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Original Article

Frequency of Congenital Sucrase-Isomaltase Deficiency by Whole Exome Sequencing: Is It Really Rare?

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ABSTRACT

Aim: Congenital sucrase-isomaltase deficiency (CSID) is an autosomal recessive disease with a mutation in the sucrase-isomaltase (*SI*) gene and disaccharide maldigestion. With limited data, the estimated incidence of this disease is 57.59/10^6 births and its heterozygosity rate is 1/132. We aimed to evaluate cases who underwent whole exome sequencing (WES) analysis in the medical genetics unit with regards to the frequency and clinic of CSID.

Materials and Methods: The patients' files who underwent WES between 2018-2023 were evaluated retrospectively. The demographic characteristics, complaints, physical examination, and laboratory findings of those patients with *SI* gene mutations were recorded. Cases with mutations were recontacted and symptom questioning was performed.

Results: Mutations were detected in 25 (8.3%) of 300 patients who underwent WES analysis. One case had a compound heterozygous mutation, while 24 cases were heterozygous. The mean age was 22.4±17.6 years (1.8-52 years) and 16 (64%) were females. Nine of the cases (36%) were symptomatic.

Conclusion: Data on the frequency of CSID are insufficient and variable in the literature. The SI heterozygosity rate in our study was higher than some studies. There were similar rates when compared to the study conducted on symptomatic pediatric patients in our country. Although the data of our study includes a heterogeneous group to evaluate the frequency of CSID, it consists of a group with suspected genetic diseases rather than healthy individuals. Therefore, large-scale population-based studies are needed.

Keywords: Heterozygous carriers, prevalence, sucrase-isomaltase deficiency, sucrose, whole exome sequence analysis

Introduction

Congenital sucrase-isomaltase deficiency (CSID), first described in the 1960s, is a disorder of carbohydrate malabsorption caused by a homozygous or compound heterozygous mutation in the sucrase-isomaltase (*SI*) gene on chromosome 3q26 (1). Due to indigestible disaccharides (sucrose and isomaltose), symptoms such as osmotic foul-smelling diarrhea, abdominal pain, gas-bloating, and vomiting are frequently seen in the clinic presentation (2). Diagnostic difficulties of this disease and clinical

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resemblance to many chronic gastrointestinal system diseases delay its diagnosis. This may cause severe time and financial losses for the patients and their relatives.

Although duodenal biopsy enzyme activity is the gold standard for diagnosing CSID, it cannot be performed in many countries such as Turkey. Therefore, genetic analysis, which is relatively easily accessible, is used in clinically suspected patients for diagnosis (3).

There are few publications on the global prevalence of this disease. In a study by de Leusse et al. (4), CSID was the most common among congenital disaccharidase deficiencies, and its estimated prevalence was reported as 57.59 per 100,000 births. Regarding the prevalence between communities, serious differences are noteworthy, for example, the frequency of this disease in Ashkenazi Jews is 247/100,000, in Non-Finnish Europeans, it is 128/100,000, while in East Asia, it is 0.36/100,000 (5). It is thought that these differences are due to the lack of diagnosis of CSID cases in many countries due to diagnostic difficulties and low awareness and so they do not reflect the true prevalence rates (6).

In recent years, the genetic and clinical structure of this disease has come to the fore again, with studies showing that cases with heterozygous SI mutations may also be symptomatic (7). de Leusse et al. (4) stated that the estimated prevalence of CSID heterozygosity was 1/132. In a recent study in Turkey, the rate of CSID in pediatric patients was 11%, while other studies were in the form of case reports (8-10).

With developments in genetics, whole exome sequence (WES) analysis can be performed in most genetics laboratories. In this way, many rare diseases which were difficult to diagnose can now be diagnosed more easily (11). At the same time, many pathogenic variants can be detected incidentally in addition to the clinically predicted diseases.

This study aimed to contribute to the literature by retrospectively evaluating the frequency and clinic presentations of *SI* gene mutations in those patients who underwent WES for any indication in the medical genetics unit of our hospital.

Materials and Methods

The results of those cases who underwent WES in our hospital for any reason between January, 2018 and October, 2023 were retrospectively analyzed. The demographic characteristics of those patients with SI mutations, reasons for performing WES, complaints, physical examination, and laboratory findings were obtained from the hospital files. Cases with mutations were contacted and symptom questioning was performed again regarding CSID.

In order to obtain genomic DNA, 2 cc peripheral venous blood samples were taken from the patients who underwent WES analysis into tubes with EDTA. From the blood taken, DNA isolation was performed manually with the application procedure of a peripheral blood lymphocyte cells DNA isolation kit (Roche) in the medical genetics laboratory. The extracted DNA was maintained at -20 degrees until it was used. After adaptor ligation was performed on the ends of the DNA sequences to recognize and separate the DNA samples obtained from peripheral blood planned to be analyzed by the next generation sequence (NGS) and WES analysis, a purification process was applied. The protocol specified in the kit instruction manual was applied for the DNA indexing stage attached to the purified adapter. The pool (library) created for the indexed sample DNAs was combined in the tube at the appropriate concentration, loaded into the device cartridge, and taken to the sequencing process with the Illumina Next Generation Sequencing device. The Illumina NextSeq platform was used for NGS. In this study, the raw data obtained by working with Twist Bioscience NGS diagnostic kits on the Illumina NextSeq platform were analyzed according to the reference genome GRCh37(h19) in a web-based bioinformatics program (https://seq.genomize.com/V.6.2.3). Clinvar, VarSome and Franklin databases were used in the evaluation of variations for pathogenicity classification, taking into account the recommendations of American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines, 2015. Variant region reads were evaluated using the Integrative Genomics Viewer program. With regards to detected variants, the ESP6500 was checked with the ExAC and GnomAD Exome study frequency values. Variants in the *SI* gene, classified as pathogenic, likely pathogenic, and variant of uncertain significance (VUS), were evaluated.

This study was conducted according to the Principles of the Declaration of Helsinki. Ethical approval for this study was obtained from the Süleyman Demirel University Local Ethics Committee (decision date: 28.09.2022, approval no.: 262). Informed consent was obtained from all participants.

Statistical Analysis

IBM SPSS (Statistical Package for the Social Sciences) version 23.0 (IBM Corp.; Armonk, NY, USA) program was used to evaluate the data in our study. For descriptive statistics, parametric tests assumptions are provided for numerical variables, standard deviations, or medians

(minimum-maximum). Categorical variables are given as numbers (n) and percentages (%).

Results

During our study period, WES was performed on 300 patients in the medical genetics unit at our center. The *SI* gene mutation was detected in 31 patients. These mutations were evaluated with the ACMG, ClinVar and Franklin Genoox databases, and those patients with benign or likely benign variants in at least one database (n=6) were excluded. A total of 25 patients, 24 heterozygous and one compound heterozygous, were included in this study (Figure 1).

While pathogenic/likely pathogenic variants were observed in 5 cases, the majority of cases (n=20, 80%) were VUS variants. Some variants were present in more than one patient. The 'c.1919A>G (p.Glu640Gly)' variant was seen in three cases, and the 'c.1730T>G (p.Val577Gly)', 'c.2864G>A (p.Cys955Tyr)' and 'c.2923T>C (p.Tyr975His)' variants were detected in two cases each (Table I).

Twenty-one different SI variants were detected in 25 cases. It was observed that 6 variants found in 6 cases were intronic, 15 variants found in 20 cases were exonic, and one case had both intronic and exonic variants.

When the WES indications of the cases with mutations were evaluated, while neurological reasons (n=9, 36%) were the primary reasons, other reasons included cancer screening (n=5, 20%), syndromic appearance (n=4, 16%), immunodeficiency (n=3, 12%), hematological (n=2, 8%) and musculoskeletal (n=2, 8%) diseases.

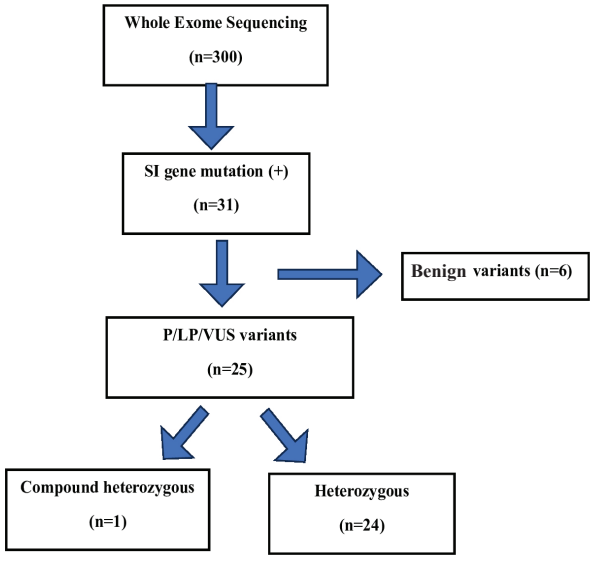

Figure 1. Algorithm of patients included in the study

The mean age was 22.4±17.6 years (1.8-52 years) and 16 (64%) were females. CSID-related symptoms were present in a total of 9 cases (36%), one with a compound heterozygote and 8 with a heterozygous mutation. Sixteen cases (64%) were in the pediatric age group and constituted the majority (7/9) of symptomatic cases.

There was only one patient with a compound heterozygous mutation and symptoms. This case was a 12-year-old male patient with *SI* gene 'c.3218G>A,p.Gly1073Asp/c.1147-177A>G' compound heterozygous mutation. The 'c.3218G>A,p.Gly1073Asp' variant was classified as "pathogenic" by the ClinVar mutation database and "likely pathogenic" by Franklin Genoox. The other 'c.1147-177A>G' variant was classified as "VUS" by the ACMG and Franklin Genoox databases, and "not available" by ClinVar. When questioned regarding CSID-related symptoms, there was intermittent abdominal pain with no nutritional relationship clearly stated.

When the symptomatic heterozygous cases (n=8) were evaluated, two adult cases had abdominal pain and dyspepsia. In the pediatric cases, growth retardation (n=3), diarrhea (n=2) and abdominal pain (n=1) were detected (Table II).

The adult patients were re-evaluated and it was stated that their symptoms were not significant and did not reduce the quality of their daily life. When the symptoms were questioned for the pediatric patients, it was observed that there were no constant complaints.

Discussion

CSID is considered an autosomal recessive inherited and rare disorder. However, more than 40 mutations have been identified recently, and sucrase-isomaltase enzyme deficiency is the most common disaccharidase deficiency (12). Due to difficulties in diagnostic testing and changes in symptoms with age, the true prevalence of this disease is likely to be underestimated and may be more common than expected (13).

The clinical presentation often occurs as severe watery diarrhea, abdominal distention, bloating, inability to gain weight, irritability, and diaper dermatitis due to exposure to sucrose or starch after complementary feeding in infancy (14). Significant symptoms in the first years of life may be intermittent diarrhea, abdominal pain, and gas-bloating, with sweet and fruit avoidance in later years. Clinical findings such as diarrhea-predominant irritable bowel syndrome can be seen in adolescents and adults (15).

More pronounced clinical symptoms in children have been associated with a shorter transit time in the small intestine and a lower absorption capacity of the colon (16). In our study, the majority of our symptomatic cases (n=7, 77%) were in the pediatric age group. Other factors affecting the symptoms include homozygous or heterozygous *SI* gene mutation, residual enzyme activity, and the amount of sugar and starch consumed (17).

Although symptoms have been reported in cases with homozygous or compound heterozygous mutations due to the inheritance pattern of the disease, there have been reports in recent years that heterozygous individuals may also be symptomatic (18). In cases with heterozygous mutations, dyspeptic complaints such as abdominal pain and nausea-vomiting can be seen rather than osmotic diarrhea, and it can be diagnosed as a functional gastrointestinal disease (19). In our study, the case with the compound heterozygous mutation was symptomatic, as expected. In addition, 8 of the 27 heterozygous cases were symptomatic. Complaints of abdominal pain and dyspepsia were observed in the adults, similar to the literature. In the pediatric cases with heterozygous mutation, the symptoms were variable. This variability can be explained by the WES indications of the patients and their primary underlying diseases. While growth retardation may be observed in cases with a syndromic appearance, diarrhea may also be present in patients with immunodeficiency. It does not seem possible to directly relate these symptoms to CSID.

Table I. Characteristics of cases with SI mutation

Case	Gender/Age	SI Variant 1/SI Variant 2	Exon/Intron	ACMG	ClinVar	Franklin Genoox	Symptom
1	M/12	**c.3218G>A/ c.1147-177A>G**	(p.Gly1073Asp)/ intronic	VUS VUS	P NA	LP VUS	+
2	M/8	**c.1730T>G**	(p.Val577Gly)	VUS	P	P	-
3	F/44	**c.1730T>G**	(p.Val577Gly)	VUS	P	P	+
4	M/16	**c.4099A>G**	(p.Arg1367Gly)	VUS	LP	LP	-
5	M/6	**c.1544G>T**	(p.Gly515Val)	VUS	NA	LP	+
6	M/11	**c.1919A>G**	(p.Glu640Gly)	VUS	VUS	VUS	-
7	M/45	**c.1919A>G**	(p.Glu640Gly)	VUS	VUS	VUS	+
8	M/46	**c.1919A>G**	(p.Glu640Gly)	VUS	VUS	VUS	-
9	F/15	**c.1-15C>A**	Intronic	VUS	VUS	VUS	-
10	F/3.5	**c.2923T>C**	(p.Tyr975His)	VUS	VUS	LB	+
11	M/16	**c.2923T>C**	(p.Tyr975His)	VUS	VUS	LB	+
12	F/28	**c.4951G>A**	(p.Val1651Ile)	VUS	VUS	LB	-
13	F/1.5	**c.170C>T**	(p.Pro57Leu)	VUS	NA	VUS	+
14	F/16	**c.1888-160_1888 159delinsGC**	Intronic	VUS	NA	VUS	-
15	M/14	**c.2864G>A**	(p.Cys955Tyr)	VUS	NA	VUS	+
16	F/6	**c.2864G>A**	(p.Ile1191Leu)	VUS	NA	VUS	-
17	F/3.5	**c.3571A>C**	(p.Cys955Tyr)	VUS	NA	VUS	-
18	F/8	**c.3562G>A**	(p.Val1188Ile)	VUS	NA	VUS	-
19	F/8	**c.3099+73A>T**	Intronic	VUS	NA	VUS	+
20	F/45	**c.374-4T>C**	Intronic	VUS	NA	VUS	-
21	F/17	**c.3149T>A**	(p.Ile1050Asn)	VUS	NA	VUS	-
22	F/45	**c.5422C>T**	(p.Arg1808Cys)	VUS	NA	VUS	-
23	F/47	**c.4457C>T**	(p.Thr1486Met)	VUS	NA	VUS	-
24	F/47	**c.5111G>A**	(p.Arg1704Gln)	VUS	NA	VUS	-
25	F/52	**c.2737-33dup**	Intronic	VUS	NA	VUS	-

SI: Sucrase-isomaltase, VUS: Variant of uncertain significance, NA: Not available, ACMG: American College of Medical Genetics and Genomics, LB: Likely benign, LP: Likely pathogenic

Table II. Characteristics of symptomatic cases

Case	Gender/Age	WES indication	Mutation	Symptom
1	M/12	Musculoskeletal	P/VUS-compound heterozygous	Abdominal pain
2	M/6	Immunodeficiency	LP- heterozygous	Diarrhea
3	M/14	Immunodeficiency	VUS- heterozygous	Diarrhea
4	F/1.5	Syndromic	VUS- heterozygous	Growth retardation
5	F/3.5	Syndromic	VUS- heterozygous	Growth retardation
6	F/8	Musculoskeletal	VUS- heterozygous	Growth retardation
7	M/16	Neurological	VUS- heterozygous	Abdominal pain
8	F/44	Neurological	P- heterozygous	Abdominal pain
9	M/45	Neurological	VUS- heterozygous	Dyspepsia

P: Pathogenic, LP: Likely pathogenic, VUS: Variant of unknown significance, WES: Whole exome sequencing

It has been stated in the literature that symptoms are not only related to *SI* gene mutation, but that clinical presentation may vary due to multifactorial reasons (2). Similarly, in our study, heterogeneity was observed in the symptom distribution of cases with the same mutation. Although it is known that symptoms are often evident at younger ages, the symptomatic cases of the 'c.1919A>G (p.Glu640Gly)' and 'c.1730T>G (p.Val577Gly) variants were adults, while the pediatric cases with the same mutation were asymptomatic. It has shown that residual enzyme activity may lead to variability and different clinical conditions in individuals with the same mutation. For this reason, in cases where enzyme activity cannot be measured, it becomes difficult to diagnose and treat CSID.

The gold standard method for the definitive diagnosis of this disease is the measurement of enzyme activity from duodenal mucosa samples. Other noninvasive tests which support the diagnosis include the sucrose challenge test or the ^{13}C-sucrose breath test (20). However, neither enzyme activity nor ^{13}C-sucrose breath tests can be performed in Turkey. In the study by Karakoyun et al. (10), five cases were diagnosed with a sucrose challenge test after a detailed nutritional history and the relationship between symptoms was determined. Another diagnostic method, *SI* gene mutation analysis, is not primarily preferred due to its high cost, but it can be used when other diagnostic methods are not available (21). Taskin et al. (8) also used next-generation sequencing (NGS) analysis for the diagnosis of CSID, referencing the recommendations of the Alaska Native Medical Center guidelines.

The use of NGS methods such as WES has become increasingly common in the detection of possible genetic diseases in cases with diagnostic difficulties. While this method is frequently used for neurological findings (35%), and multiple congenital anomalies (24%), it can also be preferred for immunodeficiency, musculoskeletal system anomalies, growth retardation, hematopoietic system, and hearing disorders (22). In our study, similar to the literature, neurological causes were among the most common WES indications. During the analysis, genetic variants related to other diseases can sometimes be detected incidentally. As a result of the WES analysis of 3,040 cases by Retterer et al. (22), secondary pathogenic variants were reported in 6.2% (n=129) of the cases (23). We detected the secondary pathogen SI variant rate as being 1.6% (5/300), but we did not evaluate this rate for other diseases. In addition to pathogenic variants, we also evaluated cases with SI mutation regarding exon and intronic variants. Examining intronic variants as well as exonic variants is performed due to the increasing number of publications in recent years indicating that intronic variants may be pathogenic (24). We observed that 2 of 6 cases (30%) with the intronic variant were symptomatic.

In our study, mutations in the *SI* gene were detected in 8.3% of those patients who underwent WES; 0.33% were compound heterozygous, while 8% had heterozygous SI variants. In a study evaluating the estimated prevalence of congenital disaccharidase deficiencies, the prevalence of heterozygous CSID was reported as being 1/132, and our results were found to be quite high compared to the limited

studies in the literature (4). The drawback of our study was that our study group may not have reflected the population in terms of specifying the frequency of CSID, since it included individuals with more genetic diseases.

Taskin et al. (8) reported the frequency of CSID as being 11.7% (1 homozygous, 10 heterozygous mutations) via the NGS method in 94 patients with chronic nonspecific diarrhea. This rate was higher than our results, which can be explained by the fact that genetic analysis was performed on symptomatic pediatric cases. We consider that the heterogeneous distribution of our study may be more consistent in reflecting the frequency of CSID.

Conclusion

In conclusion, the variable clinical presentations in heterozygotes, in contrast to the OR inheritance of CSID, may require a redefinition of the genetic pattern of this disease. However, there is still insufficient data on the prevalence of CSID. There are widely varying results in the literature. Although the data of our study includes a heterogeneous group, larger-scale population-based studies are needed in order to clarify the enzymatic activity and genotype-phenotype relationship.

Ethics

Ethics Committee Approval: This study was conducted according to the Principles of the Declaration of Helsinki. Ethical approval for this study was obtained from the Süleyman Demirel University Local Ethics Committee (decision date: 28.09.2022, approval no.: 262).

Informed Consent: Informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: F.İ.I., Concept: F.İ.I., M.A., Design: F.İ.I., M.A., Data Collection or Processing: F.İ.I., H.Ö., H.S., Analysis or Interpretation: H.Ö., Literature Search: F.İ.I., M.A., Writing: F.İ.I.

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