




Biallelic loss of *EEF1D* function links heat shock response pathway to autosomal recessive intellectual disability

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Abstract

Intellectual disability (ID) is a genetically heterogeneous neurodevelopmental disorder characterised by significantly impaired intellectual and adaptive functioning. ID is commonly syndromic and associated with developmental, metabolic and/or neurological findings. Autosomal recessive ID (ARID) is a significant component of ID especially in the presence of parental consanguinity. Several ultra rare ARID associated variants in numerous genes specific almost to single families have been identified by unbiased next generation sequencing technologies. However, most of these new candidate ARID genes have not been replicated in new families due to the rarity of associated alleles in this highly heterogeneous condition. To determine the genetic component of ARID in a consanguineous family from Turkey, we have performed SNP-based linkage analysis in the family along with whole exome sequencing (WES) in an affected sibling. Eventually, we have identified a novel pathogenic variant in *EEF1D*, which has recently been recognised as a novel candidate gene for ARID in a single family. *EEF1D* encodes a ubiquitously expressed translational elongation factor functioning in the cytoplasm. Herein, we suggest that the loss of function variants exclusively targeting the long *EEF1D* isoform may explicate the ARID phenotype through the heat shock response pathway, rather than interfering with the canonical translational elongation.

Introduction

Intellectual disability (ID) comprises a group of neurodevelopmental disorders, which are basically characterised by significant limitations in both intellectual

functioning and adaptive behaviours starting before the age of 18 [1]. ID associated with mild to severe learning and behavioural defects may be the only apparent manifestation of the disease (nonsyndromic ID) or more commonly, it may be accompanied by congenital malformations, neurological or metabolic findings (syndromic ID) [2]. Therefore, ID is an extremely heterogeneous condition that causes cognitive impairment due to both genetic and non-genetic factors. Well-established genetic causes of ID include chromosomal anomalies and monogenic diseases, while the environmental factors that may contribute to ID include foetal alcohol exposure, infections, malnutrition and insufficient health care [3]. In populations where parental consanguinity rate is high, ID is assumed to be inherited mostly as autosomal recessive [1]. Accordingly, autosomal recessive ID (ARID) is mostly caused by private mutations in families due to extreme genetic heterogeneity, which can be resolved by unbiased whole genome approaches [3, 4].

Herein, we report three sisters with ARID born to consanguineous parents from Turkey presenting with similar accompanying clinical features. Using whole genome SNP genotyping followed by linkage and whole exome sequencing

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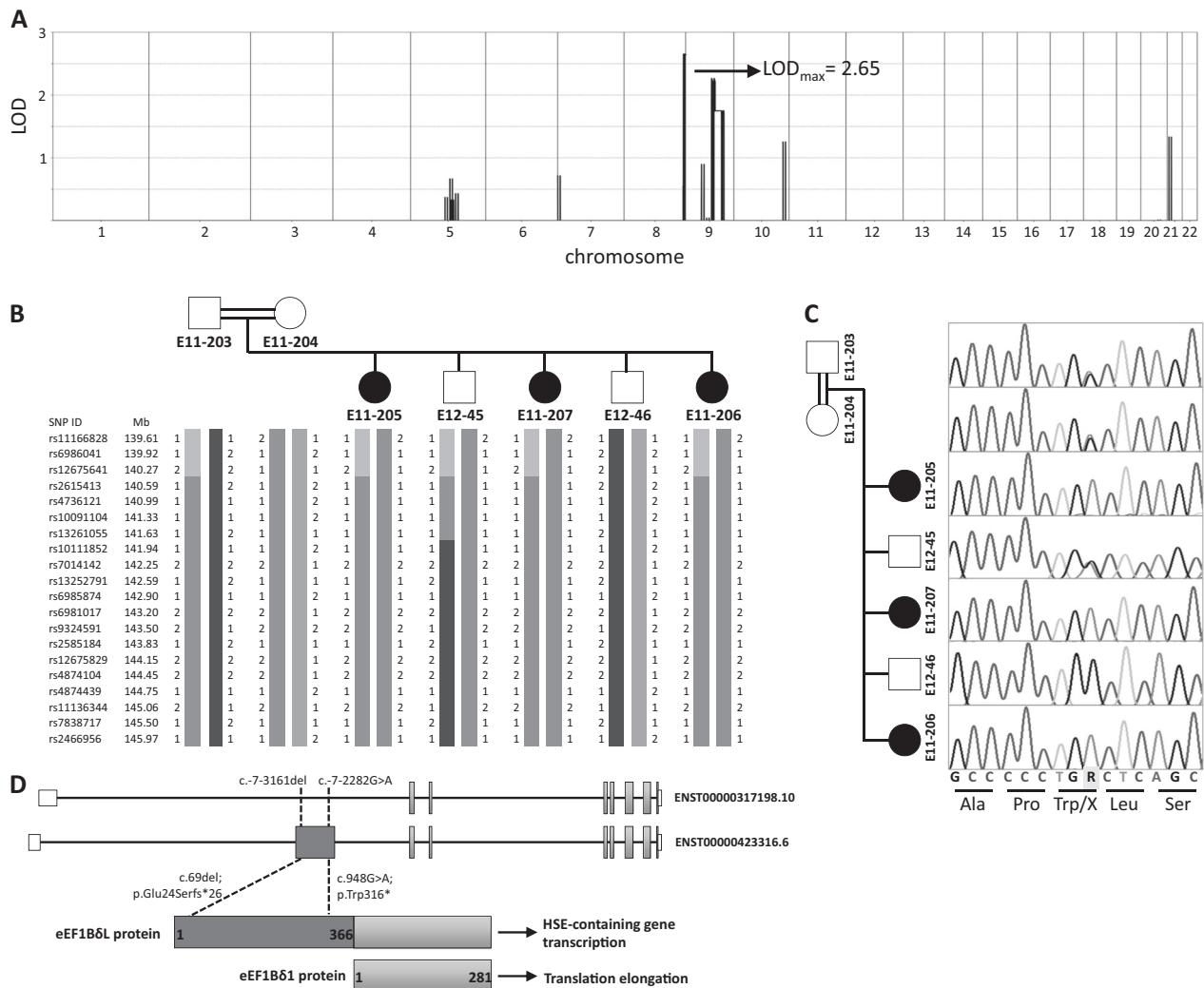


Fig. 1 Genetic studies in the ARID family. **a** Multipoint LOD scores (Allegro) of the total SNP data set along the autosomes. **b** SNP derived haplotypes on chromosome 8. Haplotypes of the parents and siblings have been shown in side-by-side alignment in order to track the IBD segregation. **c** Segregation of NM_001130053.3 (ENST00000423316.6): c.948G>A variant within the family. **d** Schematic representation of

selected *EEFID* transcripts ENST00000317198.10 and ENST00000423316.6 encoding the eEF1B δ 1 and eEF1B δ L isoforms, respectively. The exon unique to the large isoform and the protein domain encoded by this exon are shown in pink. Sites of ARID specific variations in each transcript and corresponding amino acid changes on eEF1B δ L isoform are also indicated

(WES) analyses, we have identified a novel pathogenic variant in *EEFID*, which has recently been proposed as a candidate gene for ARID in a single family [4]. We propose that biallelic loss of function variations of *EEFID* in these two families affect only the heat-shock response (HSR) pathway triggered by alternatively spliced long isoform of *EEFID*.

Materials and methods

Patients and clinical assessments

A family from Turkey with first cousin parents (E11-203 and E11-204), three affected girls (E11-205, E11-207 and E11-

206) and two unaffected boys (E12-45 and E12-46) has been clinically analysed at Harran University, Faculty of Medicine, Department of Paediatric Neurology, Sanliurfa, Turkey (Fig. 1). Physical and neurological examinations were performed for all available family members and detailed information on family history was collected. Magnetic resonance imaging (MRI) of the brain and electroencephalography (EEG) recordings were performed on the affected siblings, whenever possible. Informed consents were obtained from all family members and control individuals in accordance with Istanbul University, Istanbul Faculty of Medicine, Clinical Ethics Committee. DNA was extracted from peripheral blood samples of both children and their parents using QIAamp DNA Blood Maxi Kit (Qiagen GmbH, Hilden, Germany).

Linkage analysis and haplotyping

All seven family members were SNP genotyped using Illumina HumanCytoSNP-12 BeadChip kit and subjected to whole genome logarithm of the odds (LOD) score analysis using ALLEGRO version 1.2c software under software package easyLINKAGE plus version 5.08 assuming recessive inheritance with full penetrance [5]. Haplotypes in regions with positive linkage peaks were manually inspected for identical by descent (IBD) inheritance of the shared haplotypes in the affected children. SNP array data was also used for molecular karyotyping to detect copy number variations (CNVs) using Illumina GenomeStudio version 2011.1 and its plug-in application cnvPartition v3.1.6. CNV calling was performed with a confidence threshold value of 35 and a minimum tandem probe number of 3. Sex chromosomes were also analysed both for CNV detection and sample sex crosscheck. Copy neutral homozygous regions were detected by a default minimum value of 1 Mb.

Exome sequencing and segregation analyses

WES was performed for one of the affected girls (Patient E11-207) through the service provided by Oxford Gene Technology (OGT; Oxford, UK). Exonic DNA was captured with the SureSelect Human All Exon V4 Kit (Agilent Technologies, Santa Clara, CA, USA) and sequenced on the Illumina HiSeq2000 platform. The sample was sequenced to a mean target coverage of 50× with 90.94% of bases covered at a depth of >20×. Exome data analysis was completed in OGT's pipeline and variant lists were browsed using OGT's proprietary software (for details [6]). All rare variants in selected linkage intervals with a carrier frequency expected for an ultra rare recessive disorder (alternative allele frequency; AAF < 0.14%) were filtered [4, 7] (Supplementary information). These variants were then re-annotated with ENSEMBL Variant Effector Predictor Tool (VEP) for both detecting the consequence of these variants on current transcript versions and retrieving up-to-date population frequency data. Sequence validation and segregation analyses were performed via Sanger sequencing for each candidate variant using standard procedures. Hundred and eighty four additional individuals from Turkey with no apparent history of neurological disorders were screened via Sanger sequencing for selected variants.

Results

Clinical presentation

We have studied a consanguineous family from Turkey having three affected girls along with two unaffected boys

(Fig. 1). The patients E11-205, E11-207 and E11-206 were examined at our paediatric neurology outpatient clinic at Harran University Faculty of Medicine at the ages of 12, 8 and 4, respectively, and enrolled to this study due to global developmental delay, ID and epileptic findings. None of the patients could talk. E11-205, who was the first child of the family, was born full term after uncomplicated pregnancy by normal vaginal delivery. She had microcephaly and facial dysmorphic features including hypertelorism and micrognathia. She had weak head and neck control and she was able to sit, stand and walk only with support possibly due to hypotonia. Her deep tendon reflexes were brisk and bilateral Babinski sign was present. She had a history of generalised convulsions starting from the age of 3. According to her parents, the frequency of the seizures was once in a month, which could not be controlled due to insufficient utilisation of prescribed antiepileptic drugs. The clinical characteristics of her both younger sisters were almost identical. These two patients were available for MRI and EEG analyses. In both patients, MRI was indicative of thin corpus callosum and interictal EEG showed generalised epileptiform discharges originating from the left fronto-temporal area and spreading to both brain hemispheres. At the time of examination, Patient E11-206 was seizure free for almost one year, unlike her older affected sisters.

SNP Array, WES and segregation analyses

CNV analysis of the SNP data did not reveal any pathogenic CNVs shared in the affected individuals. Parametric linkage analysis in the family revealed two linkage peaks on chromosomes 8 and 9 with maximum LOD scores of 2.65 and 2.5, respectively (Fig. 1). Haplotype inspection in these loci revealed segregation of IBD haplotypes consistent with an autosomal recessive mode of inheritance only for the distal portion of chromosome 8 (8q24-qter). Region on chromosome 9q21.33–34.12 was eliminated as the unaffected mother was found to be homozygous between rs17088766 and rs11244140 (hg38; chr9:85,539,994–130,787,671 bp) throughout the locus encompassing the positive LOD score peaks. The linkage interval at the distal portion of chromosome 8 was marked by informative rs6578128 and contained 173 genes as listed by NCBI Genome Data Viewer (hg38; chr8:140,642,366–145,138,636 bp). WES data of E11-207 was filtered for this interval on chromosome 8q24-qter. Table 1 summarises all three variants identified *via* exome sequencing in E11-207, which were further validated by Sanger sequencing and segregation analysis. All three variants were absent from the reference cohort screened from Turkey. Amongst, *EEF1D* was selected as the most likely gene associated with the phenotype in the family as it had previously been implicated in ARID [4].

Table 1 List of variants filtered from the WES data on chromosome 8q24-qter

Variant #	hg19 and hg38 positions	AAF	Seg. ^a	Gene	Annotated transcripts	CCDS ID	cDNA variant	Amino acid change/prediction	Consequence	SIFT	PolyPhen
1	chr8:g.144,649,810T>A chr8:g.143,567,640T>A	Novel	Y	<i>MROH6</i>	ENST00000398882.7 (NM_001100878.2)	CCDS47928	c.1904A>T	p.Asn635Ile	missense_variant	D	B
2	chr8:g.144,671,304C>T chr8:g.143,589,134C>T	Novel	Y	<i>EEF1D</i>	ENST00000423316.6 (NM_001130053.3) ENST00000317198.10 (NM_001130057.3) ENST00000526838.5 (NM_001195203.2)	CCDS6404 CCDS6405 CCDS56559	c.948G>A c.-7-2282G>A c.-7-2282G>A	p.Trp316* - -	stop_gained intron_variant intron_variant	- - -	- - -
3	chr8:g.145,689,830G>T chr8:g.144,464,447G>T	rs752345868: 0.006 % (T allele in gnomAD: ALL)	Y	<i>CYHR1</i>	ENST00000528610.5 (NM_001130056.3) ENST00000438911.6 (NM_138496.1) ENST00000530374.5 (NM_001330618.1) ENST00000403000.6 (NM_032687.3)	CCDS47930 CCDS47943 CCDS83335 CCDS6426	c.-7-2282G>A c.246+13C>A c.-1793C>T c.259C>A	- no impact on splicing ^b - p.Arg87Ser	intron_variant intron_variant upstream_gene_variant missense_variant	- - - T	- - - B

Variants are annotated using distinct transcripts representing all available CCDS isoforms encoded by that gene

AAF Allele Frequency, ALL all gnomAD exomes individuals, B benign, D damaging, gnomADe gnomAD exomes, T tolerated

^aSegregation was valid for all three variants with the ARID phenotype

^bSplicing analysis of the alternative allele was performed using Human Splicing Finder v3.1 algorithm (<http://www.umhd.be/HSF3/>)

Discussion

Although, ID is collectively a common disorder with a prevalence approaching to 1% globally, almost each ID subtype comprises a rare disorder, bringing out its own needs for effective diagnosis, counselling and treatment options [8]. ARID represents a significant portion of ID, especially in populations with high rate of parental consanguinity. Unbiased genomic approaches in large numbers of ARID families have identified novel candidate genes, but pathogenicity has not been replicated for most of the ‘private genes’ with a variation segregating only in a single family [4, 9]. One such gene; *EEF1D* has recently been identified as an ARID candidate in a consanguineous family from Syria with three affected children afflicted with severe ID, microcephaly and short stature in a collaborative exome sequencing effort of 152 consanguineous families with neurodevelopmental disorders [4]. The findings presented herein add support for *EEF1D* as an important gene for ARID by identifying a novel *EEF1D* variation in a second family from Turkey with ID and developmental delay.

EEF1D can be described as a multipurpose gene in the sense that it gives rise to at least four protein isoforms in humans (ENSEMBL Gene ID: ENSG00000104529). These are grouped as short and long isoforms, where there are three short isoforms (eEF1B δ 1–3) each with 281, 262 or 257 amino acids, respectively and one long isoform (eEF1B δ L) with 647 amino acids [10]. A single, but large coding exon that is alternatively spliced out in the transcripts of short isoforms, but retained in the long ones is responsible for this drastic difference between the lengths of encoded polypeptides. The canonical eEF1B δ isoform acts as a translational elongation factor in the cytoplasm by integrating into the multi-subunit eEF1 complex [11]. The long isoform eEF1B δ L is translocated to the nucleus through its N-terminus nuclear localisation signal (NLS) sequence and act as a HSR transcription factor [12]. The two *EEF1D* variants presented herein and in the original study both target specifically the large exon retained only in the *EEF1D* transcripts encoding eEF1B δ L isoform (Table 2). This in turn may create an apparent knock-out effect for eEF1B δ L function possibly due to translation dependent nonsense-mediated decay of these particular transcripts. The exclusive locations of these two variations are possibly not affecting the transcripts encoding eEF1B δ , due to their relative deep intronic localisations (Table 2).

The selective eEF1B δ L knockout scenario possibly mimicking the effects of two *EEF1D* variations discussed herein has been studied extensively by the group that has first characterised this isoform [12]. They have shown that selective inhibition of *eEF1B δ L* expression affects cell viability under thermal stress [12]. Very recently, this group has generated an *eEF1B δ L* knock-out mouse model lacking

Table 2 Annotation of two *EEF1D* variants associated with ARID phenotype on different *EEF1D* transcripts

Annotated transcripts	Isoform type	Isoform name	CCDS ID	Previous study (Reuter et al. 2017) chr8:g.144,672,183del (hg19) chr8:g.143,590,013del (hg38)	Current study chr8: g.144,671,304C>T (hg19) chr8: g.143,589,134C>T (hg 38)
ENST00000423316.6 (NM_001130053.3)	Long (647 aa)	eEF1BδL	CCDS6404	c.69del; p.Glu24Serfs*26	c.948G>A; p.Trp316*
ENST00000317198.10 (NM_001130057.3)	Short (281 aa)	eEF1Bδ1	CCDS6405	c.-7-3161del	c.-7-2282G>A
ENST00000526838.5 (NM_001195203.2)	Short (262 aa)	eEF1Bδ2	CCDS56559	c.-7-3161del	c.-7-2282G>A
ENST00000528610.5 (NM_001130056.3)	Short (257 aa)	eEF1Bδ3	CCDS47930	c.-7-3161del	c.-7-2282G>A

Four transcripts from the Ensembl database are selected to represent all four *EEF1D* annotations in CCDS

aa amino acid

the large *EEF1D* exon [13]. Interestingly, these mice developed normal until being subjected to fear conditioning tests. After these tests, their brains were found to be decreased in weight with atrophic features in hippocampus and midbrain and reduced cortical layer thickness. Some also developed audiogenic seizures. These findings suggested an external stimuli dependent role for *eEF1BδL* in brain function.

The dual function of *EEF1D* in translation elongation and HSR pathway makes it a key element of proteostasis, by which protein homeostasis is sustained against altering physicochemical conditions and cellular stress factors [14]. Several cellular stress response pathways including HSR assist proteostasis network via spatial-temporal control of molecular chaperons, translational control and degradation of misfolded proteins, which are crucial to cellular function and survival [15]. Stress induced increase in expression of *eEF1BδL* and decrease of *eEF1Bδ* will in turn activate HSR pathway and attenuate translation in general [10]. Accordingly, homozygous loss of function variations exclusive for *eEF1BδL* will alter proper activation of the HSR pathway, while leaving the *eEF1Bδ* function intact. Consequently, eEF1BδL may be an important stress-related transcription factor that can bind to HSE and HSE-like cis-elements and modulate several cellular stress responses including heat shock and oxidative stress responses.

Regulation of stress responses is a vital process for organisms, as major neurodegenerative diseases including Alzheimer's, Parkinson's and Huntington's diseases as well as amyotrophic lateral sclerosis are characterised by protein misfolding and aggregation; a significant defect in proteostasis [16]. Additionally, several genes encoding members of heat shock stress interactome including *METTL23* (methyltransferase like 23), *SACS* (sacsin molecular chaperon), *DNAJC6* (DnaJ heat shock protein family (Hsp40) member C6) and *DNAJC12* (DnaJ heat shock protein family (Hsp40) member C12) have been implicated in ID [17–23].

The same study describing *EEF1D* has also associated a homozygous splice site variation in *TRAP1* (rs1053534094: NM_016292.2:c.1941-1G>A and NM_001272049.1:c.1782-1G>A) with ID [4]. Interestingly, *TRAP1* encodes a mitochondrial chaperone protein that is member of the heat shock protein 90 family and has recently been associated with late onset Parkinson's disease in the homozygous state (rs141984778: NM_016292.2:c.139C>T; p.Arg47* and NM_001272049.1:c.89-1798C>T) [24]. It is very interesting to note that the *TRAP1* transcript encoding the large isoform has a 159 bp coding exon that is absent from the short isoform; a similar scenario to *EEF1D* presented herein. The homozygous stop-gain variant in the late onset Parkinson's disease case lies within this particular exon leaving the short isoform intact. It is tempting to speculate that a truncating variant affecting both transcripts may lead to a more severe, early onset neurodevelopmental disorder, while a similar variant affecting targeted transcripts may be associated with a late onset neurodegenerative course.

Tissue-specific differences in expression of components of the proteostasis network could serve as the basis for the understanding of the disease pathogenesis in neuronal cells. It has previously been shown that eEF1BδL expression in adult mice on both protein and mRNA levels is restricted to brain and testis, while eEF1Bδ exhibits a more widespread expression pattern [12]. Disruption of the pivotal role of *EEF1D* during development, where there is dynamic protein synthesis coupled by active chaperone function and rapid stress response may have drastic effects on neurons and lead to severe congenital neurological conditions.

Pathogenic variants in genes encoding eEF1 subunits may cause neurodevelopmental disorders (reviewed in [11]). It seems surprising to have a rather distinct neurological phenotype when such canonical genes are altered. This dilemma may be resolved at least for *EEF1D*. The two loss of function variations associated with ID reside on a very

niche position of the gene and present an isoform and tissue-specific effect [11]. We propose that ID associated variants exert their effect through HSR related transcriptional control rather than canonical translation. Understanding the interplay between alternative mechanisms of proteostasis and the reflection of these delicate pathways on human diseases can bring new insights for better treatment targets. Isoform specific devastating variations are probably an important aspect of disease pathogenesis and may explain the targeted neurological phenotypes in ubiquitously expressed genes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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