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Novel *GUCY2D* Splicing Variants in Kurdish Leber Congenital Amaurosis Patients in Iraq

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***Abstract*—The current study examines the single-nucleotide polymorphism of the *GUCY2D* gene in blind patients with inherited Leber congenital amaurosis (LCA) from a molecular, medical, and genetic perspective. The study involved 33 patients with blindness and 11 healthy controls in Erbil Province, Kurdistan Region, Iraq. LCA is the most severe phenotype of inherited retinal diseases, marked by genetic and clinical heterogeneity. The goal of this work was to confirm and identify the underlying mutations. The visual evoked potentials (VEPs) test, fundus photography, and ophthalmic examination were the foundation for clinical studies. Each participant's genomic DNA was extracted to ascertain the frequency of mutations in the Kurdish nation. Primers were designed for the *GUCY2D* gene, encompassing exons 15, 16, and part of 17, with introns situated between those exons. Potentially pathogenic mutations were detected using Sanger sequencing analysis of the *GUCY2D* gene. Sequence data were analyzed to identify known, unknown, or novel mutations using FinchTV, BioEdit, and databases from the National Center for Biotechnology Information, including BLASTN, BLASTX, and ClinVar, along with Mutation Surveyor and MaxEntScan. Three novel splicing mutations have been found in the *GUCY2D* of blindness patients. These mutations are: c.3043+11C>T, c.3044-7G>T, and c.3043+20G>C. The patients have included nystagmus, abnormal VEPs, photophobia, hyperopia, and early infantile onset of vision loss. The three new mutations in the *GUCY2D* of Kurdish blind patients are associated with LCA disease. A family history of blindness or the presence of affected relatives may represent important risk factors for LCA, supporting its inherited genetic basis.**

***Index Terms*—Early-onset severe retinal dystrophy, *GUCY2D* gene, Leber congenital amaurosis, Single nucleotide polymorphism, Visual evoked potentials test.**

I. Introduction

On a global scale, visual impairment and blindness continue to pose significant public health burdens. As of 2020, among an estimated global population of 7.79 billion, approximately 49.1 million individuals were affected by blindness (Asimadu, Okeke and Onyebueke, 2023). Notably, around 90% of the world's blind population resides in developing countries, underscoring the disproportionate global burden (Wolfram, et al., 2019). Epidemiological analyses reveal that, on average, for every blind individual, 3.4 persons experience low vision, though this ratio varies across regions, ranging between 2.4 and 5.5. The prevalence of childhood blindness is also significant, with an estimated 1.4 million children aged 1–14 years living with blindness globally (Hussain, et al., 2019). Leber congenital amaurosis (LCA) is regarded as the most severe clinical entity of inherited retinal diseases (Cideciyan and Jacobson, 2019) and includes early-onset severe retinal dystrophy and a class of hereditary retinal illnesses (Xu, et al., 2020). It is recognized as the gravest form of retinal dystrophy (Michaelides *et al.*, 2006), with blindness developing by the completion of the first year of life, and it is characterized by significant vision impairment or blindness at birth (Georgiou, Fujinami and Michaelides, 2021).

There are 28 common causal genes causing LCA in all (Shukla, Kannabiran and Jalali, 2012; Chacon-Camacho and Zenteno, 2015; Coussa, Lopez Solache and Koenekoop, 2017; Weisschuh, et al., 2018; Bouzia, et al., 2020; Kumaran, et al., 2017, Varela, et al., 2022; Zhang and Xu, 2025). The mutations in these genes account for roughly 70–80% of LCA patients (Xu, et al., 2020), Some genes, such as *GUCY2D*, *CEP290*, *NMNAT1*, and *AIPL1*, have been identified as being very likely to be linked to LCA, as well as mutations in other genes, including *RDH12*, *RPE65*, and *LRAT* (Kumaran, et al., 2017). *GUCY2D* is also the first gene related to LCA-1, and it is one of the most famous, contributing to roughly 10–20% of cases (Bouzia, et al., 2020). The LCA-1 phenotype becomes much more severe, with loss of photoreceptor function and blindness occurring very early in life (Zobor, et al., 2023).

An early onset of severe visual impairment is a characteristic of a class of inherited retinal disorders known as LCA (Daich Varela, et al., 2022). Usually, it appears at birth or soon after (Hahn, et al., 2022). Its onset age ranges from 1 to 5 years (Kumaran, et al., 2017). LCA is

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characterized by blindness or severely reduced vision, with severe visual loss after birth or during labor (Kumaran, et al., 2017; Bouzia, et al., 2020), and the visual evoked potentials (VEPs) test is abnormal (Mohammed, Mossallam and Allam, 2021; Ibrahim and Hidayat, 2021). Other observed features comprised amaurotic pupils, sensory or wandering nystagmus, and fundus appearance that was either unremarkable or demonstrated pigmentary retinopathy, occasionally accompanied by macular atrophy (Georgiou, Fujinami and Michaelides, *et al*., 2020).

The retinal guanylyl cyclase 1 (GC1), often referred to as photoreceptor guanylate cyclase 2D in the retina, is encoded by guanylate cyclase 2D (Wimberg, et al., 2018). Guanylate cyclase 2D enzyme, found in photoreceptors, is responsible for the intracellular transport of photoreceptor excitation. This enzyme has a critical function in the recovery phase of phototransmission and is involved in the resynthesis of cyclic guanosine monophosphate (cGMP). Guanylate cyclase-activating proteins serve as the primary modulators of its regulation (Vinberg, et al., 2018). In mammal phototransduction, cGMP, an intracellular second messenger, is naturally regulated by GC1. It is considered that GC1 is found in the innermost segments and nucleus of rod and cone photoreceptor cells (Coussa, Lopez Solache and Koenekoop, 2017). Evidence indicates that GC1 functions as a calcium-modulated domain, intricately associated with phototransduction processes and retinal synaptic signaling, which are triggered by a reduction in the cell's Ca^{2+} levels (Rehkamp, et al., 2018).

The current study aimed to identify the mutation profile, including novel mutations and single-nucleotide polymorphisms (SNPs), in the *GUCY2D* gene associated with Leber congenital amaurosis (LCA). Using Sanger sequencing, exons 15, 16, part of exon 17, and their flanking intronic regions were screened to detect genetic variants and evaluate their potential pathogenicity. The analysis identified three novel splicing mutations in the *GUCY2D* gene: c.3043+11C>T, c.3044-7G>T, and c.3043+20G>C. These mutations were detected in patients presenting with clinical features including nystagmus, abnormal visual evoked potentials (VEPs), photophobia, hyperopia, and early-onset vision loss. To the best of our knowledge, this is the first study to investigate the molecular genetics and clinical characteristics of Kurdish patients with *GUCY2D*-associated LCA in the Kurdistan Region and Iraq.

II. Materials And Methods

Ethical approval for this study was obtained from several authorities: the Ministry of Higher Education and Scientific Research – Salahaddin University, Erbil (Directorate of Postgraduate Studies, (No. 3/1/7491, issued 05/10/2020); the Ministry of Labor and Social Affairs, General Directorate of Development and Social Welfare (No. 1149, issued October 05, 2020); the Directorate of Handicapped Care – Erbil (No. 449, issued October 18, 2020); and the Union of Blind and Visually Impaired Kurdistan – Higher Commission, Erbil, Kurdistan Region, Iraq. In addition, written informed consent was obtained from patients and their parents. All procedures were conducted in accordance with ethical principles to ensure the dignity, rights, safety, and well-being of the participants before initiating the study.

A. Samples

Patients group

The study included 33 individuals (male and female) registered with the Union of Blind and Visually Impaired Kurdistan–Higher Commission in Erbil, Kurdistan Region of Iraq. Participants ranged in age from 5 to 53 years. All patients had been clinically evaluated and confirmed as blind by a specialized ophthalmic committee. Before blood collection, informed consent was obtained from patients or their legal guardians. Each participant completed a structured questionnaire, which was administered through both written forms and direct interviews.

Control group

To match the patient group with the control group, 11 healthy persons of different sexes, ranging in age from 21 to 33, were randomly selected from the Erbil community as a control group.

B. Collection of blood samples

Using a sterile disposable syringe, roughly 3 mL of blood was drawn from the veins of the (11) control group and the (33) patient group who had been diagnosed and associated with LCA disease. The specimens were collected in sterile vacutainer tubes containing ethylenediaminetetraacetic acid as an anticoagulant. Samples were initially preserved in an icebox at a temperature below +10°C, and upon arrival, all samples were stored under appropriate conditions (4°C) until further processing.

C. DNA Isolation

Genomic DNA was extracted from whole blood using the AddPrep Genomic DNA Extraction Kit (AddBio, Korea), in strict adherence to the manufacturer's standardized protocol. The extraction process was conducted at the Immunogene Center in Erbil, Kurdistan Region, Iraq.

Quantitative and qualitative analysis of the extracted DNA

Thermo Fisher USA's NanoDrop was utilized to analyze the quantity and quality of isolated DNA at the IMUNOGENE CENTER in Erbil, Kurdistan Region, Iraq. About 2 μL of isolated DNA was utilized in the absorbance technique. This device calculates the DNA sample's light absorbance at particular frequencies. The wavelengths at which absorbance is measured are 260 and 280 nm. DNA purity can be estimated using the absorbance quotient (260/280). A decent absorbance quotient value is defined as $1.8 < \text{ratio (R)} < 2.0$ and is regarded as being excellent (Ghatak, Muthukumaran and Nachimuthu, 2013).

DNA storage

Following the completion of the genomic DNA extraction process, the eluted DNA was kept at −20°C to prevent degradation (Rohland, et al., 2018).

D. Sanger Sequencing

The *GUCY2D* gene was subjected to direct (Sanger) sequencing of exons 15, 16, part of 17, and their flanking intronic regions, based on the amplified polymerase chain reaction (PCR) products. Primers were chosen and designed based on a medical case report that used next generation sequencing (Ibrahim and Hidayat, 2021). The *GUCY2D* reference sequence (GenBank: NM_000180.4) was retrieved from the National Center for Biotechnology Information (NCBI), and primers were manually designed using primer design software. Primers were manually designed using primer design software. The primer design was performed using the NCBI database as a reference for the *GUCY2D* to identify the exact target region. The reverse primer sequence was generated using the Compute Reverse Complement of the Nucleotide Sequence tool (https://www.bioinformatics.org/sms/rev_comp.html), and primer characteristics were evaluated using the Oligo calculator tool (https://mcb.berkeley.edu/labs/krantz/tools/oligocalc.html). Target DNA amplification was carried out using Gradient PCR (Applied Biosystems) with assistance from the IMUNOGENE CENTER in Erbil, Kurdistan Region, Iraq. The sequencing was done in Iran. They used the 3130 genetic analyzer (Applied Biosystems, Hitachi High-Technologies, Tokyo, Japan) to sequence all PCR products in both directions. The primer sets designed for this study are presented in Table I.

E. Bioinformatics Analysis

To find known, unknown, or novel mutations, the sequence findings were analyzed using various applications, such as FinchTV, BioEdit, NCBI (BLASTN, BLASTX, and ClinVar), Mutation Surveyor, and MaxEntScan. The GenBank reference sequences [NM_000180.4] were utilized to name the mutation. The Human Genome Variation Society's (HGVS; http://www.hgvs.org) nomenclature was followed when naming and referring to the sequence variants in the text.

III. Results and Discussion

A. Clinical Description

Under the guidance of a professional medical team, the individuals underwent clinical investigation, and the ailment was identified as blindness at the time of the beginning of their lives. All affected individuals had a diverse presentation of conditions, including photophobia, dyschromatopsia, nyctalopia, retinal degeneration, retinal, and vascular attenuation based on clinical manifestations and electrophysiological findings. During this study, a number of factors that influence blindness were identified and analyzed. These factors included age, gender, the age at which blindness first manifested, parent relatives, cases of blindness in the family, cases of blindness in patient relatives, the health of the mother during pregnancy, and the ages of the mother and father. In this investigation, a questionnaire form was employed with the consent of the patients.

TABLE I
Primer Sets for Amplification of Gucy2d Coding Sequences

Reference genome		Chr17: 8,002,615-8,020,342
Positions		NM_000180.4
Exon (s) covered	15-16 and a part of 17	
Primers	Forward (5′-3′) (20 bp)	Reverse (5′-3′) (23 bp)
Sequence	5'-GGCAATCGCT TCGTGTACTC-3′	5'-CAAGTTCACGTG GATGCGGTAAG-3′
Annealing Temperature	60°C	
Polymerase chain reaction output	622 bp	

B. Bio-statistical Analysis

Following molecular genetic testing, the statistical software package version 12 (Stata) was utilized to evaluate data from the patient or case group as well as the control group. Of the 44 participants who were investigated, 33 were blind patients, and 11 were controls, as stated in Table II.

Furthermore, the majority of participants (both the patients' group and the control group) are young, with 61% of patients being between the ages of 21 and 40 (Fig. 1).

C. Molecular Genetic Screening

In Erbil City, blood was drawn for DNA extraction from 33 patients diagnosed and associated with LCA disease and 11 controls. The *GUCY2D* gene's coding sections and their flanking intronic regions, which comprised exons 15, 16, and part of exon 17, were analyzed to check for mutations.

Quantitative and qualitative evaluation of DNA

Using a NanoDrop spectrophotometer, the amounts of the extracted DNA were determined to be sufficient for the ensuing PCR reactions. Treated all 44 participants as one combined group and reported the DNA concentration and purity together; they had an average DNA concentration of 31.67 ng/μL, ranging from 20.40 to 91.70 ng/μL, and the mean DNA purity ranged from 1.84 to 1.93.

Gel electrophoresis of PCR product

Amplification of specific *GUCY2D* gene sections was done using primers and PCR on forty-four samples. PCR amplification was successful for all samples. Agarose gel electrophoresis revealed single, specific bands at the expected size (~622 bp). The intensity of the PCR products was sufficient for subsequent Sanger DNA sequencing Fig. 2.

Mutational analyses

Three variations in the *GUCY2D* gene were found by Sanger sequencing. The sequence data were examined using several software tools and databases to identify known, unknown, or novel mutations. These included FinchTV,

TABLE II
Indicate The Gender Proportion and Participation Relationship

Gender	Participants		Total
	Patients group	Control group	
Female (%)	13 (72.22)	5 (27.77)	18 (100.00)
Male (%)	20 (76.92)	6 (23.07)	26 (100.00)
Total (%)	33 (75)	11 (25)	44 (100.00)

BioEdit, and resources from the NCBI, such as BLASTN, BLASTX, and ClinVar, as well as Mutation Surveyor and MaxEntScan. They were present in 7 out of 33 patients, as shown in Table III. There are seven autosomal mutations in the positive (mutant) result. Three variations that impact splicing or protein coding were found in the total number of patients that were studied. They are novel splicing mutations that were present in the mutant patients and observed; 60.60% of males have blind patients, whereas 40.40% of females have blind patients. The ages ranged from 5 to 53 years.

Genetic analysis revealed novel splicing mutations within intron 16 of the *GUCY2D* gene among several Kurdish patients. Variants were identified in patients (1, 8, 13, 25, and 31) aged 32, 19, 28, 23, and 26 years, respectively, each exhibiting (c.3043+11C>T). In addition, patient (3) is 37 years old (c.3044-7G>T), whereas patient (10) is 47 years old (c.3043+20G>C). These findings are summarized in Table III and illustrated in Fig. 3. By altering RNA splicing, those new splicing mutations may potentially have an impact on *GUCY2D* function.

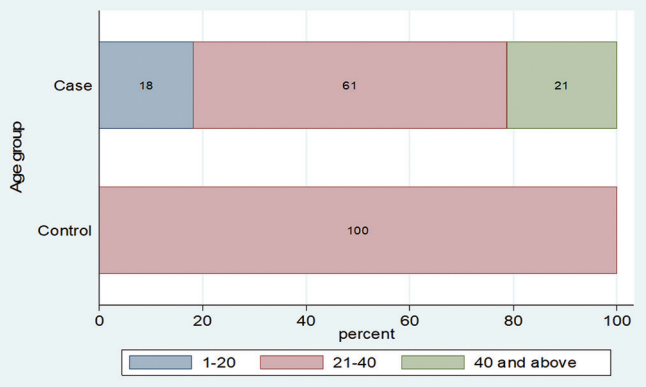

Fig. 1. The participant's age.

The patients had a 100.00% autosomal recessive mode of inheritance. The result confirms the earlier finding that the condition is similar to our result in the Netherlands (Hahn, et al., 2022).

Recent research has highlighted growing interest in genomic variants in the form of SNPs positioned within regulatory or other non-coding sequences. Notably, approximately 93% of functional SNPs cataloged in the GWAS database are situated in these non-coding sequences (Tak and Farnham, 2015 and Rwandzy, Hidayat and Sulaiman, 2024). Because they have an impact on post-transcriptional gene expression or gene regulation, these are known as regulatory SNPs (rSNPs) (Guo, et al., 2013). These rSNPs can affect gene regulation at several levels, influencing transcription factor binding and gene splicing, for example, to affect a cell's biological activity. These are also present in non-coding RNA's enhancer and promoter regions. By affecting messenger RNA (mRNA) half-life, they can reduce protein products that result in mRNA-microRNA interactions (Xue, et al., 2014).

According to this study, the percentage of positive (mutant) test results is extremely low. Table IV illustrates this, showing that the majority of patient test results are negative.

The results of this investigation confirmed t hat *GUCY2D* mutations are present in LCA instances. However, 71.4%

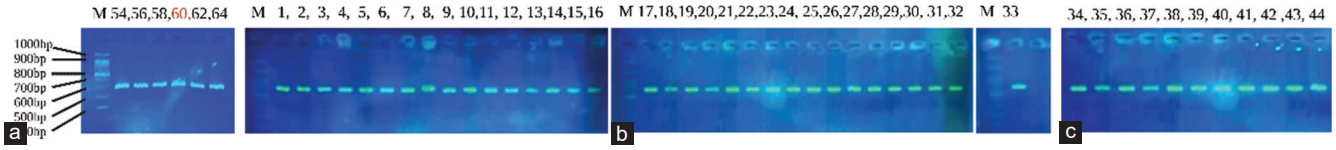

Fig. 2. Agarose gel electrophoresis of polymerase chain reaction-amplified *GUCY2D* fragments and 100 bp ladder. (a) Primer optimization showing a 622 bp band at 60°C, (b) Patient samples (lanes 1–33), and (c) control samples (lanes 35–44) all display the expected 622 bp amplicon.

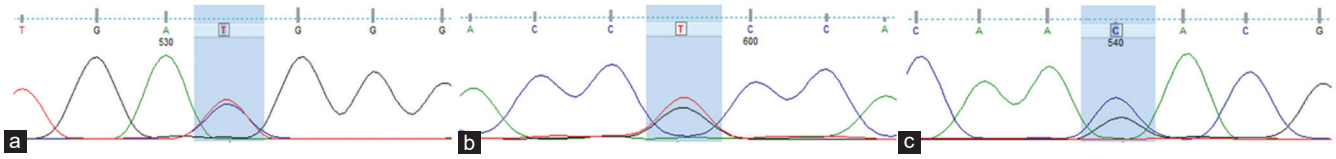

Fig. 3. The sequencing shows mutations identified in the *GUCY2D* gene. (a) In patients number 1,8,13,25, and 31, (b) Gene in patient number 3, and (c) Gene in patient number 10.

TABLE III
SNP VARIATIONS IN THE *GUCY2D* GENE WERE FOUND IN PATIENTS WITH KURDISH BLINDNESS

No of patients	Mode of inheritance	Causative variations and coexisting variations`				Molecular consequence	Type of mutation	Molecular method of searching nucleotide exon/ intron variants	Mutation state (at the Kurdish nation level)
		Nucleotide sequence							
		NC_000017.11:	NC_000017.10:	NG_009092.1:	NM_000180.4:				
1, 8, 13, 25, and 31	Autosomal recessive	g. 8015852C>T	g. 7919170C>T	g. 18183C>T	c. 3043+11C>T	Intron variant	Splicing	Sanger sequencing	Novel
3		g. 8015920G>T	g. 7919238G>T	g. 18251G>T	c. 3044-7G>T				
10		g. 8015861G>C	g. 7919179G>C	g. 18192G>C	c. 3043+20G>C				

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of the variations in the positive cases originate from the c.3043+11C>T variant, as shown in Table V.

The outcomes of the relationship between the variables in the Chi-square test. Table VI indicates that there is no significant correlation between the age group and the test result, as indicated by the p-value exceeding 0.05.

The positive results were obtained by roughly 42.86% of the male participants and 57.14% of the female participants. As observed from Table VII, there is also no correlation between gender and the test result because the p-value is over 0.05. The gender of the patients has no relation or effect on the test results; in fact, this result is comparable to that of the result has been mentioned by (Elmadina, et al., 2019).

According to Fig. 4, the average age of patients who experience blindness is 30.6 months or about 2.5 years. Either complete blindness or significantly reduced vision is found at birth or in the first several months of life. The mean age at which a patient experiences blindness in terms of months, based on test results. The average age of results that are positive and negative differs. The two samples' independent t-tests (p = 0.86) showed no statistically significant differences between them, as shown in Table VIII. The box plot also shows that there are no outliers.

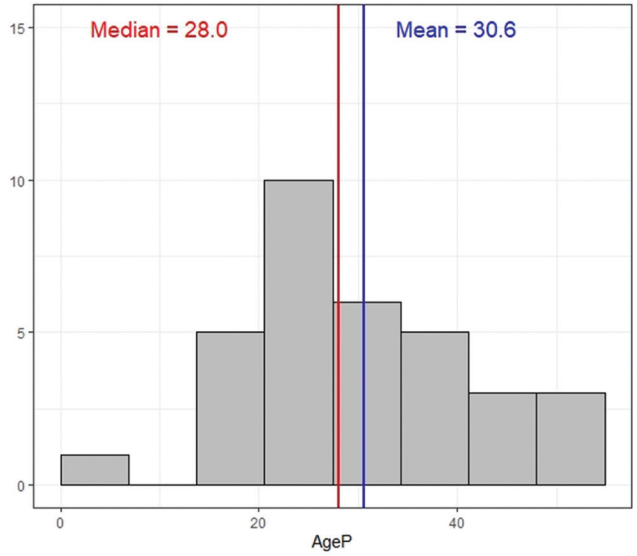

Fig. 4. The histogram displays the average age (months) at which patients experience blindness.

TABLE IV
The Number and Ratio of Mutations

Test result	Frequency	%
Negative (normal)	37	84.09
Positive (mutant)	7	15.90
Total	44	100.00

TABLE V
The Percentage of Variant Mutations

Mutation	Frequency	Percent	Cum.
c. 3043+20G>C	1	14.3	14.3
c. 3043+11C>T	5	71.4	85.7
c. 3044-7G>T	1	14.3	100.00
Total	7	100.00	-

It was discovered that 22.73% of the participants' parents were not consanguineous to one another, whereas 77.27% of the participants' parents were consanguineous to one another. Table IX shows that nearly all of the participant's consanguineous patients had positive results.

We have discovered that there is no statistical relationship between test results and parent relationships in the current study. The LCA disease is unaffected by a parent's relationship or consanguinity. This result has been reported by (Hanein, et al., 2005; Shawky, et al., 2013). However, the high rate of parents' consanguinity was positive. This result is the same as the result presented by (Lotery, et al.,

TABLE VI
Chi-Square Analysis of Age Group and Test Results

Age group (years)	Test Result		Total
	Normal (−) n (%)	Mutant (+) n (%)	
1-20	5 (83.3)	1 (16.7)	6
21-39	26 (83.9)	5 (16.1)	31
40 +	6 (85.7)	1 (14.3)	7
Total	37 (84.1)	7 (15.9)	44

Pearson χ^2 (df=2)=0.018, p=0.991, Exact test (Fisher Freeman Halton), two-sided p=1.000

TABLE VII
Chi-Square Test Results Show The Relationship Between Test Scores and Gender

Test Result	Gender		Total
	Male	Female	
Positive (mutant)	3	4	7
%	42.86	57.14	100.00
Negative (normal)	23	14	37
%	62.16	37.84	100.00
Total	26	18	44
%	59.09	40.91	100.00

Pearson χ^2 (1)=0.907, Pr=0.34

TABLE VIII
The Age of The Patient at Which They Experience Blindness in Relation to The Test Result

Group	Mean	Standard Deviation	T-test	p-value
Positive	30.6	9.4	0.1771	0.86
Negative	29.5	10.1		

TABLE IX
Quantitative and Proportional Analysis of Parent Relatives

Test result	Parent relationship		Total
	Parent consanguinity	Parent non-consanguinity	
Positive	7	0	8
%	100.0	00.00	100.00
Negative	27	10	36
%	73.0	27.0	100.00
Total	34	10	44
%	77.27	22.73	100.00

(p=0.118)

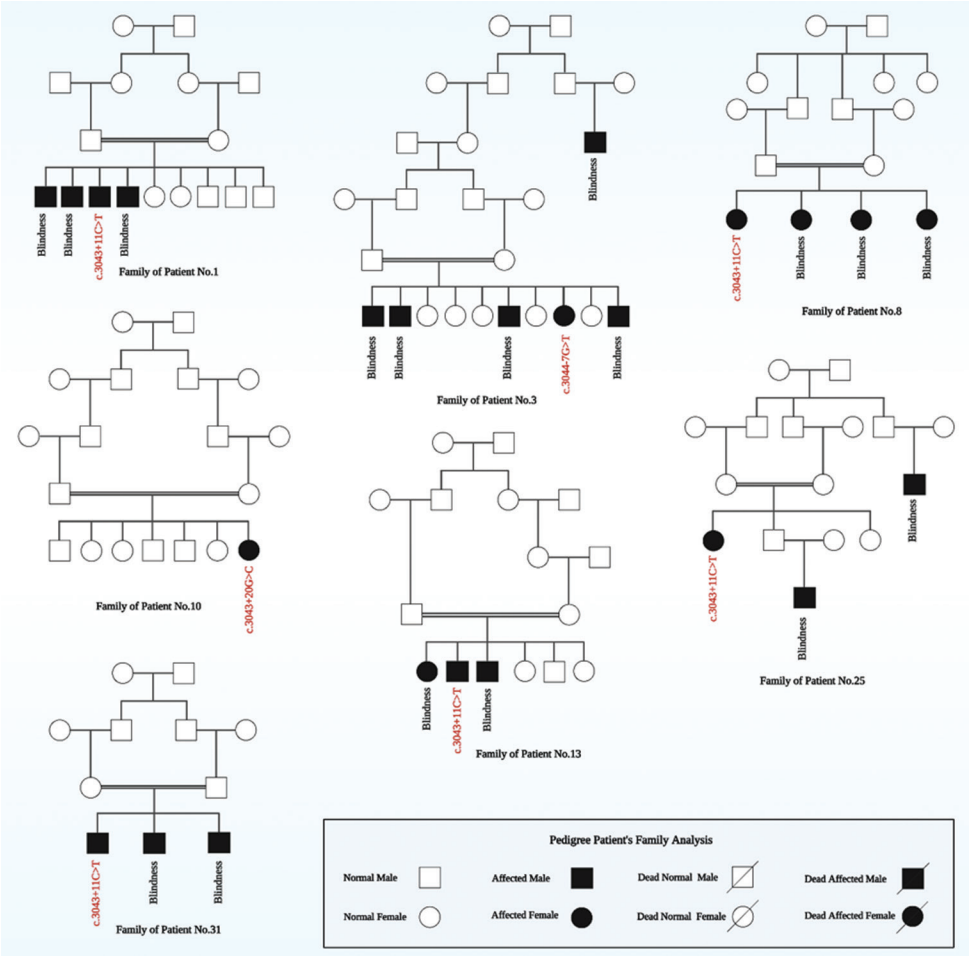

Fig. 5. A pedigree chart showing the segregation of *GUCY2D* gene mutations (autosomal recessive inheritance) in the patient's family history with Leber congenital amaurosis.

TABLE X
DESCRIBES THE PARENT/S CONSANGUINITY AND TEST RESULTS

Test results of the patient group	Parent consanguinity					Total
	1st cousin	2nd cousin	3rd cousin	2nd nephew	Non-consanguinity	
Positive (Mutant)	4	2	0	1	0	7
%	57.14	28.57	00.00	14.29	00.00	100.00
Negative (Normal)	16	9	2	0	10	37
%	43.24	24.32	5.41	00.00	27.03	100.00
Total	20	11	2	1	10	44
%	45.45	25.00	4.55	2.27	22.73	100.00

TABLE XI
PROPORTIONAL BREAKDOWN OF CASE RELATIVES IN PATIENT FAMILIES

Case relative in family patient	Test result		Total
	Positive (Mutant)	Negative (Normal)	
Both Sides [Father and Mother] (%)	0 (0.00)	2 (7.69)	2 (6.07)
Father Side (%)	2 (28.58)	9 (34.62)	11 (33.33)
Mother Side (%)	0 (0.00)	5 (19.23)	5 (15.15)
Non-relative (%)	5 (71.42)	10 (38.46)	15 (45.45)
Total (%)	7 (21.21)	26 (78.79)	33 (100.00)

TABLE XII
OVERVIEW OF MOTHER'S HEALTH STATUS IN PREGNANCY

Healthy statue	Pregnancy
Healthy (%)	5 (71.43)
Psychiatric condition (%)	2 (28.57)
Total (%)	7 (100.00)

2000). These results stand in disagreement with the evidence presented by (Liu, et al., 2020).

In individuals with blindness LCA, a positive test finding serves as confirmation of the genetic relationship between the parents. Table X illustrates the high percentage of first-degree cousins and the low percentage of third-degree cousins and non-consanguinity. To determine test results and parent consanguinity, we employed Chi-square analysis. The parent

TABLE XIII
LOGISTIC REGRESSION ANALYSIS OF RISK FACTORS FOR INHERITED LEBER CONGENITAL AMAUROSIS

Variable	β (Coefficient)	SE	Z	p-value	Odds ratio (OR)	95% confidence interval for OR
Number of blindness in family	2.605	1.088	2.29	0.022	13.53	1.61-113.65
Cases in relatives	−2.303	1.151	−2.00	0.045	0.10	0.010, 0.953
Constant	−2.631	1.109	−2.37	0.018	0.072	0.008, 0.633

relationship and test results do not statistically correlate, as we have discovered (p = 0.226). Furthermore, the family pedigrees are shown in Fig. 5, including the patients present with the LCA.

In blind patients with LCA, the kind of parent relationship indicated by the positive test result was that 28.57% were second-degree cousins, 57.14% were first-degree cousins, and 14.29% were second-degree nephews. An almost identical result was recorded by (Elmadina, et al., 2019).

As shown in Table XI, it was discovered that 28.58% of the father's side and 71.42% of the non-relatives had positive findings about the case relative among family patients.

The percentage of affected patients with a family history of blindness (28.58%), which is almost identical to the Israeli population's result (19%) (Lazar, et al., 2014) and in Europe and the USA (23%) (Kohl, et al., 2012), is lower than the results of a study carried out in Europe (40%) (Kitiratschky, et al., 2008), and in China (47%) (Jiang, et al., 2015).

As for the mother's health, it was discovered that 28.57% of them had a psychiatric condition and 71.43% of them were fit during their pregnancies, as stated in Table XII. This result agrees with the result that has been registered by (Elmadina, et al., 2019).

It has been described that certain familial factors are associated with hereditary LCA patients. Patients with a higher number of blind individuals in their family had significantly higher odds of testing positive, with an odds ratio (OR) of 13.53 (95% CI: 1.61–113.65, p = 0.022). This indicates that for each additional blind family member, the odds of a positive result increased more than thirteenfold. In this study, we investigated the possibility that having cases among family members and relatives influences the likelihood of receiving positive test results, as shown in Table XIII.

IV. Conclusion

This study provides the first retrospective research of the genetic and clinical analysis of Kurdish blind patients in Iraq with the *GUCY2D* mutation. The identification of three new splicing variations (c.3043+11C>T, c.3044-7G>T, c.3043+20G>C) suggests that these variants are likely associated with LCA disease in the *GUCY2D* gene of blind patients in this community. A high proportion of cases of blindness in family members or relatives supports a hereditary pattern and risk factor; they can increase the likelihood of receiving positive test results, even though there was no significant correlation between mutation status and age, gender, or consanguinity. These results broaden the range of known *GUCY2D* mutations and emphasize the significance of molecular diagnostics for early identification, genetic counseling, and upcoming gene-based treatment approaches.

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