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The New CIC Mutation Associates with Mental Retardation and Severity of Seizure in Turkish Child with a Rare Class I Glucose-6-Phosphate Dehydrogenase Deficiency

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Abstract
Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked recessive disease that causes acute or chronic hemolytic anemia and potentially leads to severe jaundice in response to oxidative agents. Capicua transcriptional repressor (CIC) is an important gene associated with mental retardation, autosomal dominant 45. Affiliated tissues including skin, brain, bone, and related phenotypes are intellectual disability and seizures. Clinical, biochemical, and whole exome analysis are carried out in a Turkish family. Mutation analysis of G6PD and CIC genes by Sanger sequencing in the whole family was carried out to reveal the effect of these mutations on the patient's clinical outcome. Here, we present the case of epilepsy in an 8-year-old child with a hemizygous variation in G6PD gene and heterozygous mutation in CIC gene, resulting in focal epileptiform activity and hypsarrhythmia in electroencephalography (EEG), seizures, psychomotor retardation, speech impairment, intellectual disability, developmental regression, and learning difficulties. Whole exome sequencing confirmed the diagnosis of X-linked increased susceptibility for hemolytic anemia due to G6PD deficiency and mental retardation type 45 due to CIC variant, which explained the development of epileptic seizures. Considering CIC variant and relevant relation with the severity and course of the disease, G6PD mutations sustained through the family are defined as hereditary. Our findings could represent the importance of variants found in G6PD as well as CIC genes linked to the severity of epilepsy, which was presumed based on the significant changes in protein configuration.

Keywords Seizure · Mental retardation · CIC mutation · Glucose-6-phosphate dehydrogenase deficiency · G6PD

Background

Glucose-6-phosphate dehydrogenase (G6PD) is a catalyzing enzyme (Pandolfi et al. 1995; Berg et al. 2002) whose significant role is nicotinamide adenosine dinucleotide phosphate (NADPH) and ribose 5-phosphate production in the hexose monophosphate pathway (Berg et al. 2002). NADPH is considered the key factor in protecting cell damage caused by cell damage. As the pathway is the only pathway leading to NADPH synthesis (Beutler 1978), lack of the enzyme causes physiological disorders (Takizawa et al. 1986). Deficiency of G6PD, known as favism, is an X-linked recessive disorder with prevalence of more than 400 million people worldwide (Kaplan and Hammerman 2000) and causes newborn hyperbilirubinemia, chronic hemolysis, and hemolytic crisis during infection and after ingestion of oxidative drugs or foods (Lanzkowsky 2011). The *G6PD* gene is highly polymorphic, and more than 140 mutations have been reported in *G6PD* gene (de Gurrola et al. 2008). Although G6PD deficiency is an X-linked recessive disease, it affects both the genders owing to cell level expression. In heterozygotes of this X-linked disease, the clinical manifestation of cellular disease differs by its enzyme level in each cell, reflecting the intracytoplasmic gene product of that cell's active X chromosome. In heterozygote females, half the cells have a defective X chromosome and defective enzyme activity (Luzzatto and Seneca 2014). Although individuals that were detected with deficiency in G6PD do not usually express any symptoms, additional mutations and disorders in some cases can induce massive intravascular hemolysis. The occurrence rate of the mental retardation due to deficiency in G6PD in Turkey is reported to vary from 0.25 to 18% depending on the geographical areas and/or ethnic groups studied (Altay and Gümrük 2008).

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CIC gene, known as mammalian homolog of Drosophila Capicua, is located in chromosome 19 and encodes repressor protein that belongs to high mobility group (HMG)-box superfamily of transcriptional repressors (Lam et al. 2006; Bettegowda et al. 2011). Studies suggest that the N-terminal region of this protein interacts with Atxn1 and forms a transcription repressor complex which can be altered by polyglutamine-expansion of ATXN1. Mutations in this gene have been associated with oligodendrogliomas (Lee et al. 2002). Moreover, round-cell sarcomas can be due to *CIC* gene fusions with both DUX4 and FOXO4 by translocation (Panagopoulos et al. 2014; Sugita et al. 2014). The presence of two forms of endogenous CIC was confirmed in HEK, oligodendroglioma (ODG) cells, and tumor tissues (Chittaranjan et al. 2014). The subcellular localization of CIC-S (cytoplasm) and CIC-L (nucleus) was also determined in human cells, indicating *CIC* distinct functions (Gómez-Manzo et al. 2016). Although *CIC* impairment has been detected in human diseases such as mental retardation, autosomal dominant 45, autosomal dominant non-syndromic intellectual disability, and cancers, the biology of CIC in mammalian cells remains elusive.

Here, we present an epilepsy case in a G6PD-deficient child with a CIC variant, with focal epileptiform activity and hypsarrhythmia, seizures, psychomotor retardation, poor speech, intellectual disability, developmental regression, and learning difficulties.

Materials and Methods

Clinical History

An 8-year-old boy was admitted to the Genome Center of Biruni University in Turkey. He was the third child of healthy parents born by cesarean and a birth weight of 3.4 kg. Clinical examination of the newborn revealed jaundice on the third day of birth, which was managed with clinical therapy. He had the first seizure in the form of flexor spasm at the third month of birth. However, no fever or infection was detected. He was medicated with phenobarbitals at the fifth month but had a complicated febrile convulsion at the 6th month, lasting for 10–15 s repeated for three times in an hour. The Cranial magnetic resonance imaging (MRI) and metabolic screenings were found to be normal, except a hypertrophic adenoid tissue in nasopharynx narrowing the air colon which was detected by the last MRI.

At the beginning of the seventh month, the patient suffered from febrile and afebrile partial seizures. Electroencephalography (EEG) at the eighth month showed the presence of active epileptiform encephalopathy (consisting of sharp waves and spikes in temporoparietal regions of hemispheres). At the third year of life, the patient had focal seizures up to three times in a month for a year followed by three GTC (generalized tonic-clonic) seizures at the last months of the fourth year several times in a day each lasting 1–2 min. The EEG performed at age of 3 years reported a focal epileptiform activity that consisted of sharp waves in frontotemporal lobes of left hemisphere. During seizures, diazepam, 15 mg/kg valproic acid and 30 mg/kg levetiracetam were injected. Analysis of clinical symptoms and laboratory tests classified the epilepsy as GTC seizures. The patient was treated with piracetam and levetiracetam. The patient showed reduced psychomotor development, absence of speech, seizures, and poor eye contact after 4 years of regular treatment. Thus, the biochemical and whole exome analysis (WES) (Kaushik et al. 2020) was requested.

Biochemical Data

At the seventh month of birth, the ultra-performance liquid chromatography (UPLC) of plasma of the patient presented a slightly increased level of glutamic acid (155.01 μmol/L) and suggested to be non-specific since the levels of other amino acid were in normal range. At the eighth month of birth, hematology tests presented normal level of erythrocytes (4.07×10^6/μl), but low levels of hemoglobin (9.8 g/dL), hematocrit (31.2%), mean corpuscular volume (MCV: 76.6 fL), mean corpuscular hemoglobin (MCH: 24.2 pg/cell), high level of lymphocytes (5.3×10^3/μl), and thrombocytes (417×10^3/μl). Moreover, biochemical tests of serum showed decreased level of alanine aminotransferase (ALT: 11 U/L) and increased total cholesterol (178 mg/dL). Other parameters including glucose, valproic acid, and creatinine were normal (Table 1).

At the ninth month of birth, metabolic testing of the serum showed the excretion of organic acids in the range of normal values and no organic acid-urea profile. Congenital disorder of glycosylation screening in total blood and serum demonstrated a normal profile. Tandem mass analysis in serum and total blood also revealed the amino acid and acylcarnitine profile in range of reference (Table 1).

At the age of 3 years and 8 months of life, routine blood analysis gave a high level of fasting glucose (102 mg/dL) and valproic acid (114.6 mg/L), high level of lymphocytes (3.62×10^3/μL), and low levels of creatinine (0.2 mg/dL), erythrocyte (3.79×10^6/μL), hemoglobin (11.0 g/dL), and hematocrit (33%). Investigating the plasma amino acids, only tyrosine level was low (32.2 μmol/L). However, the spot urine analysis demonstrated high values of following amino acids: lysine (1012.5 mmol/mol creatinine), taurine (816.5 μmol/g creatinine), glycine (9465.7 mmol/mol creatinine), alanine (2357.2 mmol/mol creatinine), arginine (743.6 mmol/mol creatinine), and abundantly valproic acid metabolites (Table 1). The patient's serum analysis showed G6PD deficiency at the

Table 1 All reported laboratory data of the patient

Parameter	Unit	7th month	8th month	3 years 8 months	5 years	Reference value
Glucose (fasting)	mg/dL	NR	85	102	NR	60–200
Erythrocytes	$*10^6/\mu L$	NR	4.07	3.79	4.36	3.7–5.7
Hemoglobin	g/dL	NR	9.8	11.0	13.46	10.5–14.7
Hematocrit	%	NR	31.2	33	34.9	33–43
MCV	fL	NR	76.6	87.02	80	75–97
MCH	pg/cell	NR	24.2	29.16	27.8	23–33
Lymphocytes	$*10^3/\mu l$	NR	5.3	3.62	2.43	0.9–11.3
Thrombocytes	$*10^3/\mu l$	NR	417	222	211	130–430
ALT	U/L	NR	11	16	NR	11–45
Total cholesterol	mg/dL	NR	178		NR	60–160
Valproic acid	mg/L	NR	NR	114.6	NR	50–100
Creatinine	mg/dL	NR	0.28	0.2	NR	0.17–0.42
Glutamic acid	μmol/L	155.01	NR	76	NR	10–133
Tyrosine	μmol/L	34.33	NR	32.2	NR	22–108
Lysine	mmol/mol creatinine	155.94	NR	1012.5	NR	52–196
Taurine	μmol/g creatinine	61.69	NR	816.5	NR	15–143
Glycine	mmol/mol creatinine	212.3	NR	9465.7	NR	81–436
Alanine	mmol/mol creatinine	247.38	NR	2357.2	NR	143–439
Arginine	mmol/mol creatinine	8.84	NR	743.6	NR	12–133
G6PD	U/g Hb	NR	NR	2.1	0.6	4.6–13.5

MCV mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *ALT* alanine aminotransferase, *NR* not reported

age of 3 and 5 with serum level of 2.1 U/g Hb and 0.6 U/g Hb respectively (normal value range 4.6 to 13.5 U/gHb).

Following patient's biochemical result, the siblings' G6PD levels were confirmed to be within normal range. The patient's sister (16 years old) was reported as 12.8 U/g Hb, and his brother had 13.1 U/g Hb (reference range: 7–21 U/g Hb). Hemoglobin levels of the siblings were also in normal range (Table 1).

Whole Exome Sequencing

Genomic DNA preparation, exome capture, and Illumina sequencing (NextSeq platform) were performed as recommended by manufacturer. In brief, genomic DNA was extracted from the whole peripheral blood sample using iPrep PureLink gDNA blood Kit (Invitrogen). Genome Library was prepared with Agilent SureSelect Target Enrichment system (Agilent Technologies, Inc., Santa Clara, CA, USA). An enrichment of coding exons and flanking intronic regions was performed using Agilent SureSelect Human All Exon V6 reagent following the manufacturer's protocol as previously described (Au et al. 2000), and sequencing was performed using an Illumina NextSeq500 system. Sequences were mapped to the human genome (GRC38/hg19) using the Burrows-Wheeler Aligner (version 0.6.1; algorithm "BWA-SW"; default parameters) for targeted sequencing data. The variants with a frequency of more than 1% in the population were removed from the collected data. Variants were annotated using Alamut visual and allele frequency with databases, dbSNP, and ExAC (exome aggregation consortium) variants and the 1000 Genomes Project. Disease causality was assessed using ClinVar and ESP (exome sequencing project) variants ExAC (exome aggregation consortium) variants, and ESP (exome sequencing project) variants.

Mutation Confirmation by Sanger Sequencing

To confirm mutation detected by WES, Sanger sequencing was carried out on patient, his siblings, and parents. The Exon 10 of the G6PD and CIC genes was amplified using primer by PCR, and mutation analysis was performed by direct sequencing of purified PCR products. PCR was performed with 50 ng of genomic DNA in 25 μl reactions for 35 cycles. PCR fragments were purified with 96-well PCR

filter plates (MinElute PCR purification kit, Qiagen Inc., Valencia, CA, USA), and mutation analysis was performed by BigDye Terminator Cycle Sequencing kit (version 3.1) and analyzed on ABI 3130 automated DNA sequencer (Applied Biosystems, CA, and USA).

Results

Clinical Analysis

An 8-year-old boy was admitted to the Genome Center of Biruni University in Turkey. He was the third child of healthy parents from a cesarean birth at term. He had a birth weight of 3.4 kg. Clinical examination of the newborn revealed a jaundice starting from the third day of birth till 1 month, requiring management with clinical therapy. He had the first seizure in the form of flexor spasm at the third month of birth, not accompanying with any fever or infection. He was medicated with phenobarbitals at fifth month but had a complicated febrile convulsion in the form of generalized tonic-clonic (GTC) seizure at the sixth month, lasting for 10–15 s repeated for three times in an hour. Requested cranial magnetic resonance (MR) and metabolic screenings were found to be normal, except a hypertrophic adenoid tissue in nasopharynx narrowing the air colon reported by the last MR at the fourth year of life.

At the beginning of the seventh month, the patient suffered from occasional febrile and afebrile partial seizures. The first, electroencephalography (EEG) at the eighth month showed the presence of active epileptiform potentials consisting of sharp waves and spikes in temporoparietal regions of hemispheres. At the third year of life, the patient had short-term seizures up to three times in a month for a year followed by three febrile/afebrile activations characterized as GTC (generalized tonic-clonic) seizures at the last months of the fourth year for several times in a day each lasting for 1–2 min. The second sleep EEG performed at age of 3 years reported a focal epileptiform activity that consisted of sharp waves in frontotemporal region of left hemisphere. During seizures, diazepam, 15 mg/kg valproic acid, and 30 mg/kg levetiracetam were injected. Analysis of clinical symptoms and laboratory tests diagnosed the epilepsy with GTC seizures. The patient has been medicated by Convulex 2.7 cc, Nootropile 1.5 cc (Active ingredient: Piracetam), and Keppra 100 mg/ml suspension 2.2 cc (Active ingredient: levetiracetam). Despite clinical management, after 4 years, the patient presented with reduced psychomotor development, absence of speech, seizures, and poor eye contact.

Genetic Analysis

In order to find out the exact genetic cause of disorder, whole exome sequencing (WES) (Kaushik et al. 2020) were performed by next generation sequencing (NGS) on DNA extracted from peripheral blood samples of the patient. All coding exons and exon-intron boundaries (60 Mb exome regions—targeting 99% of the regions included in CCDS, RefSeq, and genecode databases) were sequenced by covering approximately $\times 100$ using an Illumina NextSeq500 system. Typically, 97% of sequenced regions was $> \times 10$. Bioinformatic analysis was performed by using GRCh37/hg19 genome alignment, and the regions under low coverage area and variation with artifact possibilities were excluded from the evaluation. All variations related to the disorder, reported in HGMD®, ClinVar or CentroMD®, and the variations supposed to have a minor allele frequency (MAF) lower than 1% were considered under evaluation. Moreover, inton regions close to ± 20 bases of the coding exons were also analyzed. During evaluation of the variations, all potent inherited patterns, as well as the family history and clinical data, were considered. All defined variations were analyzed with regard to causality and pathogenicity, and all variations related to the patient phenotype were reported, except benign or potentially benign variations.

WES analysis demonstrated c.653C > T hemizygous variation (p.Ser218Phe) in G6PD gene and c.6961C > T heterozygous variation (p.Arg2321Cys) in CIC gene, suggesting X-linked increased susceptibility for "hemolytic anemia due to G6PD deficiency" and "Mental Retardation type 45" due to CIC variant.

Sanger sequencing results confirmed the presence of these mutations in patients. To investigate whether they are de novo or inherited, the Sanger sequencing analysis was carried out in the whole family. The results in the pedigree tree (Fig. 1) indicated that mutations are hereditary in patient as the mother is found to be heterozygous for both genes, but no mutation was detected in the father. While the sister is heterozygous for both genes and is reported as healthy, the brother is heterozygous for CIC and homozygous for G6PD gene as it is X-linked disorder. Although the heritance of both genes in the brother was similar with the patient, interestingly, clinical history and screening of the brother detected no phenotypic characteristics which are indicative for the CIC gene impairment. Based on the clinical evidence and reported cases, we assume development of the clinical symptoms further in the sibling's life.

The collected data led us to analyze detected mutation for gene variants, ExAC (exome aggregation consortium) variants, ESP (exome sequencing project) variants, ClinVar, and catalogs of somatic mutations in cancer (COSMIC) confirmed c.653C > T mutation

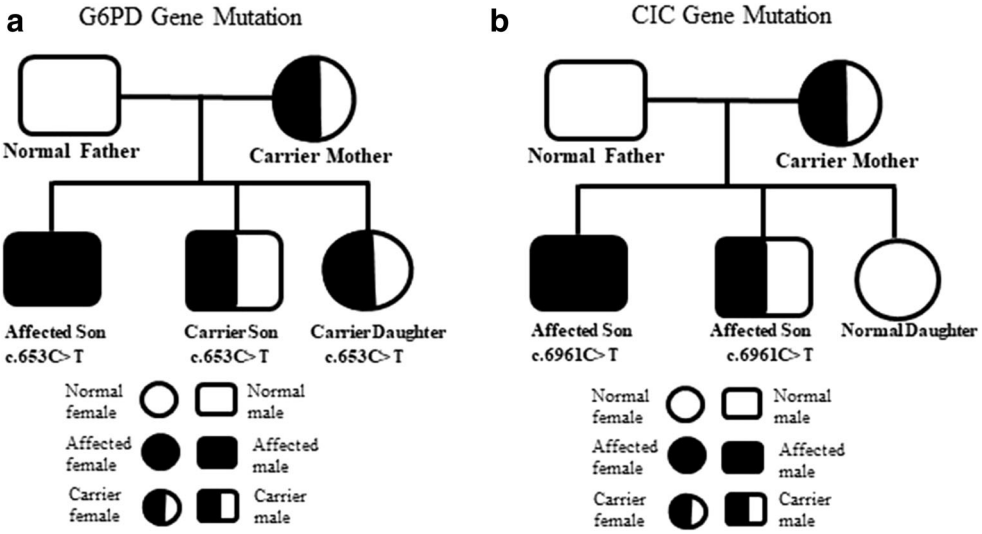

Fig. 1 Family pedigree is drawn to determine inheritance pattern of the mutations

(p.Ser218Phe) in G6PD gene to be homozygous and c.6961C > T mutation (p.Arg2321Cys) in CIC gene to be heterozygous. Computational analysis of CIC protein structure was performed by I-TASSER that carries out protein modeling using SPICKER program from structure templates identified by LOMETS from the PDB library (Zhang et al. 2017) (Fig. 2). Here, we reported substitution of arginine with cysteine in CIC gene (p.Arg2321Cys). Considering basic amino acid properties, arginine was as strongly basic and cysteine as neutral, and the position of mutation, which is located in conserved C-terminal domain, protein structure demonstrated considerable changes in ligand binding site residues changed which might indicate decline in protein binding affinity with other genes and loss of protein function (Fig. 2).

Discussion

High prevalence of G6PD deficiency is the concern to all specialists and researchers in finding probable correlation of the disorder with various genetic, environmental, and dietary factors affecting the phenotypic symptoms.

More than 217 variants on G6PD have been identified which are categorized based on their biochemical and physiochemical characteristics (Gómez-Manzo et al. 2016). The mutations have been detected to be mainly due to single nucleotide substitutions (missense variants) with 83.9% occurrence and minimally due to multiple mutations (8.7%), deletions (5.1%), and mutations affecting interns (2.3%) (Gómez-Manzo et al. 2016). G6PD deficiency types are classified with the severity of biochemical and physiological characteristics

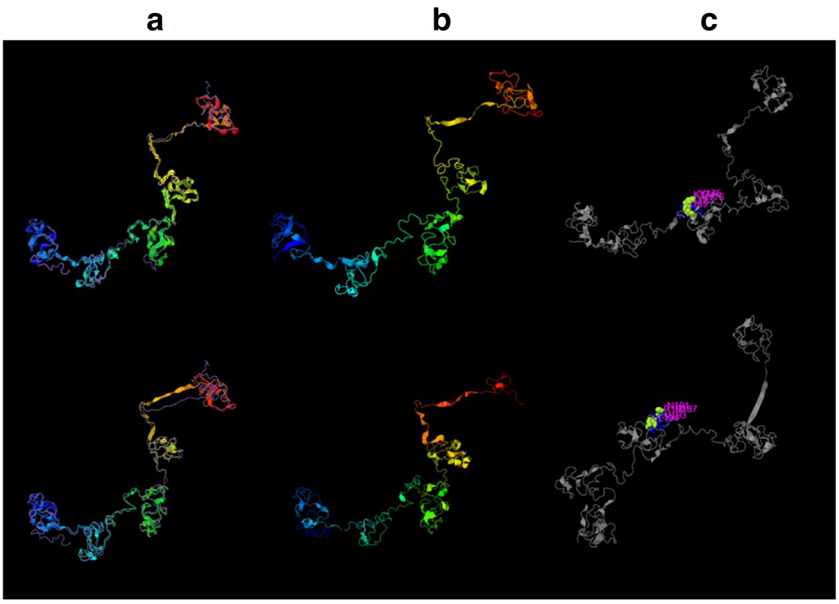

Fig. 2 Genetic impairment in CIC gene (p.Arg2321Cyc) leads to significant change in protein confirmation as illustrated in Fig. 2 a and b resulting in alteration of ligand binding sites which will presumably disrupt the protein interaction in essential metabolic pathways

based on enzyme activity and hematological parameter of the patients (Gómez-Manzo et al. 2015), ranging from the most severe manifestations with less than 5% residual activity (Class I) to the mildest form (Class V) (20). Class I mutations have the most deleterious effects on the structure and stability of the protein. Class I G6PD Zacatecas mutant (Arg257Leu) was detected in a 12-year-old boy from the Mexican state, with neonatal jaundice and hemolytic crisis and whole genome analysis detected substitution of guanine for thymine at nucleotide 770 leading to the replacement of arginine by leucine 257 (Vaca et al. 2003). Range of mutations has been detected on G6PD gene in exon 10 and 6 across the world (Vaca et al. 2003; Kordes et al. 2010; McDade et al. 2008; Mohamed and El-Humiany 2006). However, short-term overexpression of G6PD has been shown to be related to mild hypoxia. Although the same case was observed with severe hypobaric hypoxia (SH), no change in production of NADPH was observed (Vetrovoy et al. 2019). This could be due to the function of hypoxia-inducible factor-1 (HIF-1)-dependent transcriptional regulation that causes hyper-activation of glycolysis (Vetrovoy et al. 2019).

In this study, we reported Class I G6PD mutant involving a substitution of cytosine with thymine at nucleotide 653 (exon 10) (ser218Phe) in a Turkish boy with hemolytic anemia and this class is known to manifest in minority of the population that are mainly documented in males. Although numbers of mutations have been detected in exon 10 of G6PD gene, the latter region plays key role in G6PD enzyme's functional characteristics and its consistency (Au et al. 2000). Here, we presented the documented case of CIC mutation in a G6PD-deficient child. Whole exome sequencing confirmed the diagnosis of X-linked increased susceptibility for hemolytic anemia due to G6PD deficiency and mental retardation type 45 due to CIC variant, which explained the development of epileptic seizures. It is important to define the nature of mutation, de novo or hereditary, mutational analysis by Sanger sequencing showed the G6PD and CIC mutations genotype to be hereditary.

Study of protein domains in G6PD presented position of mutation in c-terminal domain which plays key role in G6PD binding site with $NADP^+$. Structural impairment due to the mutation affects lower affinity of binding with $NADP^+$ in pentose phosphate pathway, the only pathway for NADPH production, resulting in imbalance of glutathione in RBCs and vulnerability of the cells to oxidative damages.

Other genetic factors cause epilepsy and epileptic disorders by affecting signaling factors and receptors. Temporal lobe epilepsy (TLE), the most occurring symptomatic epilepsy in adults, shows alteration or impairment in 168 genes, including upregulation of SLC1A1, SLC1A2, SLC17A8, and SLC7A11 genes that encode glutamate transporter system, and the system is responsible for glutamate uptake in neurons that can control the excitability (Castro-Torres et al. 2020). Moreover, detected mutations in rho guanine nucleotide exchange factor 9 gene (ARHGEF9) that encodes guanosine diphosphate-guanosine triphosphate exchange factor collybistin (CB) indicated major role of the gene in X-linked mental retardation with commonly occurring phenotypic symptoms such as developmental delay and different degrees of epilepsy (Yao et al. 2020). Apart from genetics factors, epigenetic factors such as miRNAs play important factors in neurodegenerative diseases. Amyotrophic lateral sclerosis (ALS), the most third common neurodegenerative disease, has been related with upregulation of miR-206, whose normal level in serum is responsible for muscular regeneration at the time of nerve injury (Dardiotis et al. 2018). However, upregulation of the miR-206 has been shown to cause atrophy in ALS patients (Dardiotis et al. 2018; Tasca et al. 2016). Additionally, in mesial temporal lobe epilepsy with hippocampus sclerosis (MTLE-HS) patients, miR-145 downregulation in the brain tissues and upregulation in blood sample (Antônio et al. 2019). Nevertheless, over-expression of miR-181c in blood and normal level in brain tissues of the patients was observed (Antônio et al. 2019).

Furthermore, association of CIC mutations with human disease is restricted to the diseases affecting cells of the neural crest lineage or neurodegenerative diseases such as spinocerebellar ataxia I or a core abnormality in the majority of oligodendrogliomas (Lam et al. 2006; Yip et al. 2011).

Revealed in several cell types, CIC target genes were enriched for MAPK effector genes involved in cell cycle and proliferation. CIC binding to target genes is abolished by high MAPK activity, which activates CIC transcriptional function as repressor by sing histone deacetylases. Structural analysis of CIC genes and its protein domains indicate that independent single amino acid substitutions in oligodendrogliomas affect binding affinity of CIC with target genes. The patients' mutation position in CIC gene was found to be in C1 domain, known as highly conserved c-terminal motif, which is likely essential in transcriptional function of CIC protein (Astigarraga et al. 2007) and as our patient demonstrated, it can lead to mental retardation. However, clinical observations of our patient's brother, who carries the same mutation in CIC gene, presented no symptoms correlated with the disease. Moreover, clinical studies did not detect tumor growth or suspicious lesion in brain or clinical findings including mental and developmental retardation in motor functions.

Based on the findings, we present the following hypotheses; taking past and present studies into account, we presume symptoms' manifestations related to CIC gene in patient's sibling. Differences between mutated CIC protein expression levels may play role in the clinical outcome. Such delay in symptoms' development could be due to other physiological and metabolic conditions of the individual. Secondly, although detected mutation has been reported to defect CIC

protein function as transcriptional suppressor, further analysis is required to investigate possibilities of mutation or impairment in non-coding regions of the gene which may have affected structural complexity of the protein and its functions, as effects of non-coding genes have been previously reported in other genes (Gloss and Dinger 2018).

Conclusion

Considering CIC variant and relevant relation with the severity and course of disease, G6PD mutations sustained through the family are interpreted as hereditary. No evidential phenotypes in the patient's younger sibling need follow-up analysis which will confirm the cause, whether the expression difference, owing to age or genetic cause of non-coding regions. Taken together, our findings could represent importance of variants found in G6PD as well as CIC genes linked to severity of epilepsy.

In view of the impact of G6PD gene defined by the literature, the variations in expression or penetration of G6PD and their exact clinical impacts requires following investigation in children by further detailed studies.

Authors' Contributions Meryem Alagoz collected data, conducted the data analysis, interpreted experiments, and drafted and revised the manuscript. Nasim Kherad helped to perform data analyses and drafted the manuscript. Ezgi Gunger and Selin Kaymaz prepared the figures and helped to draft the manuscript. Adnan Yuksel participated in the preparation of patient medical information and provided genetic counseling to the parents. All the authors read and approved the final manuscript.

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Compliance with Ethical Standards

Consent Ethics statement permission was obtained from Biruni University ethics committees (2017/10-11). All procedures followed were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2013. Patients given consent to use their data in this study.

References

Altay C, Gümrük F (2008) Red cell glucose-6-phosphate dehydrogenase deficiency in Turkey. Turk J Hematol 25:1–7

Antônio LGL, Freitas-Lima P, Pereira-da-Silva G, Assirati JA, Matias CM, Cirino MLA et al (2019) Expression of microRNAs miR-145, miR-181c, miR-199a and miR-1183 in the blood and hippocampus of patients with mesial temporal lobe epilepsy. J Mol Neurosci 69:580–587

Astigarraga S, Grossman R, Díaz-Delfín J, Caelles C, Paroush Z, Jiménez G (2007) A MAPK docking site is critical for downregulation of Capicua by torso and EGFR RTK signalling. EMBO J 26(3):668–677. https://doi.org/10.1038/sj.emboj.7601532

Au SW, Gover S, Lam VM, Adams MJ (2000) Human glucose-6- phosphate dehydrogenase: the crystal structure reveals a structural NADP (+) molecule and provides insights into enzyme deficiency. Structure 8(3):293–303

Berg JM, Tymoczko JL, Stryer L (2002) Biochemistry, 5th edn. W. H. Freeman and Co, New York

Bettegowda C, Agrawal N, Jiao Y, Sausen M, Wood LD, Hruban RH, Rodriguez FJ, Cahill DP, McLendon R, Riggins G, Velculescu VE, Oba-Shinjo SM, Marie SK, Vogelstein B, Bigner D, Yan H, Papadopoulos N, Kinzler KW (2011) Mutations in CIC and FUBP1 contribute to human oligodendroglioma. Science. 333(6048):1453–1455

Beutler E (1978) Haemolytic anaemia in disorders of red cell metabolism (topics in haematology). Plenum Medical, New York, London

Castro-Torres RD, Ureña-Guerrero ME, Morales-Chacón LM, Lorigados-Pedre L, Estupiñan-Díaz B, Rocha L, Orozco-Suárez S, Rivera-Cervantes MC, Alonso-Vanegas M, Beas-Zárate C (2020) New aspects of VEGF, GABA, and glutamate signaling in the neocortex of human temporal lobe pharmacoresistant epilepsy revealed by RT-qPCR arrays. J Mol Neurosci 70:916–929

Chittaranjan S, Chan S, Yang C, Yang KC, Chen V, Moradian A, Firme M, Song J, Go NE, Blough MD, Chan JA, Cairncross JG, Gorski SM, Morin GB, Yip S, Marra MA (2014) Mutations in CIC and IDH1 cooperatively regulate 2-hydroxyglutarate levels and cell clonogenicity. Oncotarget. 5(17):7960–7979

Dardiotis E, Aloizou A-M, Siokas V, Patrinos GP, Deretzi G, Mitsias P, Aschner M, Tsatsakis A (2018) The role of microRNAs in patients with amyotrophic lateral sclerosis. J Mol Neurosci 66:617–628. https://doi.org/10.1007/s12031-018-1204-1

de Gurrola GC, Araúz JJ, Durán E, Aguilar-Medina M, Ramos-Payán R, García-Magallanes N, Pacheco GV, Meraz EA (2008) Kernicterus by glucose-6-phosphate dehydrogenase deficiency: a case report and review of the literature. J Med Case Rep 2:146

Gloss BS, Dinger ME (2018) Realising the significance of noncoding functionality in clinical genomics. Exp Mol Med 50(8):97. https://doi.org/10.1038/s12276-018-0087-0

Gómez-Manzo S, Marcial-Quino J, Vanoye-Carlo A, Enríquez-Flores S, De la Mora-De la Mora I, González-Valdez A, García-Torres I, Martínez-Rosas V, Sierra-Palacios E, Lazcano-Pérez F, Rodríguez-Bustamante E, Arreguin-Espinosa R (2015) Mutations of glucose-6-phosphate dehydrogenase Durham, Santa-Maria and A+ variants are associated with loss functional and structural stability of the protein. Int J Mol Sci 16(12):28657–28668

Gómez-Manzo S, Marcial-Quino J, Vanoye-Carlo A et al (2016) Glucose-6-phosphate dehydrogenase: update and analysis of new mutations around the world. Int J Mol Sci 17(12):2069 Published 2016 Dec 9

Kaplan M, Hammerman C (2000) Glucose-6-phosphate dehydrogenase deficiency: a worldwide potential cause of severe neonatal hyperbilirubinemia. Neo Rev 1:32–38

Kaushik, P., Mahajan, N., Girimaji, S. C., & Kumar, A. (2020). Whole exome sequencing identifies a novel homozygous duplication mutation in the VPS13B gene in an Indian family with Cohen syndrome

Kordes U, Richter A, Santer R, Schäfer H, Singer D, Sonntag J, Steuerwald U, Schneppenheim R, Janka G (2010) Neonatal cholestasis and glucose-6-P-dehydrogenase deficiency. Pediatr Blood Cancer 54(5):758–760

Lam YC, Bowman AB, Jafar-Nejad P, Lim J, Richman R, Fryer JD, Hyun ED, Duvick LA, Orr HT, Botas J, Zoghbi HY (2006) ATAXIN-1 interacts with the repressor Capicua in its native complex to cause SCA1 neuropathology. Cell. 127(7):1335–1347

Lanzkowsky P (2011) Manual of pediatric hematology and oncology, 5th edn. Elsevier, New York, pp 168–199

Lee C-J, Chan W-I, Cheung M, Cheng Y-C, Appleby VJ, Orme AT, Scotting PJ (2002) CIC, a member of a novel subfamily of the

HMG-box superfamily, is transiently expressed in developing granule neurons. Brain Res Mol Brain Res 106(1–2):151–156

Luzzatto L, Seneca E (2014) G6PD deficiency: a classic example of pharmacogenetics with on-going clinical implications. Br J Hematol 164:469–480

McDade J, Abramova T, Mortier N, Howard T, Ware RE (2008) A novel G6PD mutation leading to chronic hemolytic anaemia. Pediatr Blood Cancer 51(6):816–819

Mohamed MM, El-Humiany AU-R (2006) Molecular characterization of new variants of Glucose-6-phosphate dehydrogenase deficiency gene isolated in western province of Saudi Arabia causing hemolytic anemia. Pak J Biol Sci 9:1605–1616

Panagopoulos I, Gorunova L, Bjerkehagen B, Heim S (2014) The "grep" command but not FusionMap, FusionFinder or ChimeraScan captures the CIC-DUX4 fusion gene from whole transcriptome sequencing data on a small round cell tumor with t(4;19)(q35;q13). PLoS One 9(6):e99439

Pandolfi PP, Sonati F, Rivi R, Mason P, Grosveld F, Luzzatto L (1995) Targeted disruption of the housekeeping gene encoding glucose 6-phosphate dehydrogenase (G6PD): G6PD is dispensable for pentose synthesis but essential for defence against oxidative stress. EMBO J 14:5209–5215

Sugita S, Arai Y, Tonooka A, Hama N, Totoki Y, Fujii T, Aoyama T, Asanuma H, Tsukahara T, Kaya M, Shibata T, Hasegawa T (2014) A novel CIC-FOXO4 gene fusion in undifferentiated small round cell sarcoma: a genetically distinct variant of Ewing-like sarcoma. Am J Surg Pathol 8(11):1571–1576

Takizawa T, Huang I-Y, Ikuta T, Yoshida A (1986) Human glucose-6-phosphate dehydrogenase: primary structure and cDNA cloning. Proc Natl Acad Sci 83:4157–4161

Tasca E, Pegoraro V, Merico A, Angelini C (2016) Circulating microRNAs as biomarkers of muscle differentiation and atrophy in ALS. Clin Neuropathol 35:22–30

Vaca G, Arámbula E, Monsalvo A, Medina C, Nuñez C, Sandoval L, López-Guido B (2003) Glucose-6-phosphate dehydrogenase (G-6-PD) mutations in Mexico: four new G-6- PD variants. Blood Cells Mol Dis 31(1):112–120

Vetrovoy O, Sarieva K, Lomert E, Nimiritsky P, Eschenko N, Galkina O et al (2019) Pharmacological HIF1 inhibition eliminates downregulation of the pentose phosphate pathway and prevents neuronal apoptosis in rat Hippocampus caused by severe hypoxia. J Mol Neurosci 70(5):635–646. https://doi.org/10.1007/s12031-019-01469-8

Yao R, Zhang Y, Liu J, Wang J, Xu Y, Li N, Wang J, Yu T (2020) Clinical and molecular characterization of three novel ARHGEF9 mutations in patients with developmental delay and epilepsy. J Mol Neurosci 70:908–915

Yip S, Butterfield YS, Morozova O et al (2011) Concurrent CIC mutations, IDH mutations, and 1p/19q loss distinguish oligodendrogliomas from other cancers. J Pathol 226(1):7–16

Zhang C, Freddolino PL, Zhang Y (2017) COFACTOR: improved protein function prediction by combining structure, sequence and protein-protein interaction information. Nucleic Acids Res 45: W291–W299