HHS Public Access

Author manuscript

Am J Med Genet A. Author manuscript; available in PMC 2021 September 20.

Published in final edited form as:

Am J Med Genet A. 2021 April ; 185(4): 1288–1293. doi:10.1002/ajmg.a.62100.

Neurodevelopmental disorder in an Egyptian family with a biallelic *ALKBH8* variant

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Abstract

Alkylated DNA repair protein AlkB homolog 8 (ALKBH8) is a member of the AlkB family of dioxygenases. ALKBH8 is a methyltransferase of the highly variable wobble nucleoside position in the anticodon loop of tRNA, and thus plays a critical role in tRNA modification by preserving

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Conflict of interest

J.R.L. has stock ownership in 23andMe, is a paid consultant for Regeneron Pharmaceuticals, and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, and bacterial genomic fingerprinting. The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing conducted at Baylor Genetics (BG) Laboratories; Other authors have no potential conflicts to report. J.V.H receives royalties from chapter in UpToDate on pediatric neuroimaging.

Data Sharing

The data that support the findings of this study are available from the corresponding author upon reasonable request.

codon recognition and preventing errors in amino acid incorporation during translation. Moreover, its activity catalyzes uridine modifications that are proposed to be critical for accurate protein translation. Previously, two distinct homozygous truncating variants in the final exon of *ALKBH8* were described in two unrelated large Saudi Arabian kindreds with intellectual developmental disorder, autosomal recessive 71 (MRT71) syndrome (MIM# 618504). Here, we report a third family- of Egyptian descent- harboring a novel homozygous frame-shift variant in the last exon of *ALKBH8*. Two affected siblings in this family exhibit global developmental delay and intellectual disability as shared characteristic features of MRT71 syndrome, and we further characterize their observed dysmorphic features and brain MRI findings. This description of a third family with a truncating *ALKBH8* variant from a distinct population broadens the phenotypic and genotypic spectrum of MRT71 syndrome and firmly establishes *ALKBH8* as a novel neurodevelopmental disease gene.

Keywords

ALKBH8; tRNA modification; intellectual disability; neurodevelopmental delay

Introduction

Transfer RNA (tRNA) modifications are post-transcriptional processes that preserve the structure and function of tRNA by modifying its stability, flexibility, translational efficiency and codon–anticodon specificity during the process of tRNA maturation. Such processes include methylation, deamination, and acetylation reactions (Abedini et al., 2018).

Defects in tRNA biogenesis and tRNA modifications have been found to play a role in the etiology of neurodevelopmental disorders (Karaca et al., 2014). The anticodon loop of a tRNA molecule contains the anticodon site and accumulates greater modification diversity, especially at the wobble position where modified bases modulate codon–anticodon interactions (Abedini et al., 2018; Franz et al., 2020).

ALKBH8 (alkylated DNA repair protein AlkB homolog 8), encoded by *ALKBH8* (MIM* 613306), is an essential methyltransferase that mediates the methylation of the wobble uridine residues of tRNA. ALKBH8-mediated methylation generates 5-methoxycarbonylmethyluridine (mcm^5U) which is a prerequisite for the subsequent hydroxylation of wobble uridine in certain tRNAs (Songe-Møller et al., 2010).

Two homozygous truncating mutations in the final exon of *ALKBH8* have been shown to cause autosomal recessive intellectual disability (intellectual developmental disorder, autosomal recessive 71; MRT71, MIM# 618504) in two consanguineous Saudi Arabian families (Monies et al., 2019). Here, we describe a third family of Egyptian descent with a novel homozygous truncating variant in *ALKBH8* (c.1684delC, Arg562Alafs*56) and detail the clinical and molecular genetic features in the two affected siblings.

Clinical report

The proband (BAB13277) is a 3 years-old male born to first cousin consanguineous parents following an uncomplicated pregnancy and delivery. He has one affected sister (BAB13280, 14 years-old), and one healthy female sibling. Aside from the proband and his affected sister, there are no additional relatives with a similar condition (Figure 1a).

Both the proband and his affected sister demonstrated global developmental delay. They sat without support at 10 months, crawled between 18 and 20 months, and began to walk between 24 and 30 months of age. The proband could vocalize single syllables but could not speak any words. He could follow objects, attend to his surroundings and follow simple commands. The older affected sister's speech was limited to only a few single syllables. She is able to follow simple commands, feed herself, and has acquired sphincter control at the age of 5y, but continues to have occasional nocturnal enuresis. There is no history of epilepsy and no known history of seizure-like episodes in either the proband or the affected sister. The affected sister was enrolled in a special education daytime speech stimulation program, but with no significant improvement in speech acquisition.

Anthropometric measurements at current age were: weight 11.3 kg (z-score = −2.1) and 28 kg (z-score = −2.6), height 83 cm (z-score = −2.7) and 128 cm (z-score = −5.3), head circumference 49 cm (z-score = −0.9) and 51 cm (z-score = −2.4), in the proband and his sister, respectively. Head circumference at birth as well as serial head circumference measurements were not available to determine the exact nature of the microcephaly (postnatal progressive vs. congenital).

During examination, the proband demonstrated attention deficits, while the affected sister displayed some autistic features, and was mildly hyperactive. Both affected siblings were noted to have dysmorphic craniofacial features as described in (Figure 1b). Additionally, the male proband had bilateral undescended testes. The affected sister showed no signs of sexual maturation at age 14 (Tanner stage: B1, P1, A1). Neurological examination revealed that both siblings had muscle hypotonia, hyporeflexia, and joint hyperextensibility.

Intelligence testing was performed on both siblings and showed that the proband has an intellectual quotient (IQ) of 51 by Stanford-Binet test while his affected sister has an IQ of 42 by Wechsler Intelligence Scale for Children. The brain MRI images obtained at ages 14 months and 11 years in proband and affected sister respectively showed mild to moderate supratentorial cerebral volume loss (more pronounced in the proband, mild to moderate cerebellar vermian hypoplasia and infratentorial volume loss (more pronounced in the affected sister), a variable degree of thinning of the corpus collosum and abnormal myelination for age (Figure 1b).

Molecular analysis

The family was enrolled under an Institutional Review Board (IRB)- approved research protocol (H-29697) at the Baylor-Hopkins Center for Mendelian Genomics as part of a large cohort for novel "disease gene" discovery (Posey et al., 2019). We performed family-based research exome sequencing (ES) for the two affected siblings and both parents according

to the previously reported methods (Pehlivan et al., 2019). A novel homozygous frame-shift deletion in the last exon, of the total 12 exons, of *ALKBH8* [Chr11:g.107375703CG>C (hg19); NM_001301010.1; c.1684delC, p.(Arg562Alafs*56)] was identified in both affected siblings (Figure 1c). The variant was not present in publicly available control databases (Atherosclerosis Risk in Communities, ARIC; Grand Opportunity Exome Sequencing Project, GO-ESP; 1000 Genomes Project; and the genome aggregation database, gnomAD) or in the in-house generated BHCMG database (~13,000 exomes) in either the homozygous or heterozygous state. *ALKBH8* has a low probability of loss-of-function (LoF) intolerance score (PLI = 0). Yet, the LoF observed/expected upper bound fraction (LOEUF) score, a recently created parameter by the gnomAD group after applying their newly developed loss-of-function transcript effect estimator (LOFTEE) package, is 0.4 (0.25–0.67), suggesting that *ALKBH8* is essential for cell viability and also depleted for pLoF variation (Karczewski et al., 2020).

Subsequent segregation analysis and variant validation was performed by Sanger sequencing and showed homozygous state in the affected siblings and heterozygous state in both parents consistent with Mendelian expectations. The variant was predicted to escape nonsense mediated decay (NMD) by our inhouse developed NMD escape predictor, NMDEscPredictor (https://nmdprediction.shinyapps.io/nmdescpredictor/) (Coban-Akdemir et al., 2018). To quantitate degree of consanguinity and delineate potential identity-by-decent (IBD) intervals, unphased ES data were analyzed using BafCalculator (https://github.com/BCM-Lupskilab/BafCalculator) according to the BafCalculator algorithm described previously (Gambin et al., 2017; Gonzaga-Jauregui et al., 2020). The variant was located within 10.6 Mb and 10.8 Mb intervals of absence of heterozygosity (AOH) blocks with a total genome wide AOH size of 401.74 Mb and 287.24 Mb in the proband (BAB13277) and affected sister (BAB13280), respectively (Figure 1d).

Discussion

We report a family from Egypt with a novel homozygous frame-shift variant c.1684delC p.(Arg562Alafs*56) in the last coding exon of *ALKBH8*. Only two families with *ALKBH8* pathogenic variation have been reported to date, both from Saudi Arabia and each with likely LoF alleles, a nonsense and a frameshift mutation in the last exon, c.1660C>T p.(Arg554*) and c.1794delC p.(Trp599Glyfs*19) respectively (Monies et al., 2019). Of note, both frameshift mutations, c.1684delC and c.1794delC, occur in runs of 5C and 3C nucleotides respectively. These mutations likely occurred *de novo* in a distant member of the clan by slippage during DNA replication and were homozygosed by identity-by-descent in the family given consanguinity (Bi et al., 2006; Lupski et al., 2011; Gonzaga-Jauregui et al., 2020). All three mutant RNA likely escape nonsense mediated decay and thus make a protein mutated at the carboxy-terminus that may act as a hypomorphic allele. Both frameshift variants are predicted to cause premature truncation at the same stop codon resulting in a 616 amino acids-long protein.

The clinical neurodevelopmental and brain MRI features observed in both affected children in this family were characterized extensively. Neither had seizures, in contrast to the majority of subjects previously reported. Six out of seven affected individuals from both

unrelated Saudi kindreds reported by Monies et al. (2019) had a diagnosis of epilepsy with the latest age of onset being 2 years of age. Both affected siblings described herein display structural brain anomalies including mild to moderate cerebral volume loss, mild to moderate cerebellar vermian hypoplasia, variable degrees of thinning of the corpus collosum and abnormal myelination for age on brain MRI, whereas abnormalities on brain MRI in the form of non-specific arachnoid granulation were observed in only one out of the three affected individuals with brain imaging in Monies et al. (2019).

In conclusion, our report of an Egyptian family with MRT71, harboring a novel pathogenic *ALKBH8* variant, expands the phenotypic and genotypic spectrum of the disorder. Furthermore, the dysmorphic features described in both siblings and displayed here enrich the phenotypic characterization of the disease. Notably, all three mutations reported in association with disease thus far have PTC in the last coding exon and the variant allele mRNA is likely to escape NMD; whether they are acting as loss of function (LoF) or gain of function (GoF) alleles remains to be determined (Coban-Akdemir et al., 2018). Identification of additional families with *ALKBH8* variants and subsequent experimental work is required to further explore the mutational spectrum at this locus. This report emphasizes the importance of studying patients from different populations and ethnicities to fully understand the genetic aetiology of intellectual disability syndromes.

Acknowledgement

We would like to thank the family for their participation in this study.

Funding information

This study was supported in part by the U.S. National Human Genome Research Institute (NHGRI) and National Heart Lung and Blood Institute (NHBLI) to the Baylor-Hopkins Center for Mendelian Genomics (BHCMG, UM1 HG006542, J.R.L); NHGRI grant to Baylor College of Medicine Human Genome Sequencing Center (U54HG003273 to R.A.G.), U.S. National Institute of Neurological Disorders and Stroke (NINDS) (R35NS105078 to J.R.L.) and Muscular Dystrophy Association (MDA) (512848 to J.R.L.). D.M. is supported by a Medical Genetics Research Fellowship Program through the United States National Institute of Health (T32 GM007526–42). T.M. is supported by the Uehara Memorial Foundation. D.P. is supported by a Clinical Research Training Scholarship in Neuromuscular Disease partnered by the American Academy of Neurology (AAN), American Brain Foundation (ABF) and Muscle Study Group (MSG), and International Rett Syndrome Foundation (IRSF grant #3701–1). J.E.P. was supported by NHGRI K08 HG008986. A.K.S is supported by United States Agency for International Development (USAID) fellowship.

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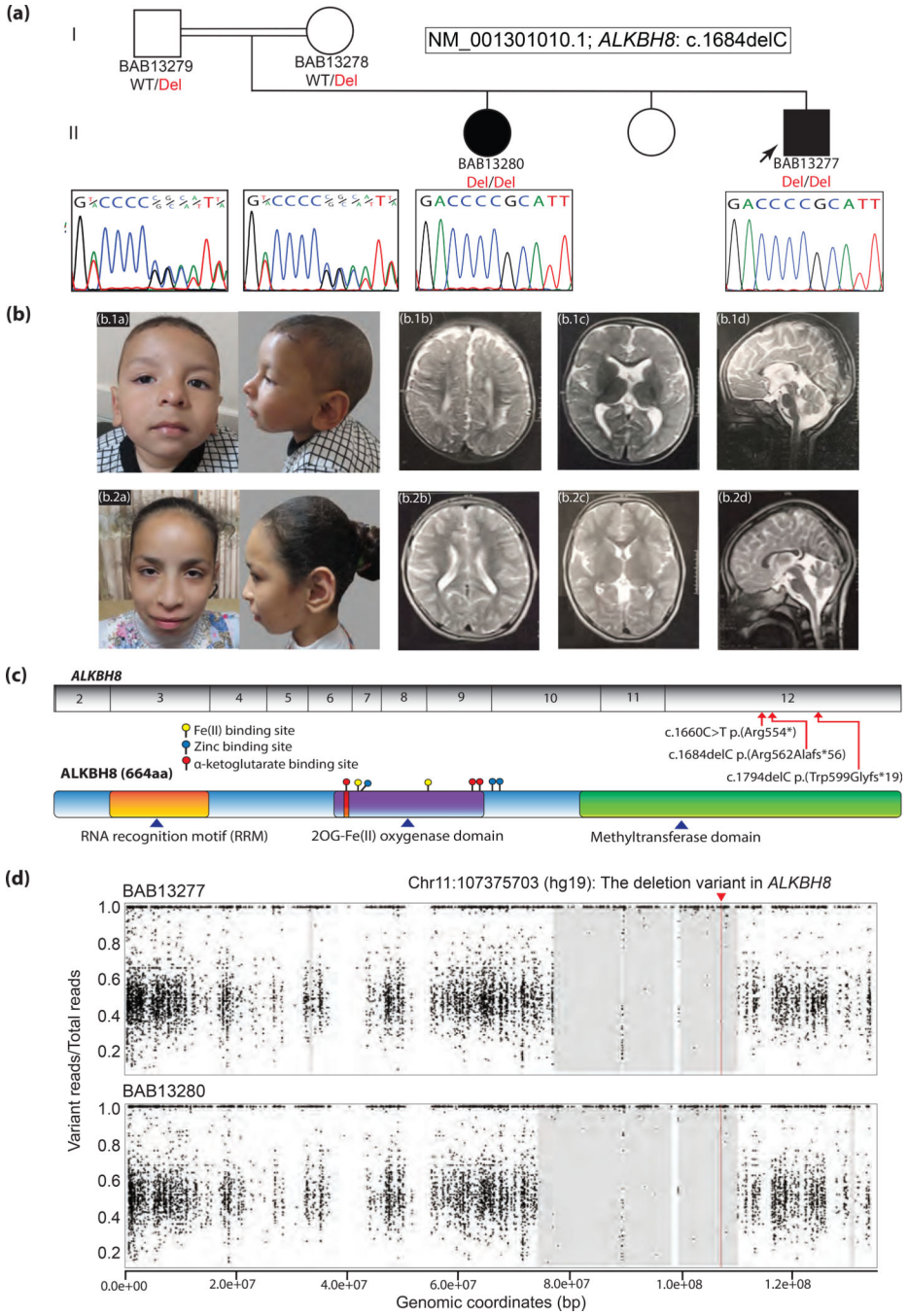

Figure 1: Family pedigree with genetic findings, facial photos and MRI images of patients, Cartoon of ALKBH8 cDNA and protein, and AOH plots.

(a) Standard pedigree symbols are shown; the proband is identified with a black arrow, affected individuals are shaded black. Identifiers and genotype along with the Sanger validation and segregation study are included below each corresponding individual on the pedigree; to the right is shown a Sanger trace of the wild type sequence.

Note the presence of a polymorphic variant, (c.1679T>A) prior to the deletion site, that appears as heterozygous in both parents and homozygous in both affected siblings.

WT: Wild Type, DEL: Deletion.

(**b.1a**) Frontal and sideview photographs of the proband (BAB13277) at 2 years of age showing craniofacial dysmorphic features including high forehead, sparse lateral eye brows, almond shaped eyes, mild ptosis, broad nasal root, prominent nose with prominent columella, long simple philtrum, long lips with V shaped upper lip and prominent lower lip with prominent vermillion, macrostomia, retruded mandible, broad chin with chin dimple and low-set large ears with prominent antihelix

(**b.1b–d**) Brain MRI images of proband (BAB13277) at the age of 14 months. (**b.1b**) Axial view (T2-weighted sequence) showing wide perivascular spaces (Virchow Robin spaces) indicating deep white matter volume loss and prominent extra-axial spaces suggestive of mild cerebral volume loss. (**b.1c**) Axial view (T2-weighted sequence) at the level of thalamus showing under-opercularization and widening of Sylvian fissures as well as thinning of the genu of corpus callosum and delayed myelination of internal capsule both of which are suggestive of delayed myelination for the age. (**b.1d**) Mid-sagittal view (T2-weighted sequence) showing moderate supratentorial volume loss, moderate to severe thinning of the corpus collosum, and mild superior cerebellar vermian volume loss.

(**b.2a**) Frontal and sideview photographs of the affected sister (BAB13280) at 14 years of age showing the same facial dysmorphic features observed in the proband and detailed in (a1); no ptosis or head tilt noted.

(**b.2b–d**) Brain MRI images of affected sister (BAB13280) at 11 years of age. (**b.2b**) Axial view (T2-weighted sequence) showing no white matter changes at this level in comparison to the proband. (**b.2c**) Axial view (T2-weighted sequence) at the level of thalamus showing widening of Sylvian fissures and abnormal myelination for the age as evident in the delayed myelination of the internal capsule. (**b.2d**) Mid-sagittal view (T2-weighted sequence) showing mild supratentorial volume loss, mild thinning and foreshortening of the corpus collosum, moderate superior cerebellar vermian atrophy, and widened of the fourth ventricle. In aggregate, these findings indicate global volume loss.

(**c**) Schematic representation of the coding exons of *ALKBH8* cDNA (NM_001301010.1) and of ALKBH8 protein (NP_001287939.2); the locations of exons are aligned relative to the ALKBH8 regions each exon encodes. Red arrows point to the locations of the two previously reported variants (c.1660C>T p.(Arg554*) and c.1794delC p.(Trp599Glyfs*19) as well as the currently described variant (c.1684delC, Arg562Alafs*56). Note all three variant alleles result in PTC mapping to the last exon and the mutant mRNA are predicted to escape NMD

(**d**) Plot for regions of absence of heterozygosity (AOH), which is marked by gray zones, on chromosome 11 in BAB13277 and BAB13280. Note that the variant in *ALKBH8* is located within a shared AOH region of 10.6 Mb in the affected siblings.