Clinical Vignette

Limitations of Whole-Exome Sequencing: Report of a Child with X-Linked Adrenoleukodystrophy

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Abstract

X-linked adrenoleukodystrophy is the most common disorder of peroxisomal beta-oxidation of very long chain fatty acids. It presents with three distinct phenotypes, including the childhood cerebral form, adrenomyeloneuropathy and Addison's disease. It is an X-linked disorder caused by pathogenic variants in the *ABCD1* gene. Our case describes the diagnostic odyssey in a six-year-old child with neuroregression in the form of progressive loss of hearing and vision, and neuroimaging features highly suggestive of adrenoleukodystrophy. This brings out the inadequacies of the apparently impregnable whole exome sequencing, due to partial coverage of the *ABCD1* gene. We also enunciate the importance of clinical acumen and the need for collaboration with experts in the field in order to reach a quicker and more accurate diagnosis, especially in treatable disorders like these.

Keywords: *ABCD1*, X-linked adrenoleukodystrophy

Introduction

X-linked adrenoleukodystrophy (ALD) is the most common disorder of peroxisomal beta oxidation with an estimated prevalence of 1 in 17000 (Bezman et al., 1998; Kok et al., 1995; Raymond et al., 1999). The disorder presents as three distinct phenotypes: the childhood-onset cerebral form, adrenomyeloneuropathy and adult-onset Addison's disease. Neuroimaging shows a characteristic pattern of symmetric enhanced T_2 signal in the parieto-occipital region with contrast enhancement (Heubner et al., 1897; Raymond et al., 1999). We present the diagnostic dilemma encountered in a six-year-old boy with adrenoleukodystrophy, highlighting the various fallacies in testing and the crucial role of clinical acumen in such situations.

Clinical report

A 6-year-old boy, fourth in birth order, presented to the genetic clinic with the chief complaints of progressive loss of ambulation and impairment of vision and hearing. Parents were married non-consanguineously. Antenatal, perinatal and neonatal periods were uneventful. The development was age-appropriate until the age of 4 years 9 months, when he started falling and bumping into objects and needed aid for ambulation. A simultaneous loss of hearing was also observed. Both hearing and vision loss were progressive and at presentation, he was unable to hear or see at all. On examination, there was no facial dysmorphism. Anthropometry was within normal limits. He was lean and average built. He was irritable and non-responsive to verbal commands. Central nervous system examination revealed spasticity in bilateral lower limbs with brisk deep tendon reflexes. Upper limb tone and reflexes were normal. Rest of the systemic examination was non-contributory. Magnetic resonance imaging (MRI) of the brain revealed characteristic T2 hyperintensities in the parieto-occipital lobe with contrast enhancement in the diffusion-weighted images (DWI), highly suggestive of adrenoleukodystrophy. Plasma lactate, ammonia and glucose were normal. Plasma very long chain fatty acids (VLCFA) analysis did not reveal any abnormalities.

Clinical diagnosis of ALD was suspected but as the VLCFA analysis was normal (repeated twice on same sample), whole exome sequencing (WES) was ordered instead of Sanger sequencing. It revealed a homozygous missense variant in the *EPRS1* gene, c.223C>T(p.His75Tyr). The clinical phenotype seemed to be consistent with that of our patient and the variant was segregated in the parents, but this remained a variant of uncertain significance according to revised American College of Genetics and Genomics

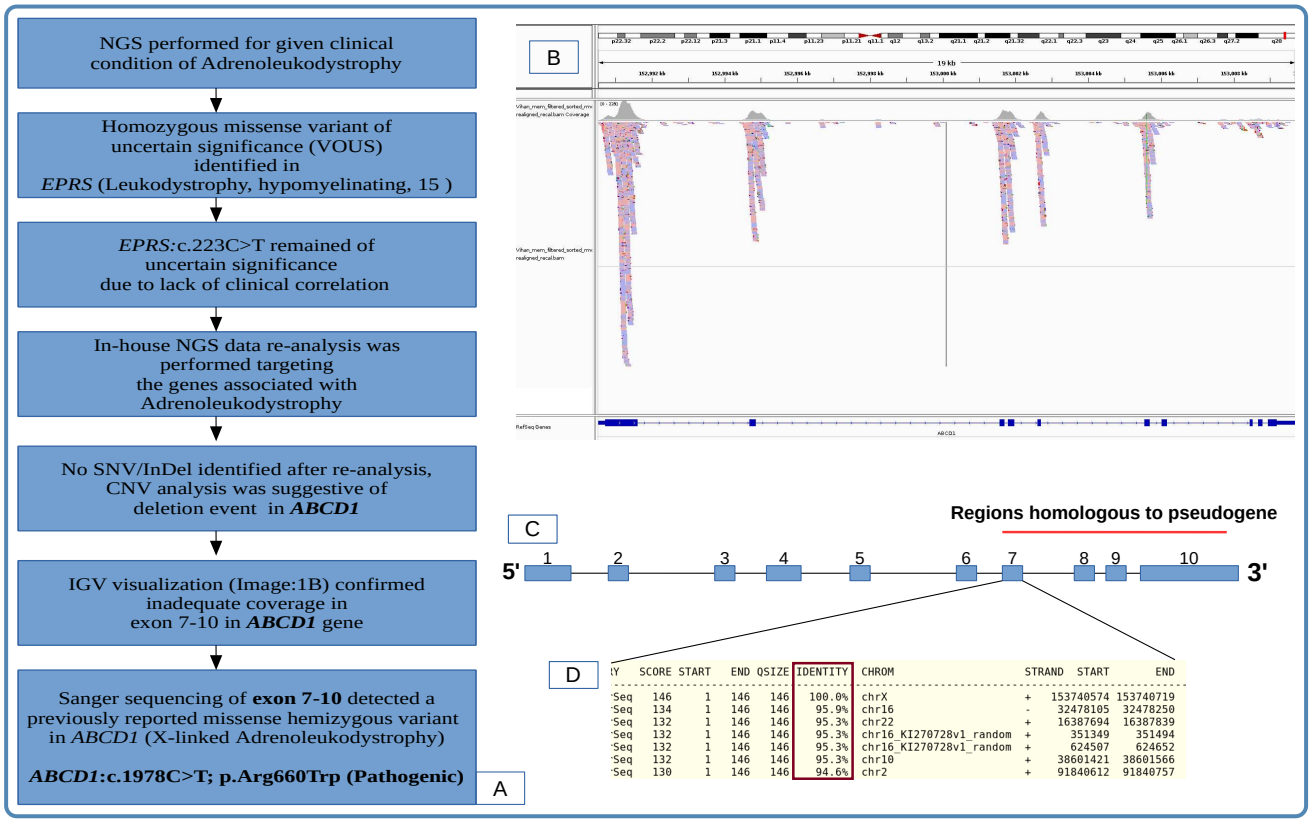

Figure 1 A) Flowchart showing the steps which led to confirmation of the diagnosis of X-linked adrenoleukodystrophy in the child. B) Integrated Genome Viewer (IGV) visualization of NGS reads mapped to ABCD1 gene. Rectangular box (red colored) indicates inadequate coverage in the exons 7-10 region. C) Diagrammatic representation of the ten (10) exons in *ABCD1* gene. Eichler et al. (1997) identified that a 9.7-kb segment encompassing exon 7-10 of *ABCD1* was duplicated to 2p11, 10p11, 16p11, and 22q11. Due to high homology between the pseudogene and the *ABCD1* exon 7-10 region, care should be taken while analyzing the *ABCD1* gene. D) Pairwise alignment of exon 7 in *ABCD1* indicating sequence homology with chr16, chr22, chr10, chr2. Results are arranged in decreasing order based on the score generated by BLAT (box).

(ACMG) criteria (Richard et al., 2015). Therefore, the diagnosis of *EPRS*-related hypomyelinating leukodystrophy was not confirmed.

Since the phenotypic features were suggestive of ALD, opinion of world experts was sought for this patient and a repeat biochemical testing was done. C26:0-lysophosphatidyl choline (C26:0-lysoPC) was performed on the dried blood spot and was elevated (653 nmol/L, ref: 29-72). This brought back the focus to ALD. WES data re-analysis detected no pathogenic variant in the *ABCD1* gene. Further, it was noted that the exons 7-10 of *ABCD1* were not covered adequately as compared to other exons of the same gene (**Figure 1B**). Hence, the possibility of a deletion at exon level could not be ruled out by looking at the data pattern. To rule out any possibility of exon level deletion, multiplex ligation-dependent probe amplification (MLPA) was performed and this was normal. Finally, *ABCD1* gene Sanger sequencing was performed in view of incomplete coverage of certain exons. A hemizygous missense, previously reported variant was identified, in exon 9 of *ABCD1*, ENST00000218104.31: c.1978C>T (p. Arg660Trp). This variant was described by Kok et al. in 1995 in an Italian proband and was classified as likely pathogenic by the revised ACMG criteria (Kok et al., 1995). The flowchart depicting the sequence of testing leading to final diagnosis is shown in **Figure 1A**.

This case describes the diagnostic odyssey in a child with ALD due to initial misleading exome results. Next-generation sequencing has brought a paradigm shift in the diagnosis of

genetic disorders. WES has greatly increased the diagnostic yield of genetic disorders, especially those with a non-specific phenotype.

Nevertheless, like every new technology, it has its own promises and pitfalls. It has been recently shown that even current WES platforms have problems in sufficiently capturing the whole exome (Meienberg et al., 2015). One of the reasons for false negative reports of WES is the presence of pseudogenes which are non-functional segments of DNA that resemble functional genes. Most arise as superfluous copies of functional genes, either directly by DNA duplication or indirectly by reverse transcription of an mRNA transcript. Due to the great degree of sequence homology with functional genes, exome sequencing data is often inaccurate in genes which have associated pseudogenes.

WES for this patient was performed as the biochemical testing was unyielding. But exome data was skewed and missed the variant due to the presence of a pseudogene. The exons 7 to 10 of the *ABCD1* gene share sequence homology with similar sequences present on chromosomes 2, 10 and 16 and 22 (pseudogenes). This resulted in alignment mismatch for some reads (**Figures 1C, 1D**). Thus, it should be borne in mind that in presence of a high clinical suspicion, targeted testing by Sanger sequencing of a gene may be a preferable modality of testing.

Although VLCFA analysis is highly sensitive and specific for X-linked ALD, pathognomonic increased plasma VLCFA values are not always found in X-ALD. Both false positive due to hemolysis of the sample, non-fasting sample, or a ketogenic diet and a false negative, due to consumption of products like rapeseed oil or mustard seed oil that are rich in erucic acid (C22:1) can lead to the lowering of C26:0 (Engelen at al., 2012). Further, now there is availability of alternate biomarkers like C26:0-lysoPC which is performed on dried blood spots and has a much higher discriminative power than the plasma VLCFA analysis (Wanders et al., 2018).

In conclusion, this case throws light on the importance of a systematic step-wise approach in establishing the correct molecular diagnosis. Elevated VLCFA levels may not always be conclusive for ALD and alternative biochemical markers such as C26:0-lysoPC should be considered. Further, WES is not the absolute end-all of genetic testing and it is worthwhile to choose the molecular test with maximum coverage for the target gene/genes. Another learning point from this patient is to not label a diagnosis on the basis of variants of uncertain significance, in the absence of a highly specific phenotype. Lastly, we emphasise on the need for collaboration and discussion of patients with experts in order to provide them with the quickest and most accurate diagnosis.

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