Multiple Mongolian spots in an individual with Kleefstra syndrome caused by a novel nonsense euchromatin histone methyltransferase 1 variant

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List of key features

Mongolian spots
Microcephaly
Tented upper lip
Highly arched eyebrows
Short chin
Developmental retardation

Introduction

Kleefstra syndrome (KS) (OMIM #610253) is caused by a heterozygous microdeletion of chromosome 9q34.3 region or a pathogenic variation in the euchromatin histone methyltransferase 1 (*EHMT1*) gene (OMIM #607001). The *EHMT1* gene located at chromosome 9q34.3 region, which contains a total of 28 exons, and the initiation of ATG occurs in exon 2 (Kleefstra *et al.*, 2006). Kleefstra *et al.* (2009) reported 16 patients with 9q subtelomeric deletion syndrome and six patients with an intragenic *EHMT1* mutation. All patients presented with the core phenotype of the deletion syndrome, and there were no phenotype-genotype correlations between size of the deletions or type of mutations and severity of clinical features, so they concluded that the haploinsufficiency of *EHMT1* gene was the basis for the phenotypic features of the deletion syndrome. There are more than 100 cases of KS reported so far, of which about 75% are caused by a heterozygous microdeletion in the 9q34.3 region containing the *EHMT1* gene, and 25% are caused by loss-of-function, intragenic *EHMT1* variants (Atik *et al.*, 2015). We report an individual with KS1 and multiple Mongolian spots (also known as congenital dermal melanocytosis) caused by a novel pathogenic nonsense variant NM_024757:exon 9:c.1468C >T(p.R490*) in the *EHMT1* gene.

Case report

An 11-month-old girl born at 37 weeks of gestational age to a 27-year-old G1P0 → 1 mother was referred to our clinics because of motor developmental delay (DD). Pregnancy and family history were noncontributory. She achieved head control by 5 months, and could sit up independently by 10 months but was not able to crawl or say simple words such as 'Mom' or 'Dad'. Growth parameters were as follows: weight 7.0 kg (<3rd centile), length 67.5 cm (<3rd centile), head circumference 41.0 cm (<3rd centile). Indifferent reaction, not easy to be amused, unable to actively look at others, no response to name. The back, buttocks, and the outside of the right thigh were diffusely distributed with bluish-brown Mongolian spots (Fig. 1). The head circumference was small (Microcephaly) and the front and back diameter of the head was short. Special facial features: highly arched

Fig. 1

The bluish-brown Mongolian spots are diffusely distributed on the back.

eyebrows, widely spaced eyes, slight antimongoloid slant palpebral fissures, low-set ears, short nose, tented upper lip and short chin (Fig. 2). The muscle tone of the limbs was low, and the muscle strength was normal. General auxiliary examinations included blood routine examination, tandem mass spectrometry analysis of amino acids/acylcarnitines of dried blood filter paper, urine organic acid analysis and head MRI showed no abnormalities.

Genetic testing: Through next-generation sequencing technology and bioinformatics analysis, a heterozygous nonsense variant NM_024757:exon 9:c.1468C >T (p.R490*) was detected in the *EHMT1* gene. This variant is novel, can cause the 490th codon of the *EHMT1* gene to change from the original codon encoding arginine to a stop codon, leading to protein truncation. Sanger sequencing confirmed the existence of this novel variant (Fig. 3). Her parents did not carry this variant. ACMG-2015 (American College of Medical Genetics) criteria were considered for pathogenicity (PVS1, PS2 and PM2) (Richards *et al.*, 2015).

Discussion

KS follows an autosomal dominant inheritance pattern, but so far almost all reported cases have occurred *de novo*. The core clinical phenotypes of KS are characterized with DD/intellectual disability, childhood hypotonia, and a recognizable pattern of facial features(brachycephaly, microcephaly, arched eyebrows, flat face, hypertelorism, short nose, anteverted nostrils and carp-shaped mouth). This can be caused by either 9q34.3 subtelomeric deletions or loss-of-function variants in the *EHMT1* gene (Hadzsiev *et al.*, 2016; Okur *et al.*, 2018). Beside the core phenotypes, the spectrum of KS-related clinical morbidity includes behavioral problems (65–70%), autism spectrum disorder (30–75%), epilepsy (20–50%), brain

Fig. 2

The special facial features: highly arched eyebrows, widely spaced eyes, slight antimongoloid slant palpebral fissures, low-set ears, short nose, tented upper lip and short chin.

imaging anomalies (50–60%), cardiovascular anomalies (40–45%), male genital anomalies (45–50%), renal issues (15–30%), hypoacusia (20–30%) and hernia (15–20%) (Ciaccio *et al.*, 2018). The *EHMT1* gene encodes lysine methyltransferase, which can methylate histones. The lack of lysine methyltransferase impairs the proper control of the activity of certain genes in many body organs and tissues, leading to the developmental abnormalities and functional characteristics of KS (Yamada *et al.*, 2018). So far, most of the KS cases reported in the literature involve submicroscopic deletion of 9q34.3, but with the development of genetic testing technology, we can detect more intragenic pathogenic variant of *EHMT1* gene responsible for KS, both result in haploinsufficiency of the *EHMT1* gene.

Neural crest cells (NCCs) are pluripotent stem cells that can differentiate into various types of cells such as chondrocytes, neurons and melanocytes. Disorders caused by NCCs abnormalities are collectively known as neurocristopathies, which are associated with developmental abnormalities in various parts of the body, including the head, face, skin and nerve tissue. Studies have shown that the lysine methyltransferase encoded by the *EHMT1* gene can methylate histones and can regulate the development, migration and differentiation of NCCs at the epigenetic regulation. Pathogenic variants in the *EHMT1* gene may affect these processes to some extent (Bronner, 2012; Hu *et al.*, 2014). It has been hypothesized that Mongolian spots are accumulation of melanocytes in the dermis caused by disrupted migration of melanocytes from the neural crest to the epidermis in human embryos (Franceschini *et al.*, 2015). The widespread abnormal distribution of Mongolian spots may be associated with some inborn errors of metabolism, the more common of which are mucopolysaccharidosis type I (Hurler syndrome, OMIM #607014) and GM1 gangliosidosis (OMIM #230500), and the others are mucopolysaccharidosis type II (Hunter syndrome, OMIM #607014), Niemann-Pick disease type A (OMIM #257200), and alpha-mannosidosis (OMIM #248500), all of which belong to lysosomal storage diseases. In this case, no pathogenic variant in corresponding genes related to these diseases was detected by next-generation sequencing technology and bioinformatics analysis, and no hepatosplenomegaly, Hurler-like coarse facial features, skeletal deformities, hearing impairment, visual impairment, epilepsy, tremor, ataxia, etc., so the diagnosis of these diseases are not considered.

The craniofacial features and autistic traits of this child with KS were typical, and the multiple Mongolian spots should be paid more attention. There has been case report of congenital hyperaccumulation of melanocytes in the local deep dermis in the child with KS (Ciaccio *et al.*, 2018), but the affected skin area was not as so extensive as this case. In this paper, we report a KS caused by a novel pathogenic variant NM_024757:exon 9:c.1468C >T (p.R490*) in the *EHMT1* gene. KS is a rare disease

Fig. 3
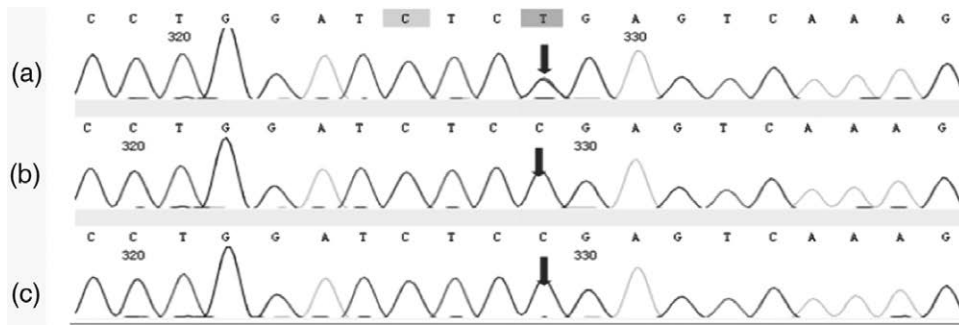
The sanger sequencing result. (a) The variant site: *EHMT1* c.1468C>T(p.R490*) was detected on the child. (b and c) The above-mentioned variant site was not found in the parents of the child.

with a very low incidence. It is of great significance to report a KS patient with a rare genotype and phenotype, and it can deepen the understanding of the disease by clinicians.

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