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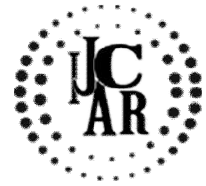

Research Article

PHF8 GENE MUTATION SCREENING FOR RS121918522 IN CLINICALLY IDENTIFIED CHILDREN WITH SIDERIUS XLID

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A B S T R A C T

Introduction: X-linked intellectual disability, Siderius type is caused by mutations in the *PHF8*. This gene provides instructions for making a protein that is found in the nucleus of cells, particularly in brain cells before and just after birth. Most *PHF8* mutations lead to an abnormally short protein that gets transportedout of the cell's nucleus. The present study screened Arg211Ter (rs121918522) nonsense mutation of *PHF8* in intellectually disabled children with cleft lip/ palate.
Materials and Methods: The selected mutation was a previously studied on patients with intellectual disability. Clinical features of the affected children was recorded. The primer sequence was designed and validated by PCR amplification successfully, followed by Sanger sequencing the mutation identification was carried out in two selected patients.
Results: In the current study, no alteration was found in two selected patients. Thus, confirmed that both children not harboring non-sense variant NM_001184897.1:c.631C>T (Arg211Ter) though they have showed exact feature of siderius type of intellectual disability.
Conclusion: The clinical representation of both children specifically described siderious XLID but selected mutation not present, the authors can be justified that another genomic mutation/s might be a cause of this clinical condition.

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INTRODUCTION

Intellectual disability (ID) is a neurodevelopmental disorder reported in 1-2% of children (Leonard and Wen, 2002). ID is defined by significant limitations in both intellectual functioning and adaptive behavior with onset before the age of 18 (American Psychiatric Association, 2013). Based on IQ level, World Health Organization categorized ID as Mild (50-69), Moderate (35-49), Severe (20-34), Profound (<20)(Division of Mental Health and Prevention of Substance Abuse World Health Organization, 1996). Genes influencing cognitive function are more often found on the X chromosome than on autosomes. X-linked intellectual disability (XLID) is a heterogeneous group and most widely studied form of intellectual disability. Occurrence of XLID is ~16% out of all cases of intellectual disability (Ropers and Hamel, 2005). XLID genes are distributed over entire X chromosome. It has been estimated there are approximately 200 genes involved; of these >100 have been identified (Llavero *et al*., 2013; Stevenson *et al*., 2012).

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Their gene products can be found in all cellular compartments are believed to involved in basic biological functions such as metabolism, DNA/RNA processing, protein synthesis and cell cycle regulation(Asensio-Juan *et al*., 2012; Kleine-Kohlbrecher *et al*., 2010).

One of the XLID proteins is PHF8 (PHD finger protein 8) which belongs to the JmjC group of proteins. The *PHF8* located on short arm (p) of X chromosome and found to cause XLID associated with Cleft Lip/Palate. The coded PHF8protein harbors two functional domains, a PHD finger and a JmjC (Jumonji-like C terminus) domain, implicating it in transcriptional regulation and chromatin remodeling (Laumonnier *et al*., 2005).

Mutations in human *PHF8* gene are associated with Siderius X-linked intellectual disability (XLID) syndrome (Siderius *et al*., 1999). *PHF8* provides guidelines for making protein that is found in nucleus of cell, particularly brain cells before and after birth. But due to mutation, it leads to formation of abnormally short protein and transported out of nucleus. Altered expression of PHF8 protein leads to intellectual disability, cleft lip and palate and other features of X-linked intellectual disability, Siderius type.

Different mutations are associated with *PHF8* causing XLID. Selected mutation (R211X) located on exon 7, which causes termination of arginine at 211 position (Laumonnier *et al*., 2005). The symptoms of Siderius XLID include, mild ID, facial dysmorphism and cleft lip/palate, implying a role for *PHF8* (JHDM1F) in midline formation and cognitive functions (Loenarz *et al*., 2010).

MATERIALS AND METHODS

Blood samples of 20 children with intellectual disability having cleft lip/palate and 20 of normal individuals were collected from Anand and Surat district of Gujarat. Clinical features and anthropometric measurements of these disease affected individuals were recorded.This study was approved by Human Research Ethics Committee, S.G. Patel Ayurveda College, New V. V. Nagar. Additionally, informed consent forms signed by parents of those children were collected before sample collection.

Genomic DNA isolation

Genomic DNA from the blood samples was isolated by using standard phenol-chloroform method, followed by quantitative and qualitative analysis using spectrophotometer and 0.8% agarose gel electrophoresis respectively.

PCR Amplification

Primers for *PHF8* were designed using Primer BLAST tool from NCBI database(Ye *et al*., 2012). Sequence for forward primer was3'-ATGCGCATTCCTACCTTGAGT-5' and reverse primer was 3'-CTCCACCCCTTCCTTGGTAGA-5'. PCR amplification was carried out in a final volume of 25 µl. Each tube contains 12.5 µl of PCR master mix, 1 µl of forward and reverse primer, 5.5 µl of nuclease free deionized water and 5 µl of genomic DNA (20ng/µl) was added to make the final volume of 25 µl. DNA samples were amplified with an initial denaturation of 95ºC for 2 min, followed by 35 cycles of 94ºC for 30 sec, 56ºC for 20 sec and 72ºC for 30 sec, and final extension at 72ºC for 5minutes.

DNA Sequencing

Amplified PCR products were sent for DNA sequencing. It was purified using Qiagen Mini elute Gel extraction kit. Before DNA sequencing, quality and quantity of PCR product were determined. Then samples were subjected to automated DNA sequencing on ABI 3730xl Genetic Analyzer. The reaction was carried out in a final volume of 20 µl. The cycling protocol consists of denaturation at 96ºC for 10 seconds, annealing at 52ºC for 5 seconds and extension at 60 ºC for 4 minutes. It was designed for 25 cycles with the thermal ramp rate of 1ºC. After cycling, the extension products were purified. Electrophoresis and data analysis were carried out on the ABI 3730xl Genetic Analyzer using the appropriate Module, Basecaller, Dyeset/Primer and Matrix files.

RESULTS

Clinical Screening

The clinical developmental screening and family history of all 20 affected children were recorded by trained pediatrician or occupational therapist. Obtained data is summarized in Table 1 and graphical representation in Figure 1. Furthermore, highly specific samples were subjected to DNA sequencing to confirm mutational change in the DNA pattern. Sample code J2 and J8 were selected for mutational screening by DNA sequencing.

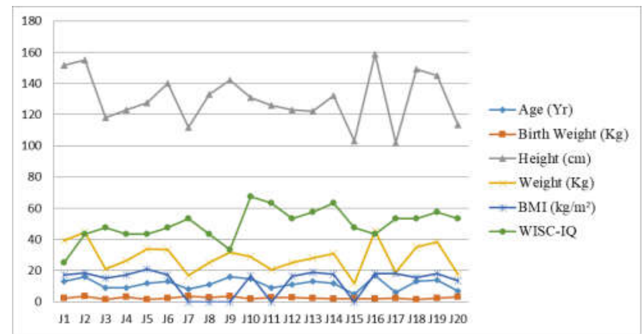

Figure 1 Graphical representation of anthropometric data of all patients

Patient J2 was male and born with 3.8 kg birth weight. He was 16-year-old with 155 cm height and 44.5 kg weight with normal BMI 18.52 kg/m^{2}at the time of examination. He has short stature and speech abnormality. He also showed flat fore head and cleft upper lip by born. Additionally, reported WISC IQ was 40-45 that showed moderate intellectual disability. The similar clinical features of other family members was found as normal.

Patient J8 was female and she was born with 2.7 kg birth weight. She was 11-year-old girl with 133 cm height and 25 kg weight at the time of examination with BMI 14.13 kg/m^{2}showed underweight. She has epileptic features andunable to produce speech sound correctly. She also has abnormal oral cavity with cleft palate and small upper lip cleft with facial dysmorphism. She has reported WISC IQ 40-45 that showed moderate intellectual disability. The similar clinical features of other family members was found as normal.

Genomic DNA analysis

DNA samples which were isolated from blood, and then subjected for qualitative and quantitative analysis. Qualitative analysis of genomic DNA carried out by 0.8% agarose gel electrophoresis and dark DNA bands were observed under UV transilluminator (Figure 2). Quantitative analysis by Nano drop® spectrophotometer revealed variable quantity of DNA in all samples between 37.2 to 219.5 ng/µl with average optical density 1.87.

Screening of *PHF8* Gene mutation for Arg211Ter

Genetic analysis was carried out to screen non-sense mutation Arg211Ter (NM_001184897.1:c.631C>T) ofPHF8 gene. Partial sequence of exon 7 of *PHF8* gene was amplified for all the samples and separated on 1.5% agarose gel using electrophoresis revealed 211 bp PCR product (Figure 3).

Furthermore, DNA sequencing of sample J2, the PCR product was carried and results revealed no nucleotide change found at NM_001184897.1:c.631C>T in the selected sample (Figure 4). Similar pattern was found for sample J8.

Table 1 Clinical screening of children with intellectual disability

Sample Code	Gender	Consanguinity	Age (Yr)	Birth Weight (Kg)	Height (cm)	Weight (Kg)	BMI (kg/m²)	WISC-IQ	ID Types
J1	Male	No	13	2.5	151.5	39.4	17.17	20-30	Severe
J2	Male	No	16	3.8	155	44.5	18.52	40-45	Moderate
J3	Male	No	9	1.65	118	21	15.08	45-50	Moderate
J4	Male	No	9	3	123	26.3	17.38	40-45	Moderate
J5	Male	No	12	1.5	127.6	34	20.88	40-45	Moderate
J6	Female	No	13	2.25	140	33.5	17.09	45-50	Moderate
J7	Female	No	8	3.75	112	17	U 13.55	50-55	Mild
J8	Female	No	11	2.7	133	25	U 14.13	40-45	Moderate
J9	Female	No	16	3.5	142	32	U 15.87	30-35	Severe
J10	Female	No	15	2	131	29	16.9	65-70	Mild
J11	Male	No	9	2.75	126	20.7	U 13.04	60-65	Mild
J12	Female	No	11	2.7	123	25	16.52	50-55	Mild
J13	Male	Yes	13	2.2	122	28	18.81	55-60	Mild
J14	Male	No	12	2	132	31	17.79	60-65	Mild
J15	Male	No	5	2	103	12	U 11.31	45-50	Moderate
J16	Male	No	17	1.8	158.5	45.5	18.11	40-45	Moderate
J17	Male	No	6	2.25	102	19	18.26	50-55	Mild
J18	Male	No	13	1.5	149	35	15.77	50-55	Mild
J19	Male	No	14	2.25	145	38.5	18.31	55-60	Mild
J20	Female	No	7	3.3	113.5	17.5	13.58	50-55	Mild

(IQ= Intelligence Quotient; Yr=Year; Kg=Kilograms; cm=Centimeter; m=meter)

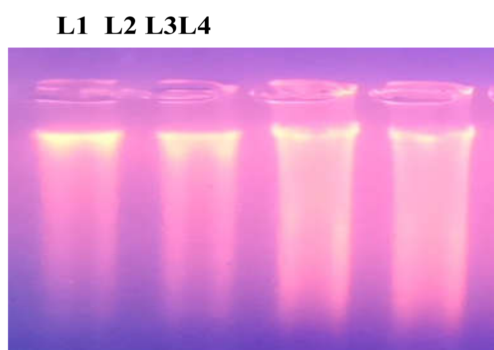

Figure 2: Genomic DNA separation using 0.8% agarose gel electrophoresis. Lane 1 to Lane 4: Samples 1 to 4

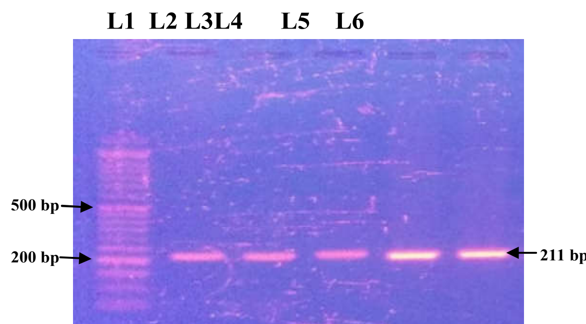

Figure 3: *PHF8* gene PCR products separation on 1.5%agarose gel electrophoresis. Lane 1: 50 bp low range ladder; Lane 2 to Lane 6: Samples J1 to J5

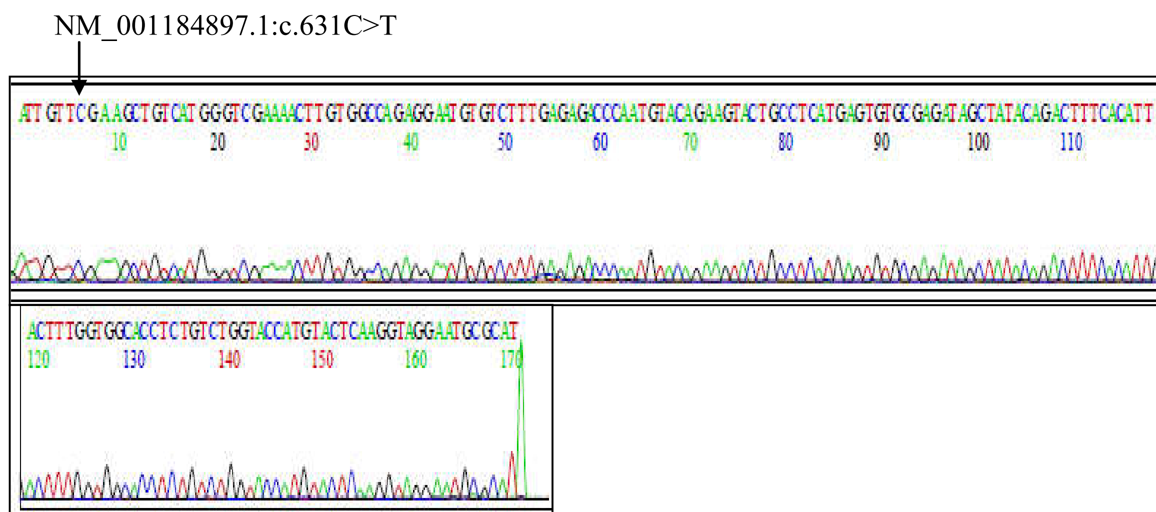

Figure 4: DNA sequencing pattern of Sample (J2) for *PHF8* gene c.631 C>T transition which is exactly at 7th position in sequencing product (Highlighted with arrow)

DISCUSSION

PHF8 gene is located on X chromosome at position Xp11.21. Mutations on this gene can cause X-linked intellectual disability with cleft lip/ palate. The genomic structure of *PHF8* comprises of 22 exons that encodes for a protein of 1024 amino acids and contains PHD zinc finger domain and JmjC domain (Kikuno *et al*., 1999).

In the present study, attempts were made to identify nonsense mutation (R211X) that results in truncation of PHF8 protein and loss of JmjC domain. The mutation resides on exon 7 and terminates arginine at 211 position (R211X). This*PHF8* nonsense mutation (c.631C>T) is linked with mild ID, mild dysmorphic features, and either unilateral or bilateral cleft lip and cleft palate.

Siderius was the first to provide evidence of the involvement of *PHF8* with intellectual disability. Later, various studies provide evidence for the association of *PHF8* with intellectual disability (Siderius *et al*., 1999). Four truncating mutations in *PHF8* have been found to cause siderius type X-linked intellectual disability with cleft lip/palate. (Laumonnier *et al*., 2005) identified a 631 C>T transition in *PHF8*, resulting in an arginine termination at 211 position (R211X). This mutation was described in 25 years old male from a family with Siderius X-linked mental retardation. Affected male has 3 deceased maternal uncles having mental retardation and/or cleft lip and palate.

Cleft lip and cleft palate are among the most widely recognized congenital malformations. Apart from *PHF8*, many genes have been identified to be associated with the formation of cleft lip/ palate like *IRF6, AXIN2, BMP4,* etc., (Rafighdoost *et al*., 2017).

Current study undertaken to screen the non-sense mutation (Arg211Ter) in the Gujarat population. However, it is not observed in selected patients who pre-diagnosed with intellectual disability. At this level, authors are recommend that study should be extended with more number of specific samples and may be with all possible mutations to be screened.

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Conflict of interest: Authorsdeclare no conflict of interest.
Note: Jasmin Patel and YashvantKhimsuriya are equally contributed in this study.

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