Title: Identification of rs797045105 in the SERAC1 gene by Whole Exome Sequencing from a patient suspicious to MEGDEL syndrome

Running title: rs797045105 is MEGDEL causing variant

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Abstract

Whole exome sequencing has been and will be increasingly utilized in genetic determinants of various inherited diseases. We identified a new variation in SERAC1 as the cause of 3-methylglutaconic aciduria (MEG), deafness (D), encephalopathy (E), and Leigh-like (L), MEGDEL syndrome using Whole Exome Sequencing (WES). At result we found an insertion (chr6, 158571484, C>CCATG), rs797045105 in SERAC1 gene with homozygous genotype in the patient and heterozygous genotype in her unaffected parents. Notably, bioinformatics analysis using mutation taster (prob>0.99) and DDIGin (prob=86.51) predicted this mutation as diseases causing. Also the variant isn’t present in our database including 700 exome files. These findings emphasize on pathogenicity of rs797045105 for MEGDEL syndrome. On the other hand our data shed light on significance of exome sequencing application as genetic test to identify and characterize the comprehensive spectrum of genetic variation and classification for the patients with neuro-metabolic disorders.

Keywords: Whole exome sequencing, rs797045105, SERAC1, MEGDEL
Introduction

3-Methylglutaconic aciduria (3-MGA-uria) is a heterogeneous group of metabolic syndromes featured by elevated urinary excretion of 3-methylglutaconic and 3-methylglutaric acids (Wortmann, Kluijtmans et al. 2012).

One subtype (type IV) of 3-MGA-uria has been introduced by a clinical presentation including neurological deterioration, central nervous system involvement, sensory-neural hearing loss, encephalopathy and dystonia, named MEGDEL (Phenotype MIM number: 614739). MEGDEL syndrome is a recessive disorder of dystonia and deafness with Leigh-like syndrome (Wortmann, Rodenburg et al. 2006).

MEGDEL syndrome has been demonstrated caused by mutations in SERAC1 (codes serine active site containing 1). Studies by Wortmann and his colleagues in fibroblasts derived from patients showed that SERAC1 is localized interface of mitochondria and endoplasmic reticulum. They showed mutations of SERAC1 can abnormally increase ratio of phosphatidylglycerol-34:1 to phosphatidylglycerol-36:1. So it is speculated that this leads to lower bis(mono-acyl-glycerol) phosphate levels and accumulation of cholesterol in the perinuclear region. Finally it is hypothesized that SERAC1 can catalyze the first enzymatic reaction in the bis-phosphate(mono-acyl-glycerol)biosynthetic pathway and has a role in the phosphatidylglycerol remodeling pathways as is involved in the transacylation-acylation reaction to produce phosphatidylglycerol-36:1. They conclude that SERAC1 encoded protein participates in both mitochondrial function and intracellular cholesterol trafficking (Wortmann, Vaz et al. 2012).

In the recent years next generation sequencing (NGS) has improved our knowledge about genetic and molecular bases of this heterogeneous group of disorders.

Here we use exome sequencing for genetic diagnosis of an Iranian patient with 3-MGA-uria, mental retardation, cerebellar atrophy, deafness, basal ganglia impairment, and spasticity symptoms which are compatible with MEGDEL syndrome.

Material and Methods

2.1. Patient/ case report
Patient with MEGDEL & his parents were recruited from southwest of Iran.

2.2. Peripheral blood samples

10 ml of peripheral blood was withdrawn from each of the enrolled patient and his parents which were collected in EDTA tubes.

2.3. DNA extraction

Genomic DNA was extracted using the standard salting out protocol. The quality and quantity of the extracted DNA samples were checked by gel electrophoresis and nanodrop.

2.4. Exome sequencing

Sample was subjected to Exome sequencing using the IlluminaHiSeq 2000 genome analyzer platform (CNAG) by Macrogen Company.

2.4. RNA extraction, Reverse transcription

Total RNA was extracted using TRIzol® reagent (Life Technologies). Complementary DNA was synthesized by PrimeScript™ RT reagent kit (Takara Bio Inc, Shiga, Japan).

2.5. Polymerase Chain Reaction

Targeted region of SERAC1 gene on DNA and cDNA were amplified by PCR using primers designed by software Oligo7 (table 1). The PCR was conducted using Master Mix (Ampliqon, Denmark). PCR thermal program was; 95 °C for 5 min, 35 cycles of 95 °C -30 sec, 60 °C -30 sec, and 72 °C -30 s, and72 °C -5 min.

Table 1

2.6. DNA Sanger-sequencing

Ampliqons were directly sequenced by the ABI PRISM 3700 Genetic Analyzer (Applied Biosystems). The sequences were analyzed with Chromas LITE 2.1.1, and then compared with the reference genomic sequence using BLASTN. Also the presence of the detected mutation was confirmed by the parent DNA analysis and bi-directional sequencing.

2.7. In-silico analysis
There are lots of various tools for prediction of pathogenicity of variations in genes coding and non-coding regions. In the present study we used some of these tools for evaluation of disease causing potentiality of the present insertion variation. The prediction of variant pathogenicity has been done using Mutation taster and DDIGin.

Result

Clinical reports for the patient

The enrolled family belonged to Lor ethnicity of Iran. The proband in family was a boy (Fig. 1, II-2) with 8 years age. Parents (Fig. 1, II-1 and II-2) were related as first cousin. First son of the family had the same symptoms as proband. The proband represented developmental delay and developmental regression, sensory-neural hearing loss, myoclonic epilepsy, and 3-methylglutaconic aciduria, mental retardation, respiratory problems, jaundice, dysphagia and lesion in basal ganglia.

Metabolic test report for the proband: The organic acids in the patient urine show a moderate excretion of 3-methylglutaric acid (16 mmol/molCreatinine, normally not detected) and 3-methylglutaconic acid (79 mmol/molCreatinine, normal<19).

MRI of the brain report for the proband: Increased signal intensity in the deep white matter of the brain in T2 weighted images are most probably representative of immaturity of neural migration however the basal ganglia are also high signal in T1 weighted images possibility of metabolic disease of the brain.
Figure 1. Family pedigree.

**Variant characteristics**

Table 1. Primers sequences.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERAC1-F</td>
<td>TGTCCTTGAAACAAAAAGTCAGTATGT</td>
</tr>
<tr>
<td>SERAC1-R</td>
<td>CCTCAATTAATTCTCCAGGAACCTTGG</td>
</tr>
<tr>
<td>SERAC1-RTprimer-F</td>
<td>AAGGCTGTGACATTAGATACTC</td>
</tr>
<tr>
<td>SERAC1-RTprimer-R1</td>
<td>AGCAAAATGGATTCCGACGTATC</td>
</tr>
<tr>
<td>SERAC1-RTprimer-R2</td>
<td>TGACTTGCTCTTGCGTAGG</td>
</tr>
</tbody>
</table>

Followed by exome data analysis we identified g.17828-17829insCATG homozygous single nucleotide variation in the patient in SERAC1 gene.

**Table 2.** Location and predicted pathogenicity of the variation. Data extracted from Mutation taster and DDIG-in.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Mutation taster</th>
<th>DDIG-in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SERAC1</td>
<td>chr6:158,571,484</td>
<td>Disease causing</td>
<td>Disease</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>C&gt;CCATG</td>
<td>g.17828-17829insCATG</td>
<td>(prob&gt;0.99)</td>
<td>(prob=86.51)</td>
</tr>
</tbody>
</table>

There are 25 reported variants in clinvar database (https://www.ncbi.nlm.nih.gov/clinvar/) for SERAC1 gene; including 8 benign, 3 likely benign, 3 likely pathogenic and 11 pathogenic variants (table 3). The present variant, rs797045105 (table 2, g.17828-17829insCATG) reported in clinvar as likely pathogenic variant provided by clinical testing.

SERAC1 protein (NP_116250) has 654 aminoacids including; 32-54 as proposed transmembrane region, 386-592 as Pimeloyl-ACP methyl ester carboxylesterase and 396-540 as Esterases and lipases regions. Up to now most of reported mutations locations are in the Esterase_lipase region.
2. Sanger sequencing analysis of genomic amplification

We have proved the result of exome sequencing of the patient using Sanger sequencing. Also allele segregation has been checked by parents’ genome amplification and sequence assessment of
targeted region included the variation. Results showed patient was homozygous and his parents were heterozygous for the present variant (figure 2).

**Figure 2.** Chromatograms of the affected child and his parents’ genome with focusing on the targeted region including the variation. A. father, B. Mother and C. affected child.

3. cDNA analysis
We demonstrated insertion of nucleotides in the transcripts of SERAC1 using RT-PCR and Sanger sequencing of cDNA product of SERAC1 transcripts (figure 3).

Figure 3. Chromatogram of cDNA sequence.

4. ORF finder

We used ORF Finder to search potential protein encoding segments of wild type and mutant cDNA of SERAC1. As the result of ORF Finder shows the mutant sequence cannot produce correct protein coding frame.

Conclusion

Neurometabolic disorders are complex because they are genetically heterogeneous, with different genetic defects that give rise to clinically indistinguishable phenotypes. Finding more pathogenic mutations could help knowing more the disease characteristics and shed light on the way of diagnosis and its subsequent steps Whole exome sequencing is a powerful approach to open the blind knots in this complexity (Kaname, Yanagi et al. 2014, Rabbani, Tekin et al. 2014, Tarailo-Graovac, Shyr et al. 2016).

3-MGA-uria type IV, a heterogeneous group of inborn metabolic errors with primary mitochondrial and endoplasmic reticula impairment leads to a spectrum of multisystem defects. Various enzymes, structural proteins, cellular transport proteins, and other constituents are

Recently SERAC1 mutations have been found to cause a disease belongs to 3-MGA-uria type IV; MEGDEL syndrome. It seems that the symptoms for the disease are variable covering; central nervous system involvement, neurological deterioration, microcephaly, sensori-neural hearing loss, encephalopathy, dystonia, optic atrophy, abnormality in range of urine 3-MGA and plasma lactate, alanine and cholesterol (Wortmann, Vaz et al. 2012, Tort, Garcia-Silva et al. 2013). SERAC1 has role in phospholipids biosynthetic process and seems is an important player in the mitochondria and endoplasmic reticulum functions.

In the present study we carried out exome sequencing to investigate the causative genetic mutations in an Iranian patient which suspected to have MEGDEL syndrome. Followed by data analysis we identified a framshift variation, g.17828-17829insCATG (which reported before as rs797045105) in the patient in homozygous manner. This variant was heterozygous in parents. Also the identified variant isn’t present in our 700 exome files sequenced from 700 referred patients of southwest of Iran to our laboratory.

In conclusion, rs797045105 which have been reported clinically in Clinvar database as likely pathogenic variant could be considered as pathogenic variant causing MEGDEL syndrome. The present variant location is in the boundary of exon 4 and intron 4-5. Interestingly Sanger sequencing of SERAC1 cDNA confirmed the insertion of nucleotides in the spliced transcripts of the gene so it is predicted to be pathogenic due to the production of frame shift, Gly89fs. SERAC1 encodes for a protein with a serine-lipase domain. This variation disrupts the potential of SREAC1 mRNAs for coding this domain.

Our results in line with many recently studies, show that whole-exome sequencing could be a powerful tool for the diagnostic of highly heterogeneous neuro-metabolic disorders.

Conflict of interest statement

There is no conflict of interest regarding the publication of this article.

References


