Kleefstra Syndrome: The First Case Report From Iran

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Abstract- Kleefstra Syndrome is characterized by severe mental retardation, brachycephaly, microcephaly, epileptic seizures, distinct facial features, and infantile weak muscle tone and heart defects. Deletion of *EHMT1* is the main player in 75% of cases. Because of blurriness in genotype-phenotype correlation through clinical and molecular features of both 9q34.3 microdeletion patients and those with an intragenic *EHMT1* mutation in Kleefstra Syndrome, genetic characterization of patients with clinical symptoms of such spectrum is desirable. We report the first Kleefstra Syndrome patient in Iran characterized through genetic approaches. Our report could improve KS diagnosis in Iran and prepare PND and PGs options for involved families.

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Keywords: Kleefstra syndrome; Iran; *EHMT1*; Deletion

Introduction

There are reports of a mental retardation syndrome which is associated with submicroscopic subtelomeric deletions of chromosome 9q (1-4). The disorder was previously known as 9q subtelomeric deletion syndrome (9qSTDS) (5). Severe mental retardation without speech development, brachycephaly and microcephaly, epileptic seizures, distinct facial features, infantile weak muscle tone and heart defects are core syndromic features of these patients (2,6). Thereafter, such patients were characterized with 9q deletions and haploinsufficiency for the EHMT1 (Euchromatic Histone MethylTransferase 1) gene is introduced as a driver for pathogenesis and phenotypic main features (7).

Kleefstra syndrome (KS) (OMIM #610253) is an Autosomal Dominant (AD) or Isolated Cases(IC) syndrome (www.omim.org), characterized by symptoms mentioned above (8,9). Haploinsufficiency of the EHMT1 gene (HGNC: 26540) due to microdeletions of 9q34.3 fulfills its etiology in approximately 75% of affected individuals. For the remaining 25% of cases, there are some explanations about intragenic EHMT1 mutations (7,10,11). Amongst disorders with manifestations of intellectual disability and childhood hypotonia and a characteristic facial appearance, KS is one of the most frequent syndromes of subtelomeric deletion (12).

There is no clear observation about the genotype-phenotype correlation through clinical and molecular features of both 9q34.3 microdeletion patients and those with an intragenic EHMT1 mutation in Kleefstra Syndrome (8). Thus genetic characterization of patients with clinical symptoms of such spectrum is necessary. Here, we report the first Kleefstra Syndrome in Iran characterized through genetic approaches.

Case Report

A 3-year-old female with chief compliments of hypotonia, hearing impairment, and developmental delay was referred to the Genetic Counseling. This patient was born through the second pregnancy of a non-consanguineous marriage, while the first pregnancy was spontaneously aborted (Figure 1).

Clinical findings

The patient's birth weight was 2350 g, length 40 cm, and head circumference 30.8 cm. She experienced seizure in her early days and third year of life. The pregnancy and delivery were uneventful for the patient. The proband represents the acronym CHOMS (craniofacial features, hypotonia, obesity, microcephaly and speech delay) in addition to mental retardation, behavioral and psychiatric disorders and inability to walk.

Other manifestations of the patient include

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conotruncal heart defects, unilateral dislocation of the kidneys, recurrent infections and umbilical hernia. The proband had myopia, normal brain CT, flat face, midface hypoplasia, coarse facies, synophrys, upslanting palpebral fissures and anteverted nostrils.

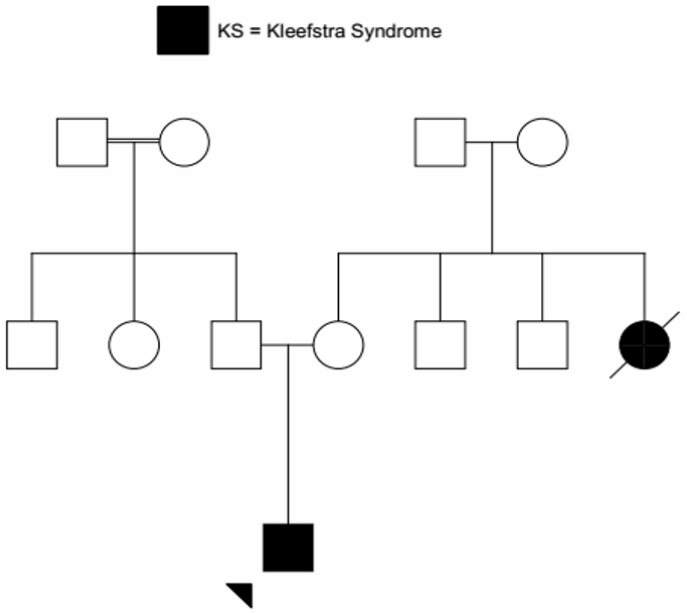

Figure 1. The pedigree of the family, proband is indicated by the arrow.

Genetic characterization

Patient's Informed consent was obtained from the parents and blood sampling performed for the affected person to be used as a source of lymphocytes and cell culturing. Cells were cultured, and karyotyped practiced in standard 440-460 bph resolution.

Blood samples were collected from the antecubital veins of the patient and his parents. Subsequently, genomic DNA was obtained from peripheral blood lymphocytes according to the chelex method (13). Subsequently, submicroscopic subtelomeric deletions were detected using routine subtelomeric Multiplex Ligation-dependent Probe Amplification (MLPA) kit for all family members (SALSA MLPA probemix P036-E1 HUMAN TELOMERE-3, MRC-Holland). Genscan analysis was performed on ABI 3130 genetic analyzer (Applied Biosystems, USA).

RNA isolation was carried out in the patient's blood using the PAXgene™ Blood RNA kit (PreAnalytiX, Switzerland). Subsequently cDNA was constructed using a standard method. Moreover, using specific primers for exon 3 of EHMT1 gene, qPCR was applied to evaluate gene dosage at the transcription level. The primers were designed by the Primer3 algorithm (http://bioinfo.ut.ee/primer3-0.4.0/), and the expected PCR product was 105 bp (Table 1).

Table 1. Primers applied to evaluation of *EHMT1* gene dosage

Primer ID	Primer sequence	Product size (bp)
EHMTF	5'-GGATGGCACCAACACACTAAC-3'	105
EHMTR	5'-GTCTGCACAAAGTCGTCGG-3'	

Genetics findings

Patient's karyotype was 46, XX compatible with apparently a normal female, from a cytogenetic point of view. Our genetic studies with MLPA kit revealed microdeletion in the 9q34.3 region in the patient in the heterozygous state but not in her parents (Figure 2). To confirm MLPA findings, qPCR was used, and the results show more confidence about the haploinsufficiency and LOH in *EHMT1* gene (Figure 3).

A

GNB2L1 probe 3319-L02737	05q	338	05-180.6 GNB2L1	10	0.91
IRF4 probe 1724-L02048	06p	165	06-000.3 IRF4	11	1.09
PSMB1 probe 1746-L01304	06q	346	06-170.8 PSMB1	12	0.97
CENTA1 probe 2275-L02049	07p	172	07-000.7 CENTA1	13	1.1
VIPR2 probe 1747-L01303	07q	354	07-158.4 VIPR2	14	0.99
FBXO25 probe 2397-L01845	08p	179	08-000.4 FBXO25	15	1.09
KIAA0150 probe 1748-L01302	08q	362	08-144.6 KIAA0150	16	0.98
DMRT1 probe 1727-L02050	09p	186	09-000.9 DMRT1	17	1.15
EHMT1 probe 8205-L08170	09q	370	09-139.8 EHMT1	18	0.98
KIAA0934 probe 2277-L01768	10p	194	10-000.5 KIAA0934	19	1.16
PAO probe 9142-L09953	10q	378	10-135.1 PAO	20	0.91
RIC-8 probe 3315-L02733	11p	202	11-000.2 RIC-8	21	1.13
KIAA0056 probe 1751-L01299	11q	386	11-133.6 KIAA0056	22	0.98
SLC6A12 probe 2276-L01767	12p	208	12-000.2 SLC6A12	23	1.12

B

CENTA1 probe 2275-L02049	07p	172	07-000.7 CENTA1	13	1.12
VIPR2 probe 1747-L01303	07q	354	07-158.4 VIPR2	14	0.99
FBXO25 probe 2397-L01845	08p	179	08-000.4 FBXO25	15	1.14
KIAA0150 probe 1748-L01302	08q	362	08-144.6 KIAA0150	16	0.99
DMRT1 probe 1727-L02050	09p	186	09-000.9 DMRT1	17	1.07
EHMT1 probe 8205-L08170	09q	370	09-139.8 EHMT1	18	1
KIAA0934 probe 2277-L01768	10p	194	10-000.5 KIAA0934	19	1.15
PAO probe 9142-L09953	10q	378	10-135.1 PAO	20	0.92
RIC-8 probe 3315-L02733	11p	202	11-000.2 RIC-8	21	1.13
KIAA0056 probe 1751-L01299	11q	386	11-133.6 KIAA0056	22	0.99
SLC6A12 probe 2276-L01767	12p	208	12-000.2 SLC6A12	23	1.09
ZNF10 probe 2687-L02154	12q	394	12-132.3 ZNF10	24	0.95
PSPC1 probe 2399-L01847	13p	218	13-019.2 PSPC1	25	1.09

C

31	PSMB1 probe 1746-L01304	06q	346	06-170.8 PSMB1	12	Normal (1.02)
32	CENTA1 probe 2275-L02049	07p	172	07-000.7 CENTA1	13	Normal (1.12)
33	VIPR2 probe 1747-L01303	07q	354	07-158.4 VIPR2	14	Normal (0.9)
34	FBXO25 probe 2397-L01845	08p	179	08-000.4 FBXO25	15	Normal (1.15)
35	KIAA0150 probe 1748-L01302	08q	362	08-144.6 KIAA0150	16	Normal (1.09)
36	DMRT1 probe 1727-L02050	09p	186	09-000.9 DMRT1	17	Normal (0.91)
37	EHMT1 probe 8205-L08170	09q	370	09-139.8 EHMT1	18	LOH (0.54)
38	KIAA0934 probe 2277-L01768	10p	194	10-000.5 KIAA0934	19	Normal (1.17)
39	PAO probe 9142-L09953	10q	378	10-135.1 PAO	20	Normal (0.99)
40	RIC-8 probe 3315-L02733	11p	202	11-000.2 RIC-8	21	Normal (1.07)
41	KIAA0056 probe 1751-L01299	11q	386	11-133.6 KIAA0056	22	Normal (0.98)
42	SLC6A12 probe 2276-L01767	12p	208	12-000.2 SLC6A12	23	Normal (0.95)

Figure 2. Results of MLPA test for the family members. Highlighted rows are referring to *EHMT1* region. A) MLPA results for the patient's father, the green row is addressed to 9q34 region. B) MLPA results for the patient's mother, the green row is addressed to 9q34 region. **C)** MLPA results for the patient, the pink row is addressed to 9q34 region and especially *EHMT1* gene.

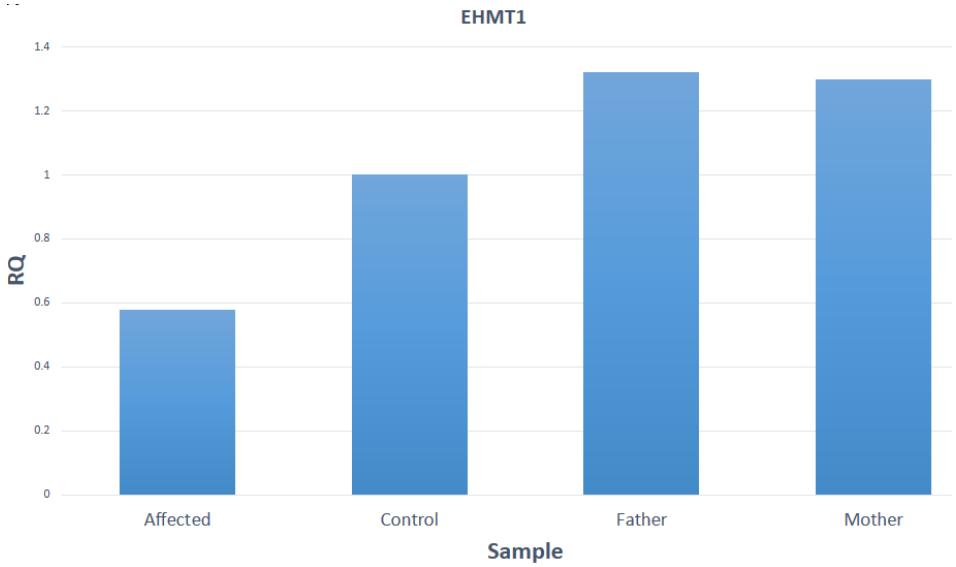

Figure 3. Confirmation of LOH in the patient by qPCR Quantification of *EHMT1* gene transcripts in family members using primers described before. *EHMT1* gene dosage is equal as behalf of her parent's levels.

Discussion

Here we reported the first Kleefstra syndrome in Iran and described the chief culprit in the presentation of mental retardation and behavioral phenotype in one Iranian female patient suspected to be suffering from KS. Our findings strengthen the hypothesis that EHMT1 is a driver for disturbances in neurodevelopmental processes in the described proband.

Kleefstra Syndrome has been well documented (9), described in more details (7), and some reports available regarding KS descriptions (14-17). Maternal somatic mosaicism for interstitial 9q34.3 microdeletions is registered in familial cases (18) while donor splice site mutation in EHMT1 (19) and ring chromosome 9 (20,21) are the other scenarios responsible for KS. The majority of studies persist on the loss of functions or disruption of EHMT1 gene as the cause of Kleefstra syndrome (11,22,23). The encoded protein is a histone methyltransferase which methylates the histone H3 in Lys-9 and tags it for transcriptional repression and silencing of MYC- and E2F-responsive genes and therefore could play a role in the G0/G1 cell cycle transition (http://www.ncbi.nlm.nih.gov/gene/79813) (24).

These findings suggest the importance of EHMT1 in epigenetic regulation and undesirable alterations fulfill many disease conditions such as cognitive diseases (25). Epimarkers may be employed for diagnosis and management of diseases like cancer (26). Accordingly, EHMT1 deletion and its haploinsufficiency might disturb some cell signaling pathways, epigenetic instability and features of 9q deletion syndrome, also known as Kleefstra Syndrome, are common consequences.

The EHMT1 gene contains 28 exons, and its initiation occurs in the ATG exon 2 (11). The breakpoints of EHMT1 are characterized in intron 9 of the gene elsewhere (10). In addition, the existence of Alu-type repetitive DNA elements -which are prone to non-homologous recombination- in EHMT1 is a possible explanation for breakpoints and subsequent duplication/deletions in EHMT1 gene in a patient with KS (8,23,27). These changes impair the protein function and representation of Kleefstra syndrome symptoms eventually.

As mentioned above, 9q34 microdeletion in the patient suggests that haploinsufficiency of EHMT1 gene is a key player in KS. The present case is the first report of KS from Iran and improving the diagnosis of such patients with hypotonia, hearing impairment and developmental delay in Iran. The present report is advantageous for the management of families who have a KS case with no history in their family. With regards to recommendations in terms of follow-up and diagnosis of KS patients (8), we studied the cardiac health of the patient due to the potential occurrence of cardiac arrhythmias. Moreover, this family would be considered as a candidate for PND or even PGS approach for their next pregnancy.

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