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CLINICAL REPORT

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Novel Homozygous *DEAF1* Variant Suspected in Causing White Matter Disease, Intellectual Disability, and Microcephaly

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DEAF1 encodes a transcriptional binding factor and is a regulator of serotonin receptor 1A. Its protein has a significant expression in the neurons of different brain regions and is involved in early embryonic development. In addition, its role in neural tube development is evident from the knockout mouse as many homozygotes have exencephaly. Heterozygous mutations of this gene have been linked to intellectual disability in addition to the gene's involvement in major depression, suicidal tendencies, and panic disorder. In this clinical report, we describe two children from a consanguineous family with intellectual disability, microcephaly, and hypotonia. The brain MRI of both patients showed bilateral and symmetrical white matter abnormalities, and one of the patients had a seizure disorder. Using whole exome sequencing combined with homozygosity mapping, a homozygous p.R226W (c.676C>T) mutation in *DEAF1* was found in both patients. Furthermore, sequencing analysis confirmed complete segregation in tested family members and absence of the mutation in control cohort (n = 650). The mutation is located in a highly conserved structural domain that mediates DNA binding and therefore regulates transcriptional activity of its target molecules. This study indicates, for the first time to our knowledge, a hereditary role of *DEAF1* in white matter abnormalities, microcephaly and syndromic intellectual disability.

Key words: DEAF1; homozygous p.R226W; syndromic intellectual disability; white matter abnormality; microcephaly

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INTRODUCTION

Neurodevelopmental disorders (NDs) comprise a group of highly heterogeneous disorders mostly caused by alterations during early brain development. Various NDs share similar features such as brain dysfunction and cognitive impairment [van Loo and Martens, 2007]. The recent advances in massively parallel-sequencing techniques have led to numerous gene breakthroughs in inherited and sporadic NDs related to intellectual disability. However, the full picture of pathogenesis of NDs is not fully understood.

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Deformed epidermal autoregulatory factor 1 (DEAF1)—also known as "nuclear DEAF-1-related transcriptional regulator"—is a trans-acting, trans-regulator element that regulates the serotonin 1A receptor (5HT1A) in the brain. *DEAF1* is known to be highly conserved among different species with significant expression in all the neurons of different brain regions. It is important in early embryonic development and regulates immunity gene expression in drosophila [Veraksa et al., 2002; Reed et al., 2008]. In addition, its role in neural tube development is evident from the mouse knockout as many homozygotes have exencephaly [Hahm et al., 2004]. The gene longest transcript yields 565-amino acid-long polypeptide that is critical for transcriptional regulation of serotonergic synapses. 5HT1A is widely expressed in serotonergic neurons and brain, and co-localizes with DEAF1 in serotonergic raphe cells and hippocampal and cortical neurons [Lemonde et al., 2003]. DEAF1 selectively suppresses somatodendric 5HT1A autoreceptor expression in serotonergic synapses in an allele-specific manner [Lemonde et al., 2004]. Besides its proposed role in psychiatric disorders [Mann, 1999; Czesak et al., 2006; Jans et al., 2007] and carcinoma [Carr et al., 1990; LeBoeuf et al., 1990, 1998; Ban and LeBoeuf, 1994; Manne et al., 2001], the action of DEAF1 in regulating the serotonin system further suggests a causative role in mental disorders [Czesak et al., 2012]. *DEAF1* has been previously linked to nonsyndromic sporadic intellectual disability (ID) [Vissers et al., 2010; Rauch et al., 2012] and is suggested to have a role in major depression, suicidal tendencies, and panic disorder [Albert and Lemonde, 2004; Lemonde et al., 2004].

Due to high consanguinity rate (reaching to nearly 80% in some regions) and the large family size, countries like Saudi Arabia (SA) allow great opportunities for geneticists who focus on inherited NDs. Altogether, these disorders in SA are estimated to be around 3–5:100. Despite the discovery of numerous disease-causing genes, many clinically suspected inherited NDs cases remain molecularly unidentified. In order to identify novel genetic causes of hereditary NDs, we performed autozygome analysis using high-density SNP arrays combined with exome sequencing as a positional cloning strategy that led to the identification of a novel missense mutation in *DEAF1* in a consanguineous Saudi family.

METHODS

Two patients and their family members from a consanguineous family were ascertained under KFSHRC IRB-approved protocols (RAC#2120 022) after signing the written informed consents. Whole blood samples were collected and DNA isolations were performed using PureGene DNA Purification Kit (Gentra Systems, Inc., Minneapolis, MN). Human Mapping Axiom arrays (Affymetrix, Inc., Santa Clara, CA) were used for SNP genotyping. The assay preparation, scanning, image processing, genotyping, and preliminary data analysis were all done according to manufacturers' protocols and guidelines. Exon Capture and library construction were done according to Agilent's SureSelect Protocol Version 1.2 (Agilent, Inc.). Enrichment was carried out using SureSelect protocols. Sequencing was performed on the Illumina HiSeq2000 platform using TruSeq v3 chemistry (Illumina, San Diego, CA). Runs of homozygous (ROH) blocks using SNP calls were determined by identifying loss of heterozygous regions (LOH) calculated by AutoSNPa [Carr et al., 2006] and HomozygosityMapper [Seelow et al., 2009]. Exon specific primers for *DEAF1* were designed using publicly available Primer3 algorithm [Rozen and Skaletsky, 2000]. PCRs were performed according to standard protocols using BigDye Terminators (Thermo Fisher Scientific Inc., Waltham, MA) on an ABI 3100 sequencer. Sequence chromatograms were analyzed using SeqMAN software version 1.1 (DNASTAR, Inc., Madison, WI). Eleven different bioinformatics tools were utilized to predict the functional consequences of the p.R226W (Supporting Information File 1). DEAF1 related sequences from various species were obtained from Ensembl and multiple sequence alignment (MSA) was performed using ClustalW and then visualized with JalView. Protein Knowledgebase (UniprotKB) was searched for DEAF1. Uniprot output O75398 (DEAF1_HUMAN) was used for 3-D structural analysis for SAND domain. Array Comparative Genomic Hybridization (aCGH) was performed as part of routine diagnostic cytogenetic protocols using Agilent 180K arrays (Agilent, Inc., Santa Clara, CA) according to manufacturer's instructions and guidelines.

CLINICAL REPORT

Phenotypic Analysis of the Patients

We ascertained a two-branched consanguineous Saudi family from a farming community in central Saudi Arabia. The clinical features of the patients are given in Table I. The prominent clinical features of the two patients include microcephaly, ID, and white matter abnormalities. Index Patient II-5 had a sister who died at the age of 3 years with similar features (DNA was not available in this research). Extensive metabolic investigation was performed on the deceased patient (Patient II-4) including plasma amino acids,

TABLE I. Clinical and Laboratory Findings

Patients	II-2	II-5
Age	2½ years	2 years
Gender	Male	Male
Age at presentation	Neonatal	2 Months
Birth weight	2.3 kg	Not available
Hypotonia	+	+
Feeding difficulties	+	+
Growth	Poor	Poor
OC	44 cm (2½ years)	44 cm (at 22 months)
Vision	Normal	Normal
Hearing	Normal	Normal
Dysmorphism	None	None
Motor delay	Moderate	Moderate
Speech delay	Severe	Severe
Cognitive delay	Severe	Severe
Seizure	+	−
Hyperactivity	−	−
Karyotype	46 XY	46 XY
Array CGH	Normal	Normal
Metabolic screen	Unremarkable	Unremarkable
Brain MRI	Abnormal (see text)	Abnormal (see text)
Electroencephalogram	Normal	Not available

MRI, magnetic resonance imaging; OC, occipitofrontal circumference.

acylcarnitine profile, urine organic acid analysis, screening for congenital; glycosylation defects and array CGH were all unremarkable.

Briefly, index Patient II-2 was referred to us at the age of 2 years with history of hypotonia, seizures, and psychomotor retardation. His weight was 12.2 kg (<3rd centile), length was 83 cm (10th centile) and occipitofrontal circumference (OFC) was 43 cm (<3rd centile). There were no distinctive facial dysmorphic features and systemic examination was unremarkable. The tone and power were decreased, and the deep tendon reflexes were preserved. The other patient (Patient II-5) was referred at the age of 22 months with severe hypotonia, psychomotor retardation, poor feeding, and excessive irritability. He had microcephaly (OFC was 44 cm; <3rd centile) but weight and length were normal. The axial and peripheral tone and power were decreased. The deep tendon reflexes were depressed. There was no facial dysmorphism and the systemic examination was intact. Plasma amino acids, acylcarnitine profile, urine organic acid analysis, screening for congenital glycosylation defects, and aCGH on both patients were normal.

Brain MRI of Patient II-2 showed bilateral and symmetrical T2 high-signal intensity changes affecting the peritrigonal deep white matter in the parieto-occipital regions. In addition, an abnormal gyral pattern was noted in the 3D volumetric study affecting the cortex around the sylvian fissures and in the parietal lobes. The MR spectroscopic pattern demonstrated relatively high choline peak in the parietal white matter and the basal ganglia with a normal NAA peak suggesting disturbed myelination. The brain MRI of Patient II-5 showed the same findings with bilateral and symmetrical T2 high-signal intensity changes. MRS peaks were normal (Fig. 1).

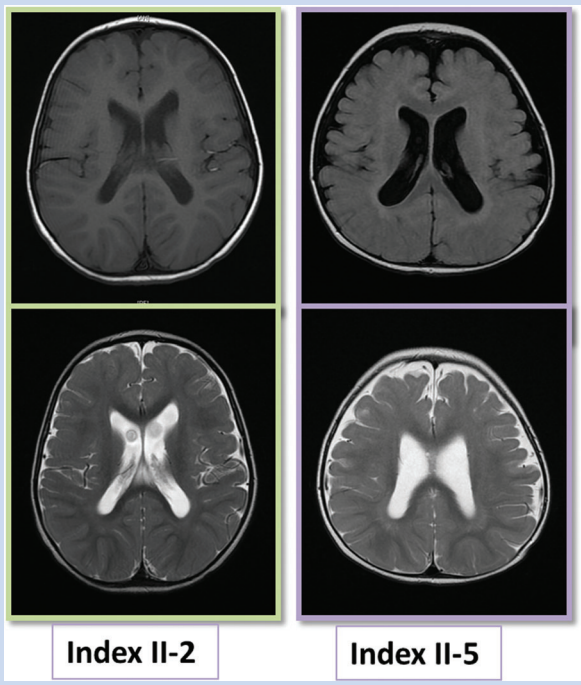

FIG. 1. Brain MRI images of Patients II-2 (left) and II-5 (right) are presented in the figure showing signal intensity changes affecting the peritrigonal deep white matter in the parieto-occipital regions. In addition, an abnormal gyral pattern was noted in the 3D volumetric study affecting the cortex around the sylvian fissures and in the parietal lobes.

A basic evaluation of the immune system was performed. For Patients II-2 and II-5, complete blood and differential white blood cell counts were normal. For Patient II-2, the Immunoglobulin IgG was 8.50 (Ref 3.5–12.4 g/L), IgA was 1.14 (Ref 0.40–1.20 g/L), and IgM 0.78 (Ref 0.43–1.7 g/L). Flow cytometry for lymphocyte markers showed B lymphocytosis with intact expression of MHC Class II antigen on B lymphocytes. The activated T cells (CD3+DR+) were 7.3%, whereas for patient II-5, the immunoglobulin IgG was 6.83, IgA was 1.48, and IgM was 1.36. Flow cytometry for lymphocyte markers showed T and B lymphocytosis with intact expression of MHC Class II antigen on B lymphocytes. The activated T cells (CD3+DR+) were 3.3%. Because *Deaf1* has been implicated in immune function [Yip et al., 2009], the measured indices of immune function were all in the normal range in patients carrying the mutation.

Genetic Analysis

Initially, we performed exome sequencing on Patient II-5 followed by genome-wide homozygosity screening on the family (Fig. 2A) using high-resolution SNP custom arrays (Affymetrix, Inc.) reasoning that the family based on both parents' consanguinity would show homozygous, biallelic mutations embedded within larger blocks of homozygosity inherited from a common ancestor. To achieve that axiom SNP calls were entered into AutoSNPa software after genotypes were generated by the GeneChip scanner and annotated using SNP-Annotator software. During the analysis default settings of AutoSNPa were not changed and three runs of homozygosity blocks were detected on chromosomes 11, 15, and 20; the longest one is being on chromosome 11 (6.3 Mb; Fig. 2B). The remaining blocks were 2.3 Mb (Megabase) (chromosome 15) and 0.8 Mb (chromosome 20) in size. We subsequently searched sequence-variant databases, such as dbSNP, Ensembl, and the National Heart, Lung, and Blood Institute (NHLBI) Exome Variant Server (EVS), 1,000 genome project and filtered out previously known SNPs; and did not found the variant in these databases. Furthermore, we ranked and later focused on only homozygous changes that are present only in the patient specific ROH. List of the identified homozygous changes by exome sequencing is given in Supplementary Information File 2 (see Supporting Information Online). This analysis identified a novel homozygous missense mutation in *DEAF1* on Chromosome 11 in Patient II-5. The same mutation was also present in Patient II-2, his cousin from the other branch. Their asymptomatic parents (Individuals I-1, I-2, I3, and I-4) were all found to harbor the mutation in the heterozygous state (Fig. 2C). We subsequently screened for ethnically matching control cohort for p.R226W (NM_021008; c.676C>T) (n = 650) and confirmed the novelty. Moreover, 11 different bioinformatics tools predicted harmful functional consequences of the p.R226W (c.676C>T) (Supplementary Information File 1 see Supporting Information Online).

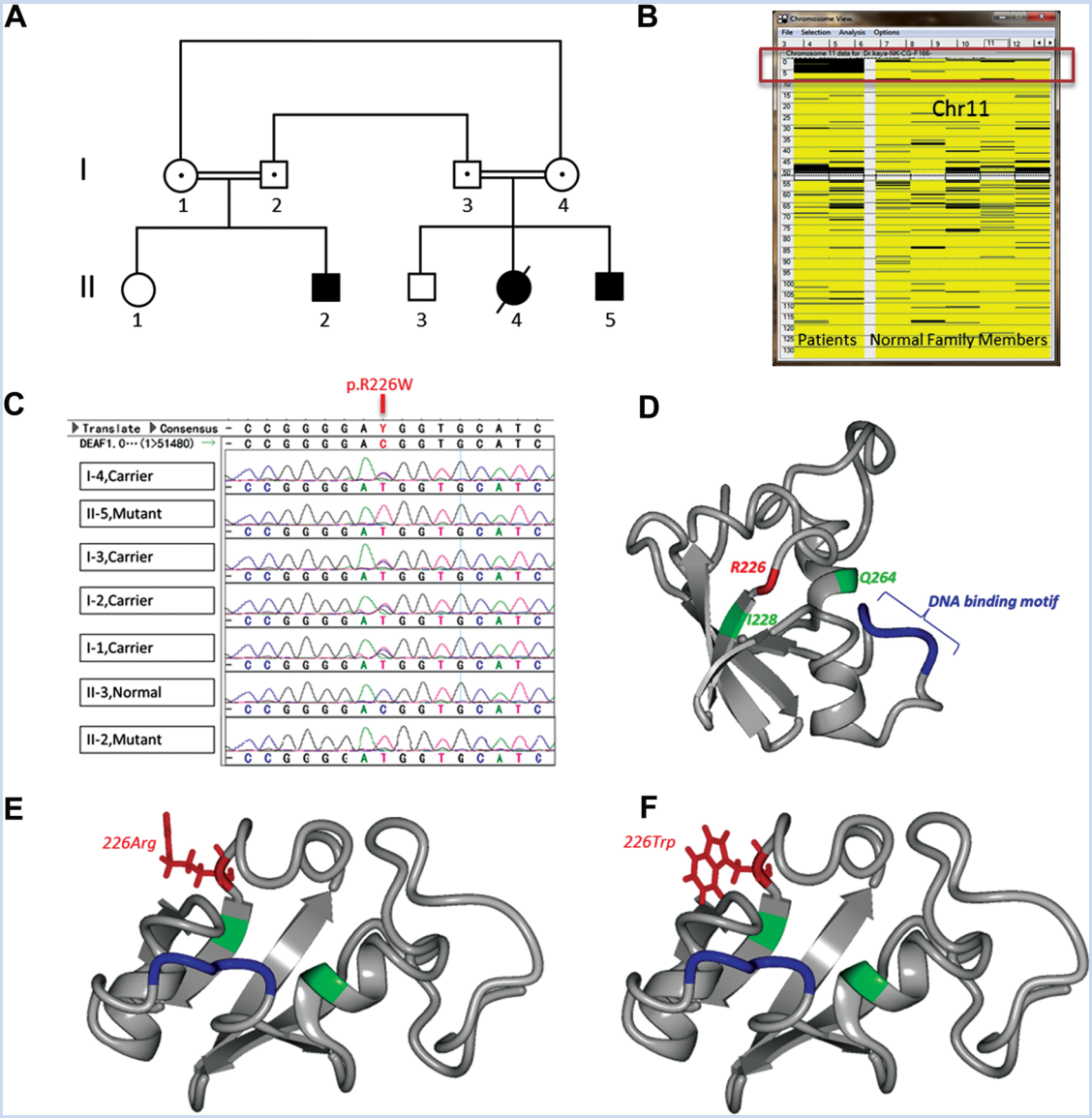

FIG. 2. Pedigree (A) indicates a consanguineous family with affected individuals. All the members were tested in the family except for Patient II-1and Patient II-4 whose DNA was not available for analysis. AutoSNPa result (B) for chromosome 11 reveals a shared ROH (boxed in red) and patients were presented in the left panel. DNA chromatograms (C) indicate the position of the mutation (p.R226W) in the family. Protein modeling (D) is based on 1h5p (PDP; Protein Data Bank Entry), representing the SAND domain, presented as a ribbon structure. The DNA binding motif, KDWK, is highlighted in blue, whereas the locations of published de novo mutations are indicated in green. The homozygous mutation identified in our family is shown in red. The wild type arginine (E) is replaced by larger Tryptophan (F) depicted in the 3-D structure.

DISCUSSION

Recently, DEAF1 has received considerable attention due to its structural properties, its regulatory role in serotonin related pathways and synapses. Structurally, the protein has two main domains (SAND and MYND), Ala-rich and pro-rich motifs, and transmembrane domains. Overall SAND domain is a highly conserved ~80-amino acid-long structure playing a role in mediating DNA binding thus regulating transcriptional activity of its target molecules. The SAND is placed in a close proximity to the N-terminus whereas MYND domain resides towards C-terminus. Both domains are acronyms for the initial letters of genes/proteins sharing such structures. SAND stands for ***Sp100***, ***AIRE-1***, ***NucP41/75***, ***DEAF1*** whereas MYND is called after **My**eloid translocation protein, **N**ervy protein, and **D**eaf1.

The mutation we found in this family, p.R226W, is located towards the end of LGSGGRGRC motif which is completely conserved among 40 different species (Supplementary Information File 3 in Supporting Information Online). Through case-parent trios, two patients with nonsyndromic ID were recently found to

have de novo mutations (The first is c.683T>G, p.Ile228Ser; and the second is g.686871T>G, Gln264Pro) in *DEAF1* in heterozygous state that are likely to be pathogenic [Vissers et al., 2010; Rauch et al., 2012]. The site of the mutation in this report in relation to the previously known de novo mutations is depicted on 3-D structure (Fig. 2D). Previously known somatic mutations are given in Supplementary Information File 4 (see Supporting Information Online). The mutation, p.R226W, does not seem to directly interfere with the DNA binding domain and located distantly from the binding site; however, it is reasonable to consider that the large tryptophan may stereotypically hinder normal protein folding (Fig. 2E,F).

Interestingly, DEAF1 regulates its targets by a number of different mechanisms. It is able to bind its own promoter as well as promoters of other genes, and has capacity to regulate its targets without promoter binding perhaps through protein–protein interactions [Huggenvik et al., 1998; Michelson et al., 1999]. It is also known that it acts on retinoic acid response element and may be involved in arresting cells in the G0 or G1 phase [Manne et al., 2001]. DEAF1 has also been shown to reduce activity of the serotonin autoreceptor 5HT1A through allele-specific manner [Lemonde et al., 2003, 2004; Albert and Lemonde, 2004].

Taken together, our results implicate an inherited *DEAF1* defect in a syndromic form of ID and opens up new research possibilities into the intricate involvement of SAND domain related defects in human neurodevelopment, brain and synaptic function.

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