Case report

A New Variant of the IER3IP1 Gene: The First Case of Microcephaly, Epilepsy, and Diabetes Syndrome 1 from Turkey

Söbü E et al. Microcephaly, Epilepsy, and Diabetes Syndrome 1

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What is already known on this topic?
MEDS1 manifests as microcephaly with simplified gyral pattern in combination with severe infantile epileptic encephalopathy and early-onset permanent diabetes.

What this study adds?
This is the first case reported from Turkey, differs from other cases due to the absence of a typical simplified gyral pattern on early brain MRI, the late onset of diabetes, and the presence of a new genetic variant.

Abstract
Microcephaly, Epilepsy, and Diabetes Syndrome 1 (MEDS1) is a rare autosomal recessive disorder caused by defects in the IER3IP1 (Immediate Early Response 3 Interacting Protein 1) gene. Only 9 cases have been described in the literature. MEDS1 manifests as microcephaly with simplified gyral pattern in combination with severe infantile epileptic encephalopathy and early-onset permanent diabetes. A simplified gyral pattern has been described in all cases reported to the date. Diagnosis is made by demonstration of specific mutations in the IER3IP1 gene. In this study, we present an additional case of a patient with MEDS1 who is homozygous for the c.53C >T p.(Ala18Val) variant. The case, the first to be reported from Turkey, differs from other cases due to the absence of a typical simplified gyral pattern on early brain MRI, the late onset of diabetes, and the presence of a new genetic variant. The triad of microcephaly, generalized seizures and permanent neonatal diabetes should prompt screening for mutations in IER3IP1.

Keywords: Developmental delay, diabetes mellitus, epilepsy, IER3IP1, MEDS1.
expression in the cerebral cortex and in beta cells. As the patient’s seizures did not respond to the initial treatment, other drugs were introduced in the following order: levetiracetam, topiramate, clonazepam, and clobazam. Brain Computed tomography (CT) performed at the age of 9 months revealed increased distance in the CSF space, particularly remarkable in the frontal lobe (Figure 5). While there were no clinical signs of hypoglycemia that might occur with MEDS1, thyroid function tests revealed thyroid stimulating hormone (TSH) level of 0.89 IU/ml (normal range: 0.27-4.2) and free T4 level of 0.96 ng/ml (normal range: 0.87-1.76). At the age of 18 months, he presented with rapid breathing, and further tests revealed a blood glucose level of 311 mg/dl, insulin level of 4.9 mU/L, C-peptide level of 0.43 µg/L, and HbA1c level of 7.09%; upon establishing a diagnosis of diabetes, insulin therapy was started at a dose of 0.4 U/kg. Diabetes antibodies (anti-glutamic acid decarboxylase, anti-insulin, and islet antibodies) were negative. There was no acidos or ketonuria. Medical history was negative for polydipsia, polyuria, and significant weight loss. This variant has not been previously reported. Both parents were found to be heterozygous for the mutation.

The patient’s parents provided informed consent for publication of this case report.

Preparation for Genetic Analysis
Genomic DNA extraction was performed according to manufacturer’s instruction (Maxwell RSC Blood DNA kit, Promega, USA) using Maxwell RSC Instrument (Promega, USA). 30 µl of Proteinase K (PK) Solution was added into 200 µl blood sample. 300 µl of Lysis Buffer was added to blood and PK mix and incubate at 56°C for 20 minutes. After this step, each blood lysate sample was transferred into the cartridges. At the end of assay in the instrument, 50 µl DNA was eluted. The concentration of DNA was determined spectrophotometrically by measurement of the absorbance at 260/280 nm using a Nanodrop 1000 apparatus (Thermo Fisher Scientific). The concentration of DNA samples for libraries were determined by using Qubit 3.0 (Thermo Fisher Scientific). The sequencing libraries for exome sequencing were prepared according to Twist Human Core Exome Kit protocol (Twist Bioscience, USA). Paired-end 150 bp read sequencing was performed on a NovaSeq system (illumina, USA).

Results
Raw data were uploaded to the Sophia DDM (Sophia Genetics-Lausanne-Switzerland) platform and for further analysis, which detected homozygous c.53C>T p.(Ala18Val) (NM_016097) variation in IER3IP1. This detected variant could not be found in any literature report or in the healthy population database (gnomAD: https://gnomad.broadinstitute.org). However, in silico prediction databases (MutationTaster, PROVEAN, SIFT) stated, in consensus, that the variation was “deleterious.” The American College of Medical Genetics (ACMG) 2015 criteria qualified the variant as “Class 3 - variant of uncertain clinical significance” (10). The segregation analysis for the variation was performed with Sanger sequencing and Integrative Genomics Viewer (IGV) using samples collected from the patient’s parents, and both parents were found to be heterozygous carriers of the mutation (Figure 4, Figure 5). Because the patient’s clinical findings were similar to the expected symptoms of the “Microcephaly, epilepsy, and diabetes syndrome 1 (OMIM: 614231)” phenotype caused by homozygous pathogenic variants in IER3IP1, this mutation was thought to account for the patient’s phenotypic features.

Discussion
This case report presented a male patient with homozygous variation in IER3IP1, this is the first case reported from Turkey and the 10th case in the literature. It differs from other cases due to absence of a typical simplified gyral pattern on brain MRI and later onset of diabetes compared with other reported cases.

MEDS1 syndrome was first reported by Poulton et al. in 2011 in two cases from two unrelated families. Common findings in these cases were microcephaly with simplified gyral pattern in combination with severe infantile epileptic encephalopathy and early-onset permanent diabetes. An autopsy specimen from one patient showed increased apoptosis in the cerebral cortex and pancreatic beta cells, implicating premature cell death as the pathogenetic mechanism (5). Microcephaly, epilepsy, and diabetes syndrome type 1 (MEDS1) (OMIM: 614231), which shows an autosomal recessive pattern of inheritance, results from a defect in the production of immediate early response 3 interacting protein 1 (IER3IP1) expressed in beta cells of the cerebral cortex and pancreas beta cells, implicating premature cell death as the pathogenetic mechanism (5,7). Microcephaly, epilepsy, and diabetes syndrome type 1 (MEDS1) (OMIM: 614231), which shows an autosomal recessive pattern of inheritance, results from a defect in the production of immediate early response 3 interacting protein 1 (IER3IP1) expressed in beta cells of the cerebral cortex and pancreas. IER3IP1 is localized to the endoplasmic reticulum (ER) and is thought to play a role in the transport of proteins between the ER and Golgi apparatus and involved in the ER stress response (5). The association of neonatal diabetes with IER3IP1 mutations suggests that IER3IP1 regulates β-cell survival and/or function. Increased apoptosis in the cerebral cortex and pancreatic beta cells in autopsy samples with IER3IP1 mutation points at early apoptosis as the pathogenic mechanism (5,7). Neonatal diabetes refers to diabetes that is associated with a monogenic defect and is usually diagnosed in the first 6 months of life. The age at diagnosis of diabetes in the reported MEDS1 cases ranges from 14 days to 2 months (Table 1). In our case, however, diabetes emerged at age 18 months, a later than in other reported cases (6,7,9). Although current studies have shown that monogenic diabetes usually occurs in the first 6 months, recent studies have shown that it can rarely occur at the age of 12 or even 24 months (3,11,12). The important characteristics of the cases reported in the literature are summarized in Table 1.

The detection of c.62T>G and c.233T>C variants in all but one of the cases reported to date, and the fact that most of the cases are in Middle Eastern and North African countries or in countries receiving immigration from these regions, indicate that these variants are probably not mutational hotspots, but rather are rare ancestral variants unique to these regions. In the case of Shalev et al., the common c.62 T G frameshift variant and the novel c.79delT frameshift variant were compound heterozygous, and although this novel variant was a frameshift variant, the patient was more mildly affected than previously reported ones and survived to 8 years of age (8). This shows that variants other than two common mutations can cause different phenotypes. The c.62 T>G (p.Val21Gly) variant affects the first transmembrane hydrophobic domain of the protein, and the c.233T>C (p.Leu78Pro) variant affects the second transmembrane hydrophobic domain of the protein, impairing the protein's expression and/or function. The variant in our patient affects amino acid at position 18 in the first transmembrane hydrophobic domain, probably its mechanism of action is similar to c.62T>G, which affects amino acid at position 21. In in silico analysis, the c.62T>G variant is adjacent to the protein cleavage site. The milder phenotype of our case may be due to the fact that our variant is not so close to this cleavage site (5). Another piece of evidence supporting the pathogenicity of the variant in our patient is that the residues affected by both our patient's variant (18th residue) and the c.62T>G (21st residue) variant are located within a highly conserved 12-mer residue region among the species as shown below.

<table>
<thead>
<tr>
<th>Species</th>
<th>AALLCVNAIAVHHEERFLKNIG</th>
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<tbody>
<tr>
<td>Human</td>
<td>AALLCVNAIAVHHEERFLKNIG</td>
</tr>
<tr>
<td>Mouse</td>
<td>AALLCVNAIAVHHEERFLKNIG</td>
</tr>
<tr>
<td>Zebrafish</td>
<td>TAILTNAIAVHHEERFLKIG</td>
</tr>
<tr>
<td>Cow</td>
<td>AALLCVNAIAVHHEERFLKNIG</td>
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In differential diagnosis, Wolcott–Rallison syndrome has been reported to be the most common cause of neonatal diabetes in families with consanguine marriages (13). This syndrome, which results from a homozygous mutation in IER3IP1, is characterized by insulin-dependent diabetes mellitus before 6 months of age, skeletal dysplasia after 6 months of age, and liver failure. This syndrome manifests as renal failure, microcephaly, epilepsy, and central hypothyroidism and it must be ruled out in the differential diagnosis of MEDS1 (14). Increased ER stress, and thus beta-cell death, constitutes the pathogenesis of the disease, and management requires insulin replacement (15). This syndrome may also include episodes of liver failure and skeletal anomaly in later ages, indicating the importance of early genetic diagnosis.

A study that presented 4 cases of MEDS1 reported that three of the patients had skeletal findings, including osteoporosis, metaphyseal changes, craniosynostosis, pathologic fractures, and poor modeling of long bones (6). Our patient had no skeletal anomaly. IER3IP1 has an unclear role in the development of the cortex and in the pathogenesis of epilepsy and diabetes, but it is thought to be required during early stages of neural development, for instance, during neural progenitor proliferation. Presence of microcephaly with simplified gyration has a distinctive role in differential diagnosis and already exists during gestation. Severe infantile epileptic encephalopathy is highly unusual in primary microcephaly and has been reported only in patients with WDR62 mutations (16). In addition, a rare combination of primary microcephaly and severe infantile epilepsy in patients with PANK2 mutations has been reported (17).

Because our patient had refractory epilepsy, microcephaly, and axial hypotonia at the time of presentation, no specific complication at birth and was born of parents who were first cousins, he underwent WES early and was diagnosed with MEDS1. The patient’s family was asked to be vigilant about symptoms of potential diabetes with blood glucose being monitored. Diabetes emerged later than reported in other cases in the literature, but early genetic analysis allowed for diagnosing diabetes before acidosisis developed. The patient did not present with microcephaly at birth, but with increasing age, it became evident. It was remarkable that the simplified gyral pattern, which was detected in all other cases, was absent on early MRI. Continued apoptosis in the postnatal period was thought to be the cause of magnetic resonance imaging (MRI) findings and microcephaly. This hypothesis was supported by increased distance in the CSF space detected on brain CT performed at the age of 9 months.

In conclusion, this is the first case of MEDS1 reported from Turkey and shows a variant that has not been previously described in the literature. Although the simplified gyral pattern, which co-occurs with the triad of microcephaly, epilepsy, and diabetes, may guide the diagnosis of MEDS1, manifestation of the symptoms may sometimes take time. Early genetic counseling should be considered in families where consanguine marriage is accompanied by epilepsy and microcephaly.

Acknowledgements
None

Ethics Informed Consent
The patient’s parents provided informed consent for publication of this case report.

References

Table 1. The important characteristics of the cases reported in the literature.

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<tbody>
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<td></td>
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<td>Family 1578</td>
<td>Case 1</td>
<td>Case 2</td>
<td>Case 3</td>
<td>Case 4</td>
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<td>M</td>
<td>F</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>MRI</td>
<td>simplified gyral pattern</td>
<td>simplified gyral pattern</td>
<td>simplified gyral pattern and agenesis of corpus callosum with mild cerebellar vermis hypoplasia</td>
<td>cerebra l atrophy with simplified gyral pattern and agenesis of corpus callosum with normal cerebellum</td>
<td>simplified gyral pattern</td>
<td>simplified gyral pattern</td>
</tr>
<tr>
<td>EEG</td>
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<td>hypsarrhythmia</td>
<td>polymyoklonus slow waves with burst suppression</td>
<td>general ized epileptiform abnormalities with sharp and slow waves</td>
<td>burst suppression pattern</td>
<td>burst suppression pattern</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>c.233 T &gt; C p.Leu78Pro Homozygous</td>
<td></td>
<td>c.53C&gt; T p.(Ala18Val) Homozygous</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
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<tr>
<td><strong>Skeletal findings</strong></td>
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<td>NA</td>
<td>osteoporosis, metaphyiscal changes</td>
<td>pathologic fractures</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Death</strong></td>
<td>18 months</td>
<td>27 months</td>
<td>5 1/2 years</td>
<td>26 months</td>
<td>31/2 years</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1. General characteristics of MEDS patients
MRT: magnetic resonance imaging, EEG: electroencephalogram, NA: not available, M: Male, F: Female.

**Figures**
Figure 1. Patient's facial appearance.
Figure 2. Brain MR images of the patient at the age of three months old.
Figure 3. Brain CT at the age of 9 months old.

Figure 4. Results of Next-generation sequencing at the mutation locus c.53C>T p.(Ala18Val).
Figure 5. Integrative Genomics Viewer were used to analyse the characteristics of the mutated MEDS1 protein.