 11-year-old chimpanzee showed remarkably similar features to juvenile onset cerebral X-ALD in humans including demyelination of frontal lobes and corpus callosum on MRI, elevated plasma levels of C24:0 and C26:0, and identification of the c.1661G > A *ABCD1* variant.

Conclusions: This case study presents the first reported case of a leukodystrophy in a great ape, and underscores the fidelity of MRI pattern recognition in this disorder across species.

1. Introduction

X-linked adrenoleukodystrophy (X-ALD) is a rare, X-linked disorder that primarily affects the white matter of the brain and the adrenal cortex [1,2]. It is caused by a mutation in the *ABCD1* gene which encodes the ATP binding cassette transporter protein ABCD1 (also known as ALDP) embedded within the peroxisomal membrane [1,2]. ALDP transports very long chain fatty acids (VLCFA, carbon length > 22) across the peroxisomal membrane [1,2] after which they are metabolized to coenzyme A derivatives by a very long chain acyl-CoA synthetase [1,2] and subsequently degraded by substrate-specific β-oxidation enzymes in the peroxisomal matrix. Mutations in *ABCD1* disrupt this process and lead to an accumulation of VLCFA. Males hemizygous for *ABCD1* pathogenic mutations are at risk for developing childhood cerebral X-ALD, adrenomyeloneuropathy (AMN), or Addison disease [3]. There is no genotype-phenotype correlation, since *ABCD1* mutations can be associated with both childhood cerebral X-ALD and AMN in the same family and discordant phenotypes in monozygotic

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twins [4,5]. X-ALD is the most common peroxisomal inborn error of metabolism, with an incidence estimated between 1:16,800 and 1:50,000 [1]. It does not have a significantly higher incidence in any specific ethnic groups.

Ideally X-ALD is diagnosed in the presymptomatic phase based on family screening and newborn screening, allowing timely intervention including prevention of adrenal insufficiency [6]. Unfortunately, many patients are still diagnosed based on neurologic symptoms and specific brain MRI abnormalities. Childhood cerebral X-ALD most commonly presents as difficulty in school and is often mistaken for attention deficit hyperactivity disorder (ADHD) [3]. As the disease progresses, more obvious neurological symptoms manifest such as seizures, cognitive changes, fine motor abnormalities, and gait dysfunction. Early symptoms overlap with many disorders, and MRI often provides the first clue to diagnosis. In contrast, AMN is an adult onset disorder that initially impacts the spinal columns, although about 20% of AMN patients will ultimately experience cerebral demyelination as well. Finally, Addison disease results in adrenocortical insufficiency presenting at any age from toddlerhood to adulthood, with a peak around seven and a half years [3].

Childhood cerebral X-ALD entails a devastatingly rapid process of cerebral demyelination that most often begins in the splenium of the corpus callosum and spreads to the parieto-occipital regions and is thought to be due to brain endothelial dysfunction [7,8]. A subset of individuals with X-ALD, primarily juveniles or young adults, show demyelination in the genu of the corpus callosum and frontal white matter either initially or as a progression of their disease following parieto-occipital abnormalities [3,8,9].

X-ALD is diagnosed by measurement of VLCFA levels in plasma. Affected individuals will have increased plasma levels of VLCFA, especially C22:0, C24:0, and C26:0 [3,10,11]. Diagnosis of X-ALD can then be confirmed by molecular analysis of *ABCD1*, usually with DNA sequencing, but in some cases by dosage testing such as Southern blot analysis or Multiplex ligation-dependent probe amplification (MLPA).

We report here a chimpanzee in captivity who presented with classic clinical and MRI signs of cerebral X-ALD, with ultimate confirmation of the diagnosis *via* VLCFA analysis and *ABCD1* sequencing.

2. Methods

2.1. Magnetic resonance imaging (MRI)

A 1.5 T brain MRI of the 11-year-old male chimp, CLD_01.0, was obtained by the Save the Chimps organization. Non-contrast and contrast-enhanced T1-weighted sequences were performed. In addition, fluid-attenuated inversion recovery (FLAIR) and T1- and T2-weighted spin-echo MR sequences were obtained (Fig. 1). Images were evaluated for the presence of contrast enhancement, as well as abnormal T2-hyperintensity present on both the FLAIR and T2-weighted sequences [9].

2.2. Biochemical measurements

Plasma total lipid VLCFA were measured as previously described [12]. Plasma from healthy chimpanzees was tested alongside the affected individual to assess species specific norms. Additionally, samples were compared to VLCFA levels from both humans affected with adrenoleukodystrophy and unaffected humans.

2.3. Sequencing of ABCD1

Sanger sequencing of the *ABCD1* gene was performed as previously described [13], except that four amplification primers were re-designed to account for differences between the human and chimpanzee genomes (e1aF-chimp: 5′-TGTAAAACGACGGCCAGTACAACAGGCCCAGGGTCAGC-3′; e6F-chimp: 5′-TGTAAAACGACGGCCAGTGCCATAGGGTGCGGGAAGCG-3′; e7R-chimp: 5′-CAGGAAACAGCTATGACCCTTCCCTAGAGCACCCAT-3′; and, e10R-chimp: 5′-CAGGAAACAGCTATGACCGCGGGGTGCGTGCGTGGGTGG-3′). The forward and reverse primers contain 5′-TGTAAAACGACGGCCAGT-3′ and 5′CAGGAAACAGCTATGACC-3′ tails, respectively. There are 11 nucleotide differences in the reference cDNA of human and chimpanzee, but 9 of these are synonymous. The two amino acid “substitutions” (p.Gln430His and p.Met598Val) in chimpanzee compared to human have never been reported as human mutations. The p.Gln430His alteration also has not been previously reported in reference datasets of human variation (GnomAD, ExAC, 1000 Genomes) or in X-ALD mutation databases. The p.Met598Val variant has been seen in 2/930 alleles in Finnish Europeans (ExAC database http://exac.broadinstitute.org/). Overall, this region of the *ABCD1* gene does not have good coverage in the exomes submitted to ExAC (only about one-third of the possible 90,000 alleles). The variant is also present in 7 of 174,488 alleles in the gnomAD dataset (http://gnomad.broadinstitute.org), including two hemizygotes, and was reported in 1/145 (0.7%) alleles from African Caribbean's in Barbados in the 1000 Genomes dataset (http://grch37.ensembl.org). Both SIFT and PolyPhen predict the human p.Met598Val variant to be benign and tolerated, respectively.

3. Results

Here we describe an 11-year-old male chimpanzee, CLD_01.0, who developed cerebral X-ALD. He was infected with Hepatitis C virus (HCV) 5 years previously for research purposes, but tested negative for HCV by polymerase chain reaction at the onset of neurological symptoms. Initial symptoms that were noted included leg weakness, drooling, occasional erratic behavior, and inability to focus. As symptoms progressed, he had difficulty swallowing, dragged both legs when locomoting, and developed liver failure. Eventually, he was euthanized for human reasons.

Prior to his death, a brain MRI was performed that triggered his diagnosis of X-ALD. The MR examination demonstrated a peripherally enhancing leukodystrophy affecting the bilateral frontal lobes and corpus callosum, similar to the juvenile onset presentation of X-ALD (Fig. 1) [8,9,14]. The genu of the corpus callosum was involved and there was relatively symmetric involvement of the bifrontal periventricular white matter. Peripheral contrast enhancement of the T2-signal abnormalities was noted which is typically seen in human X-ALD.

Relative to a different colony of healthy captive chimpanzees, the test subject's plasma C24:0, C26:0, and C26:1 VLCFA levels were highly elevated (Table 1), consistent with a defect in peroxisomal β-oxidation. Since elevated plasma VLCFA levels are also observed in a subset of patients with peroxisome biogenesis disorders (PBDs), we investigated red blood cell (RBC) plasmalogen, phytanic acid, and pristanic acid levels, which are reduced in a subset of PBD patients, but not X-ALD (Supplemental Table 1).

Genomic DNA isolated from EDTA blood samples was used to Sanger sequence the *ABCD1* gene in the affected chimpanzee and a healthy control chimpanzee. The *ABCD1* variant c.1661G > A (p.Arg554His) was identified only in the candidate X-ALD affected chimpanzee. Interestingly, this *ABCD1* missense mutation has been reported > 40 times in unrelated humans (X-ALD Mutation Database, http://www.x-ald.nl/). The nucleotide c.1661 is part of a CpG dinucleotide and thus is more susceptible to sequential methylation and deamination, likely explaining the relative frequency of this mutation in humans and in this chimpanzee. This arginine residue is highly conserved across species (*ABCD1* orthologues) and within the human homologs ALDR and PMP70. This substitution is not reported in the ExAC database (http://exac.broadinstitute.org/), or the gnomAD database (http://gnomad.broadinstitute.org/) despite good coverage through this region of *ABCD1*. It is associated with an absence of ABCD1 protein in cultured skin fibroblasts harboring the *ABCD1* p.Arg554His allele and has been reported as *de novo* in at least one family [15]. Additionally, the mutation has been shown to reduce ALDP

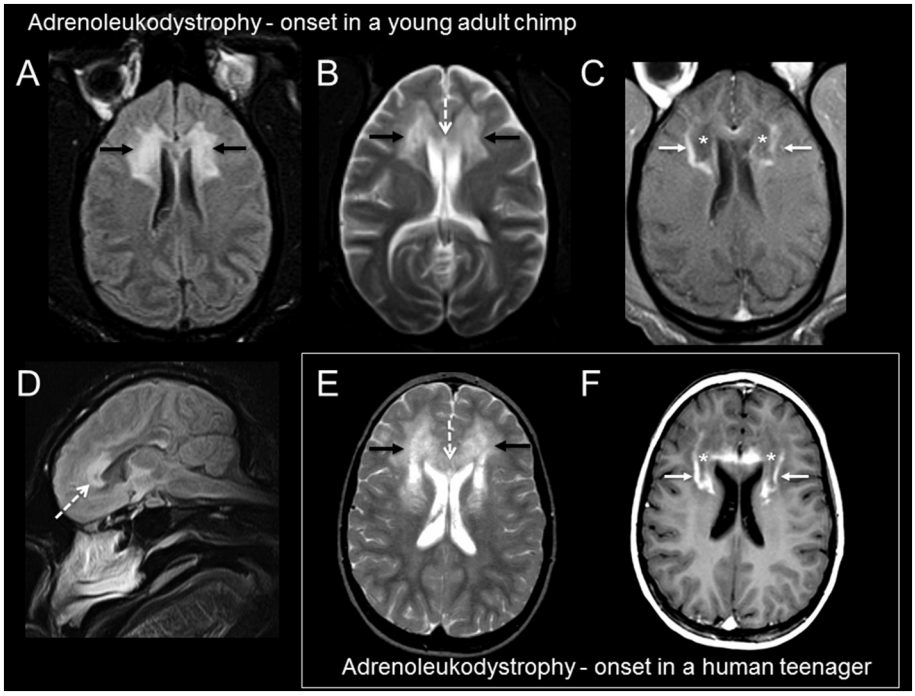

Fig. 1. MR in Adrenoleukodystrophy (ALD): Axial and mid-sagittal FLAIR imaging (A and D, respectively), axial T2-weighted images (B, E), and axial post-gadolinium-enhanced T1-weighted images (C, F) in the chimpanzee (A–D) and human (E, F). Note abnormal, relatively symmetric, bilateral, frontal lobe white matter hyperintensity on the FLAIR and T2-weighted mages in both individuals (black arrows). Involvement of the genu of the corpus callosum is demonstrated on B, D, and E (dotted white arrows). Hypointensity of the central portion of the white matter lesion is noted on the T1-weighted images (asterisks), and contrast enhancement of the advancing edge of the lesion (white arrows) are typical for actively demyelinating X-ALD. While involvement of the parieto-occipital white matter, including the splenium of the corpus callosum, predominates in childhood onset X-ALD, initial involvement of the frontal region, including the genu of the corpus callosum, is characteristic of juvenile and adult patients with X-ALD.

Table 1
Biochemical measurements.

Metabolite	CLD_01.0	Healthy chimpanzee controls[a]				Healthy human controls[b]				X-ALD hemizygote[c]			
		Mean	SD	Low	High	Mean	SD	Low	High	Mean	SD	Low	High
C22:0 (μg/ml)	16.09	14.39	3.02	10.82	22.85	20.97	6.27	8.43	33.51	18.5	5.1	8.3	28.7
C24:0 (μg/ml)	**35.93**	16.92	3.88	11.44	27.78	17.59	5.36	6.87	28.31	**32.25**	**8.2**	**15.85**	**48.65**
C26:0 (μg/ml)	**3.4**	0.27	0.11	0.17	0.62	0.23	0.09	0.05	0.41	**1.3**	**0.45**	**0.4**	**2.2**
C26:1 (μg/ml)	0.75	0.22	0.06	0.14	0.37	0.18	0.09	0	0.36	0.34	0.16	0.02	0.66
C24/C22	**2.23**	1.18	0.12	0.97	1.53	0.84	0.1	0.64	1.04	**1.71**	**0.23**	**1.25**	**2.17**
C26/C22	**0.21**	0.02	0.01	0.01	0.05	0.01	0.004	0.002	0.018	**0.07**	**0.03**	**0.01**	**0.13**

SD standard deviation.
Bold text signifies measurements that are changed between the controls and the X-ALD hemizygote.
[a] Plasma data from 17 healthy captive chimpanzees.
[b] Plasma data from 7818 healthy controls.
[c] Plasma data from 1084 X-ALD hemizygote.

levels to 1% compared to normal [16]. Overall, *ABCD1* p.Arg554His meets ACMG guidelines [17] to be classified as a pathogenic variant. The mother of the chimpanzee with cerebral X-ALD is deceased and there is no genomic DNA available, so testing could not be performed to determine if this *ABCD1* mutation was inherited or *de novo*. We do note recent studies have established that ~80% human female carriers of *ABCD1* mutations can develop a wide range of neurologic abnormalities [3,18].

4. Discussion

There have been prior examples of non-human primates (NHP) exhibiting human neurologic conditions, that correlate with human pathologic, MRI, and behavioral findings. For example, there is a naturally occurring model of Krabbe disease (globoid cell leukodystrophy) in rhesus macaques that has severe early-onset and displays neurologic and behavioral symptoms similar to those of affected humans [19,20].

The 11-year old chimpanzee described herein represents the first case of a leukodystrophy identified in a great ape and, to our knowledge, the first sequence-verified Mendelian genetic disorder described in a great ape. This chimpanzee presented with all the classic findings used to diagnose juvenile onset X-ALD in humans including characteristic MRI features, elevated plasma VLCFA levels, neurologic symptoms, and identification of a pathogenic *ABCD1* variant.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgme.2017.08.012.

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