

Hypomelanosis of Ito and *De novo* Interstitial 15q11.2q13.3 Triplication in Bulgarian Family

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Abstract

Here we report a case of Hypomelanosis of Ito (HI) and *de novo* interstitial 15q11.2q13.3 triplication. The HI is typically associated with various chromosomal anomalies, that is why a karyotyping was selected as a first choice in genetic approach. The obtained result showed a pathological karyotype: additional material on the long arm of chromosome 15, (15)(q11q13) bands. To confirm this extra material on the long arm of chromosome 15, our subsequent step was Multiplex Ligation-dependent Probe Amplification analysis (MLPA), which detected abnormal copy numbers, corresponding to duplication, along the targeted region 15q11.2 (genes *SNRPN* and *UBE3A*). In order to further clarify the duplication boundaries the aCGH was performed, which revealed arr[GRCh37] 15q11.2q13.3(22,558,697-30,366,124)x4, 15q13.2q13.3(30,652,489-32,462,701)x3. As a final step we conducted segregation analysis within the family by QF-PCR of polymorphic loci to identify the origin of the chromosomal rearrangement, which turned out to be maternally inherited. Based on the results from aCGH, in our opinion the reported here chromosomal rearrangement is an interstitial triplication of chromosome 15, resulting in very rare case of tetrasomy within the targeted region of chromosome 15q. Review of the literature showed that, here we report a first genetically proven case of Hypomelanosis of Ito caused by a *de novo* interstitial 15q11.2q13.3 triplication.

Keywords: Hypomelanosis of Ito; Interstitial 15q11.2q13.3 triplication; *De novo*

Introduction

Hypomelanosis of Ito (HI) formerly called incontinentia pigmenti achromians is a rare neurocutaneous syndrome. The incidence and prevalence of HI was estimated to be between 1 in 7540 births and 1 in 82,000 in different studies [1]. McKusick's catalogue of inherited diseases classified IH as an autosomal dominant disorder, although evidence for this mode of inheritance, or for any genetic etiology, is inconclusive [2,3]. The clinical manifestations of HI are variable, but the most remarkable clinical markers are distinct patterns of skin-hypopigmentation along the lines of Blaschko. It is now known that HI is a systemic disease with other manifestations arising predominately within the central nervous system and the musculoskeletal system [4-7] in combination with craniofacial, cardiac, renal, and gonadal abnormalities it occurs primarily in females and on occasion in males. The female:male ratio is 20:1 [8,9]. Many cases are associated with genetic mosaicism and sporadic gene mutations. However, in many patients the condition arises from genetic irregularities. Specific chromosomal abnormalities have been identified in certain cases of hypomelanosis of Ito including ones affecting chromosome 9q33, chromosome 15q11-q13, chromosome Xp11 and Xp21.2. Chromosomal abnormalities have been identified in approximately 60 percent of cases of hypomelanosis of Ito. The specific gene(s) involved in the development of hypomelanosis of Ito have not been identified so far.

Clinical data

Here we report the clinical phenotype of 3 years old girl with skin lesions (characteristic linear unilateral hypopigmentations following the lines of Blaschko), delayed motor and neuropsychological development, normal Computed Tomography (CT) of the brain and lack of family history. From the anamnestic data the child was born from pathological pregnancy, during which the mother was treated with cephalosporins. The child was born by a scheduled c-section. The newborn had normal

weight and APGAR (Appearance, Pulse, Grimace, Activity, and Respiration) score, with transient tachypnea, maternal-to-fetal infection but with a normal transfontonele ultrasonography in the neonatal period. After the delivery, linear hypopigmentations in the right side of the body in the area of the thigh, scapula, arm and back and linear hypopigmentation in the area of the sternum have been noticed. A delay in the early motor and speech development has been observed with a gradual improvement over time-head control was achieved after 6 months of age, unsteady sitting-after 11 months of age, unsteady walking with a wide-based gait-at the age of 1 year 10 month. The child was treated with Nootropil for a short period of time. An EMG and brain CT showed normal result. CPK, ASAT, and ALAT were within the normal range.

At the time of our study, the girl shows an expressive language disorder-she uses only 10 to 15 words and could mimic the sounds that animals made. Head circumference was 46 cm at the lower limit for this age and sex. She was able to perform easy tasks and did not play with other children. The gait was unstable and the purposeful movements were with mild dysmetria. The child demonstrated mild to moderate mental retardation (IQ 50). No seizures were reported. There is no family history for skin lesions and mental retardation.

The diagnosis hypomelanosis of Ito was discussed because of

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the combination of skin lesions (characteristic linear unilateral hypopigmentations following the lines of Blaschko) (Figure 1), delayed motor and neuropsychological development, normal CT of the brain and lack of family history. Cytogenetic and genetic testing for this diagnosis was recommended.

Methods

Editorial policies and ethical considerations

The study was approved by the Ethics Committee of Sofia Medical University. Written informed consent was obtained from patient's father prior to genetic testing.

Cytogenetic and molecular genetic methods

Karyotyping was performed on cultivated peripheral blood lymphocytes using Lymphogrow medium following a standard protocol with Colcemid (at a final concentration of 0.1 µg/mL) and fixative (freshly prepared 3 parts Methanol: 1part Acetic acid)[10].

Genomic DNA was extracted from blood leukocytes by standard salting out procedure [11].

Multiplex Ligation-dependent Probe Amplification-MLPA® is the gold standard for DNA copy number quantification and is used to study both hereditary disorders and tumours. We used MLPA analysis (SALSA MLPA P374 Microdeletion syndrome) to detect abnormal copy numbers. The MLPA reaction can be divided in five major steps:

1. DNA denaturation and hybridization of MLPA probes
2. Ligation reaction

3. PCR reaction
4. Separation of amplification products by electrophoresis
5. Data analysis

Array comparative genomic hybridization is a molecular cytogenetic method for analyzing submicroscopic chromosome imbalances. We used Agilent SurePrint G3 4x180k slides, and Agilent Complete Labelling kit, following the manufacturer's protocol.

Quantitative Fluorescence PCR (QF-PCR) analysis includes amplification, detection and analysis of chromosome-specific DNA sequences known as genetic markers or Small Tandem Repeats (STRs). The genetic markers/STRs may vary in length between individual chromosomes and subjects, depending on the number of repeated STRs. The relative copy number of each allele is assessed by calculating the ratio of the peak areas of both alleles in the heterozygous profile. The ratio 1:1 is considered to be normal.

For determination of the parental origin of the detected duplication, we used the following STR markers along the (15)(q11q13) chromosomal region: D15S1513, D12S657, D15S643, D15S659, D15S128 and D15S822.

Results and Discussions

The HI is typically associated with various chromosomal anomalies, so karyotyping was selected as a first choice in molecular genetic approach. The obtained result showed a pathological karyotype: additional material on the long arm of chromosome 15, (15)(q11q13) bands (Figure 2).

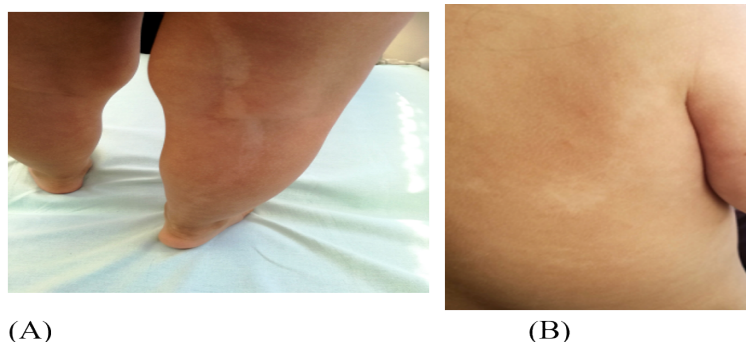


Figure 1: Characteristic linear unilateral hypopigmentations following the lines of Blaschko (A) on the right leg; (B) on the back.

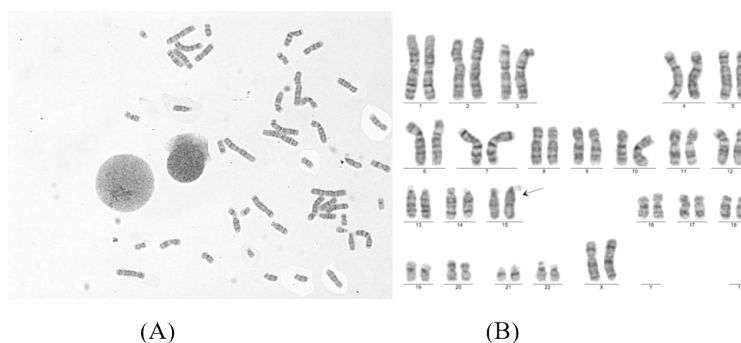


Figure 2: (A) Microscope photo of mitosis of dup (15)(q11.2q13.3); (B) Karyotype of dup (15)(q11.2q13.3) indicated with arrow.

To confirm this extra material on the long arm of chromosome 15, our subsequent step was MLPA analysis along the targeted 15q11-15q13 region. The obtained copy number profile showed duplication on the long arm of chromosome 15q11.2 (genes *SNRPN* and *UBE3A*) (data available upon request). In order to further clarify the duplication boundaries the Microarray-based Comparative Genomic Hybridization (aCGH) was performed (Figure 3).

The duplication borders were specified as: (15)(q11.2q13.3)(22,558,697-30,366,124)x4, 15q13.2q13.3(30,652,489-32,462,701)x3.

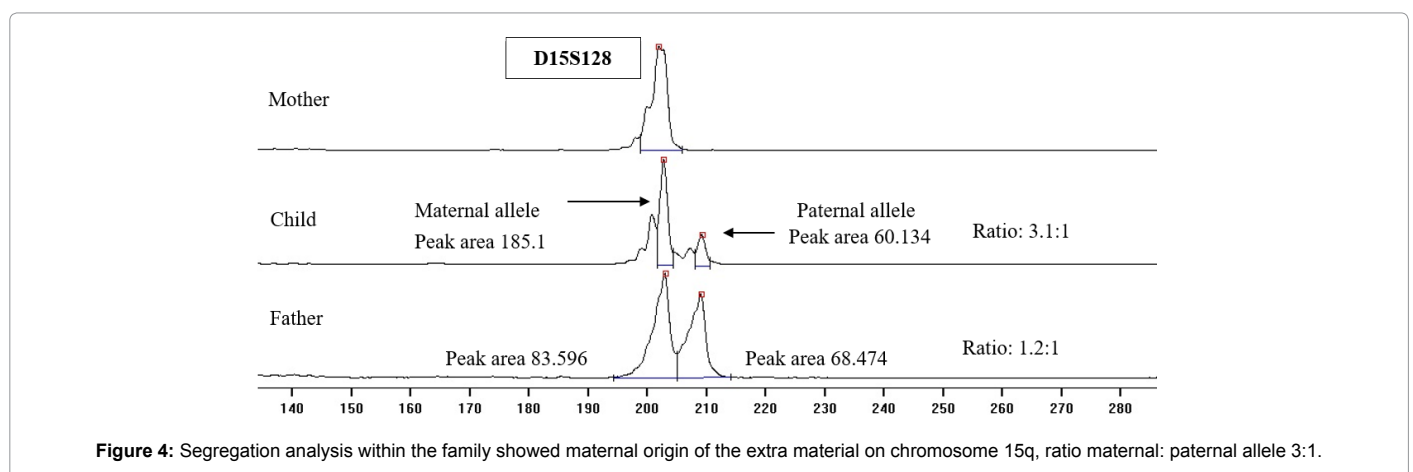
As a final step we conducted segregation analysis within the family by QF-PCR of polymorphic loci to identify the origin of the chromosomal rearrangement. The following markers were chosen for the analysis: D15S1513, D12S657, D15S643, D15S659, D15S128 and D15S822. The first four turned out to be non-informative, while the last two showed maternal origin of the extra material on chromosome 15q (Figure 4).

The proximal long arm of human chromosome 15 is prone to cytogenetic abnormalities, and gives rise to deletions, duplications, triplications, translocations, and inversions, as well as inv dup (15)

supernumerary markers (i.e. inverted duplications of chromosome 15) [12]. Large dup (15) chromosomes are exclusively from maternal origin [13,14].

In our opinion, the results from aCGH, showed that the reported here chromosomal rearrangement is interstitial triplications of 15q11.2q13.3 region, resulting in tetrasomy (Figure 4, allele ratio 3:1). Tetrasomy along the chromosome 15 (15q11.2– 15q13.3 region) has to be very rare. Review of the literature showed that there are only few cases of Hypomelanosis of Ito reported, but they are associated with other molecular pathologies, which do not include interstitial triplications of 15q11–q13 region [15-24]. Triplications of about 6.8Mb, located between the BP2 and BP4 Low Copy Repeats (LCRs) of chromosome 15, result in tetrasomy of the involved region [25]. The proximal long arm of chromosome 15 contains a cluster of Low Copy Repeats (LCRs), located at breakpoints BP1–BP5. These repeated motifs mediate various deletions and duplications *via* non-allelic homologous recombination [26].

The detected duplication in the present study is located within BP2-BP3 the Prader-Willi/Angelman syndromes critical region, both of them caused by deletions or uniparental disomy within the targeted region.



The literature review showed that the cases with Hypomelanosis of Ito and hypopigmentation of the skin are exclusively associated with deletions in this region (15)(q11.2q13.3) [27-29].

Based on the results from aCGH, in our opinion the reported here chromosomal rearrangement is interstitial triplication of chromosome 15, resulting in very rare case of tetrasomy within the targeted region of chromosome 15q. Review of the literature showed that, here we report a first genetically proven case of Hypomelanosis of Ito caused by a *de novo* interstitial 15q11.2q13.3 triplication.

Conclusion

Given the range of genetic, clinical features, it seems that HI is not an isolated entity but a clinical syndrome encompassing multiple cutaneous disorders as demonstrated by our patient. Review of the literature showed that, here we report a first genetically proven case of Hypomelanosis of Ito caused by a *de novo* interstitial 15q11.2q13.3 triplication.

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