Array CGH - A Powerful Tool in Molecular Diagnostic of Pathogenic Microdeletions - Williams-Beuren Syndrome - A Case Report

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ABSTRACT: Williams-Beuren syndrome (WBS) (OMIM 194050) is caused by interstitial deletions or duplications of the 7q11.23 chromosomal region and characterised through a complex phenotype. We described a case diagnosed clinically and genetically confirmed through aCGH. Genetic assessment identified three microdeletions with a total size of 1.35 Mb located at 7q11.23. The deleted regions encompasses more than 30 genes including several protein coding genes such as ELN, LIMK1, FZDS, WBSCR22, WBSCR27, WBSCR28, STX1A, CLDN3, CLDN4, LAT2, ABHD11 or EIF4H.

KEYWORDS: array CGH, 7q11.23 microdeletion, Williams-Beuren syndrome, global developmental delay

Introduction

Submicroscopic deletions and duplications are involved in the pathogenesis of multiple clinical conditions characterised through global developmental delay (GDD), intellectual deficiency (ID), multiple congenital anomalies and dysmorphic features. Resolution of conventional cytogenetic methods does not allow detection of this type of chromosomal rearrangements, but development of array comparative genomic hybridization (aCGH) techniques has facilitated the identification and molecular characterization of submicroscopic and subtelomeric deletions and duplications. Furthermore, it allows identification of genomic breakpoints and genes content of the chromosomal regions affected by deletions or duplications [1,2].

Since 2010, genetic testing through aCGH for detection of microdeletions or microduplications, also known as copy number variants (CNVs), is recommended by ISCA (International Standards for Cytogenomics Arrays) consortium to be used as first-line test in the assessment of individuals with GDD, ID, multiple congenital anomalies and dysmorphic features [3].

Williams-Beuren syndrome (WBS) (OMIM 194050) with an incidence of 1/7500 – 1/20000 live births and characterised through neurodevelopmental delay, DI, dysmorphic features, cardiovascular and connective tissue abnormalities, growth, endocrine and behavioral problems is caused by interstitial deletions or duplications of the 7q11.23 chromosomal region [4-6]. The first clinical cases were described in 1956 and 1961 by Schlesinger, respectively Williams and Beuren, while the genetic substrate of this condition represented by CNVs located on long arm of chromosome 7 (7q11.23) was unknown until 1993 [4,7-11].

Here we report a 5-year-old boy with 3 microdeletions in the region 7q11.23 involving multiple genes, including ELN. The main clinical features in this boy are neurodevelopmental delay, cardiac abnormalities and specific dysmorphic features. We performed aCGH to identify the breakpoints and genes content of the deleted regions. Based on this powerful method, we present the molecular characterisation of 3 de novo microdeletions (7q11.23 (72,755,027-73,494,802) x1dn; arr 7q11.23 (73,508,114-73,952,117) x1 dn; arr 7q11.23 (73,980,005-74,142,690) x1dn) of the WBS critical region identified in a patient with a severe and complex phenotype.

Methods

The extraction of genomic DNA was performed from blood lymphocytes of the patient using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA), following the manufacturers protocol. DNA quantity and quality was assessed using the Agilent 2010 Bioanalyzer (Agilent Technologies Inc., US). High-resolution microarray analysis was realized with the 135K oligonucleotide aCGH platform, on a 3×720K slide (Roche NimbleGen, Madison, WI, USA).
software (Roche NimbleGen, Madison, WI, USA) was used to data extraction. Copy number data was analyzed with Nexus 6.1 software (Nexus BioDiscovery, El Segundo, CA).

**Clinical Report**

The proband is the first child of healthy unrelated Caucasian parents. He was born at 39 weeks of gestation by caesarean delivery due to breech presentation, after a normal full-term pregnancy. Apgar score was 7, 9 at 1, 5 min, respectively and birth parameters where at mean: (birth weight: 2800 g; birth length: 48 cm; and head circumference: 35 cm). From the first month of life, the child was diagnosed with a severe congenital cardiac malformation: coarctation of the aortic arch associated with pulmonary artery stenosis and patent ductus arteriosus. So that, he required surgical interventions performed at age of 3 and 8 months, respectively.

The boy had a delayed psychomotor development: he started walking and emitted his first syllables at 16 months. At the age of 3 years was referred to the Pediatric Psychiatry Service because of speech delay and behavioral disorder. Physical and clinical evaluation revealed a reduced growth (L = 100 cm (25th percentile), W = 15 kg (25th percentile) and HC = 47 cm (less than 3rd percentile)) associated with mild ID (IQ = 49) and speech delay (his vocabulary consisted of only 10 simple words). The boy presented additional problems such as hyperkinetic episodes with self-aggression, autistic spectrum features, namely stereotypes and repetitive play, transient hypercalcemia, strabismus and distinctive facial dysmorphism characterised through broad forehead, flat nasal bridge, short upturned nose, long philtrum, and wide mouth with full lips and small jaw.

Based on the severe and complex phenotype of unknown etiology, the child was admitted for evaluation to Medical Genetics ambulatory, Clinical Hospital of Craiova, Romania. Genetic assessment through aCGH identified three microdeletions with a total size of 1.35 Mb located at 7q11.23 (chr7: 72,755,027-73,494,802; 73,508,114-73,952,117 and 73,980,005-74,142,690, National Center for Biotechnology Information coordinates) (Fig.1).

![Fig.1. Array CGH result on chromosome 7. Image generated with Nexus 6.1 software (Nexus BioDiscovery, El Segundo, CA). Data extraction (signal intensities) was performed with DEVA software (Roche Nimblegen)](image1)

![Fig.2. Molecular kayotype of the patient generated with Nexus 6.1 software (Nexus BioDiscovery, El Segundo, CA).](image2)

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According to the aCGH analyses result, the molecular karyotype of the patient was arr 7q11.23 (72,755,027-73,494,802) x1 dn; arr 7q11.23 (73,508,114-73,952,117) x1 dn; arr 7q11.23 (73,980,005-74,142,690) x1 dn (Fig.2).

The deleted regions encompasses more than 30 genes including several protein coding genes such as FKBP6, ELN, LIMK1, FZD9, TBL2, WBSCR22, WBSCR27, WBSCR28, STX1A, CLDN3, CLDN4, LAT2, ABHD11, RFC2, CLIP2, GTF2IRD1, GTF2I, TRIM50 or EIF4H (Fig.3).

Discussion

Since 1993, 7q11.23 region is known as the WBS region. It was reported that CNVs (microdeletions / microduplications) located in this region are responsible for the WBS complex phenotype characterised through multi-system involvement and variable expressivity [4].

The main clinical features reported as pathognomonic signs for WBS are distinctive facial characteristics (broad forehead, short upturned nose, long philtrum, or small jaw), cardiovascular abnormalities (supravalvular aortic stenosis, pulmonary artery stenosis, coarctation or aortic arch hypoplasia, ventricular septal defects, tetralogy of Fallot or aortic arch hypoplasia), connective tissue anomalies, growth and endocrine problems, neurological impairment (hyperreflexia, cerebellar dysfunction, strabismus, sensorineural hearing loss, hypotonia or brain malformations) and characteristic neurocognitive and behavioral phenotype (mild-moderate ID, impaired capacity to reorient in the environment and detect social threats, high sociability, strong verbal abilities associated with lack of depth in understanding, anxieties, depression, autism, sleep difficulties, attention deficit) [4-6,12-36]. The present case showed many clinical signs reported as being specific for WBS, namely severe cardiovascular anomalies (coarctation of the aortic arch associated with pulmonary artery stenosis and patent ductus arteriosus), transient hypercalcemia, strabismus, growth difficulties, particular behavior and distinctive facial dysmorphic features like broad forehead, flat nasal bridge, short upturned nose, long philtrum, wide mouth with full lips and small jaw.

The clinical assessment together with the results of the genetic testing indicate that is the first case of classic WBS clinically diagnosed and genetically confirmed through aCGH in the South-West region of Romania. The most widely used genetic method to confirm the clinical diagnoses of WBS is FISH with elastin gene as probe performed on metaphase chromosomes. This technique is not very expensive, but has several important disadvantages: does not detect the exact size and genes content of the detected CNVs (microdeletion / microduplication), is labor-intensive and time-consuming and it cannot be used in the cases harboring atypical CNVs [4]. Conversely, methods like qPCR, MLPA (multiplex ligation-dependent probe amplification) or aCGH that detect small CNVs through DNA analysis are very sensitive, map the size of the microdeletion / duplication, offer valuable information regarding the affected genes (aCGH) and allow the processing of multiple samples from different patients within the same run [37-39]. Thus, aCGH testing of our patient detected three microdeletions located in the 7q11.23 region and also provided information regarding their size, the breakpoints.
and genes affected by the deletions: ELN, LIMK1, FZD9, TBL2, WBSCR22, WBSCR27, WBSCR28, STX1A, CLDN3, CLDN4, LAT2, ABHD11, RFC2, CLIP2, GTF2IRD1, GTF2I, TRIM50 and EIF4H. Details that would not have been revealed if only FISH evaluation had been performed. This results revealed that the microdeletions carried by our patient are located in the genome region commonly affected in WBS. It also shown evidences that the deletions involved the elastin gene (ELN) which may partly explain the severe cardiac anomalies identified in this case. Since 1993, has been established that haploinsufficiency of ELN that comprises 33 exons is responsible for the cardiovascular and connective tissue abnormalities identified in WBS [10,40]. FKBP6, BAZ1B and WBSCR22 are other genes contained in the deleted region, strongly expressed in the cardiovascular structures, that might explain the pathogenesis of the described cardiac defects. Duplication of FKBP6 gene, component of the synaptonemal complex, was reported to be associated with congenital heart defects [41]. Furthermore, knock-out animal studies showed that hetero/homozygous deletions of the BAZ1B gene are associated with cardiac anomalies like multiple atrial and muscular ventricular septal defects or hypertrophy of both ventricles. These data outline the important role of BAZ1B gene in the normal development and function of the heart [4]. Recently, it was shown that the haploinsufficiency of this gene might be also involved in the neurodevelopmental impairment and specific dysmorphism described in patients diagnosed with WBS [42,43]. There are no data available in the literature regarding the association of WBSCR22 gene mutations with cardiovascular abnormalities.

Another gene affected by the microdeletion detected in our case was LIM kinase 1 (LIMK1) that is mainly expressed in the central nervous system and involved in organization of the actin cytoskeleton by modulating actin depolymerization [4]. Based on the fact that actin remodeling was associated with the formation and maintenance of memory and learning [44], LIMK1 impairment by the microdeletions reported in our case could partly explain the abnormal neurobehavioral phenotype identified characterised through stereotypes, repetitive play and impaired detection of social threats [45,46]. These clinical findings might be also explained by the functional impairment of FZD9, STXI, GTF2I, GTF2IRD2 or EIF4H genes contained in the affected genomic region. These genes are highly expressed during the development and differentiation of central and peripheral nervous system structures [4,47,48]. Accordingly, several studies linked the GTF2I (general transcription factor II) gene with the pathogenesis of autism spectrum disorders described in WBS [49-51]. Haploinsufficiency or genetic variations such single nucleotide polymorphisms (SNPs) of GTF2I were reported to be associated with the cognitive-behavioral phenotype of WBS [49,52]. Furthermore, experimental studies showed that the transmembrane cell surface receptor encoded by the FZD9 gene is involved in hippocampus development and defects of this gene are associated with learning and memory disorders [43,53]. Our clinical report sustains the hypotheses raised by previous studies that the genes mentioned above gene might be candidate genes responsible for the development of the WBS neurocognitive profile, namely the autistic-like features. Also, other genes (TBL2, CLDN3, CLDN4, LAT2, ABHD11, RFC2, CLIP2 or TRIM50) located in the region affected by the microdeletions detected in our case was shown to have important roles in the development and functions of the nervous system or other organs [4,54]. Thus, all these findings could explain the complex phenotype described in our patient diagnosed with WSB.

In summary, we describe a typical case of WBS genetically confirmed through aCGH testing. The result of the aCGH testing also contributed through the detection of the affected genes to establish a proper follow-up of the patient, mainly focused on the prevention of possible severe complications related to the severe cardiovascular phenotype identified such as sudden death or portal hypertension [55] or to the defects of the tumor suppressor genes contained in the deleted region (BCL7B, CLDN3 or CLDN4) [4,56,57] that could threaten the patient's life.

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Ioana Streţă and Simona Şerban-Şoșoi contributed equally to this work.

F. Mixich and M. Ioana contributed equally to this work.

References

1. Shinawi M, Cheung SW (2008) The array CGH and its clinical applications. Drug Discov Today 13: 760-770.
2. Rosenberg C, Knijnenburg J, Bakker E, Vianna-Morgante AM, Sloos W, et al. (2006) Array-CGH detection of micro rearrangements in mentally retarded individuals: clinical significance of

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imbalances present both in affected children and normal parents. J Med Genet 43: 180-186.

3. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, et al. (2010) Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 86: 749-764.

4. Merla G, Brunetti-Pierri N, Micale L, Fusco C (2010) Copy number variants at Williams-Beuren syndrome 7q11.23 region. Hum Genet 128: 3-26.

5. Pober BR (2010) Williams-Beuren syndrome. N Engl J Med 362: 239-252.

6. Amenta S, Sofocleous C, Koliaelexi A, Thomaidis L, Giouroukos S, et al. (2005) Clinical manifestations and molecular investigation of 50 patients with Williams syndrome in the Greek population. Pediatr Res 57: 789-795.

7. Schlesinger BE, Butler NR, Black JA (1956) Severe type of infantile hypercalcaemia. Br Med J 1: 127-134.

8. Williams JC, Barratt-Boyes BG, Lowe JB (1961) Supravalvular aortic stenosis. Circulation 24: 1311-1318.

9. Beuren AJ, Apitz J, Harmjanz D (1962) Supravalvular aortic stenosis in association with mental retardation and a certain facial appearance. Circulation 24: 1235-1240.

10. Ewart AK, Morris CA, Atkinson D, Jin W, Sternes K, et al. (1993) Hemizygosity at the elastin locus in young adults with the syndrome. Am J Med Genet 45: 1311-1318.

11. Sharma P, Gupta N, Chowdhury MR, Phadke SR, Supravalvular aortic stenosis. Pediatr Int. 57: 789-795.

12. Shiohama T, Fujii K, Ebata R, Funabashi N (2010) Williams-Beuren Syndrome: a T1-weighted MRI study. Neuroreport 21: 1311-1318.

13. (2001) American Academy of Pediatrics: Health care supervision for children with Williams syndrome. Pediatrics 107: 1192-1204.

14. Axelsson S, Bjornland T, Kjaer I, Heiberg A, Debets E, Bregman J, et al. (2004) Multisystem study of 20 older adults with Williams syndrome. Am J Med Genet A 131: 255-264.

15. Stagi S, Bindi G, Neri AS, Cariello A, Losi S, et al. (2005) Thyroid function and morphology in patients affected by Williams syndrome. Acta Endocrinol (Oxf) 63: 456-460.

16. Ferrero GB, Howald C, Micale L, Fusco C (2010) Copy number variants at Williams-Beuren syndrome 7q11.23 region. Hum Genet 128: 3-26.

17. Partsch CJ, Siebert R, Caliebe A, Gorochow A, Wessel A, et al. (2005) Detection of 5q deletion in 22 Williams-Beuren syndrome patients. Pediatr Res 57: 789-795.

18. Partsch CJ, Japing I, Siebert R, Caliebe A, Gorochow A, Wessel A, et al. (2005) Thyroid function and morphology in patients affected by Williams syndrome. Acta Endocrinol (Oxf) 63: 456-460.

19. Cambiasso P, Orazi C, Digilio MC, Loche S, Capolino R, et al. (2007) Thyroid morphology and subclinical hypothyroidism in children and adolescents with Williams syndrome. J Pediatr 150: 62-65.

20. Cherniske EM, Carpenter TO, Kliman C, Young E, Bregman J, et al. (2004) Multisystem study of 20 older adults with Williams syndrome. Am J Med Genet A 131: 255-264.

21. Stagi S, Bindi G, Neri AS, Cariello A, Losi S, et al. (2005) Thyroid function and morphology in patients affected by Williams syndrome. Acta Endocrinol (Oxf) 63: 456-460.

22. Del Pasqua A, Binelli G, Tosocono V, Iacobelli R, Digilio C, et al. (2009) New findings concerning cardiovascular manifestations emerging from long-term follow-up of 150 patients with the Williams-Beuren syndrome. Cardiol Young 19: 563-567.

23. Eronen M, Peippo M, Hiippala A, Raatikka M, Arvio M, et al. (2002) Cardiovascular manifestations in 75 patients with Williams syndrome. J Med Genet 39: 554-558.

24. Bouchireb K, Boyer O, Bonnet D, Brunelle F, Decramer S, et al. (2010) Clinical features and management of arterial hypertension in children with Williams-Beuren syndrome. Nephrol Dial Transplant 25: 434-438.

25. Nakamoto S, Saga T, Shinohara T (2003) Williams syndrome associated with complete atrioventricular septal defect. Heart 89: e15.

26. Pankau R, Gorochow A, Wessel A (1993) Cytogenet Genome Res 146: 187-194.

27. Dykens EM (2003) Anxiety, fears, and phobias in persons with Williams syndrome. Dev Neuropsychol 23: 291-316.
36. Leyfer OT, Woodruff-Borden J, Klein-Tasman BP, Fricke JS, Mervis CB (2006) Prevalence of psychiatric disorders in 4 to 16-year-olds with Williams syndrome. Am J Med Genet B Neuropsychiatr Genet 41b: 615-622.
37. Edelmann L, Prosnitz A, Pardo S, Bhatt J, Cohen N, et al. (2007) An atypical deletion of the Williams-Beuren syndrome interval implicates genes associated with defective visuospatial processing and autism. J Med Genet 44: 136-143.
38. Howald C, Merla G, Digilio MC, Amenta S, Lyle R, et al. (2006) Two high throughput technologies to detect segmental aneuploidies identify new Williams-Beuren syndrome patients with atypical deletions. J Med Genet 43: 268-273.
39. Schubert C, Laccone F (2006) Williams-Beuren syndrome: determination of deletion size using quantitative real-time PCR. Int J Mol Med 18: 799-806.
40. Guemann AS, Andrieux J, Petit F, Halimi E, Bouquillon S, et al. (2015) ELN gene triplication responsible for familial supravalvular aortic aneurysm. Cardiol Young 25: 712-717.
41. Kriek M, White SJ, Szuhai K, Knijnenburg J, van Ommen GJ, et al. (2006) Copy number variation in regions flanked (or unflanked) by duplicons among patients with developmental delay and/or congenital malformations; detection of reciprocal and partial Williams-Beuren duplications. Eur J Hum Genet 14: 180-189.
42. Lalli MA, Jang J, Park JC, Wang Y, Guzman E, et al. (2016) Haploinsufficiency of BAZ1B contributes to Williams syndrome through transcriptional dysregulation of neurodevelopmental pathways. Hum Mol Genet.
43. Fusco C, Micale L, Augello B, Teresa Pellico M, Menghini D, et al. (2014) Smaller and larger deletions of the Williams Beuren syndrome region implicate genes involved in mild facial phenotype, epilepsy and autistic traits. Eur J Hum Genet 22: 64-70.
44. Nimchinsky EA, Sabatini BL, Svoboda K (2002) Structure and function of dendritic spines. Annu Rev Physiol 64: 313-353.
45. Meng Y, Zhang Y, Tregoubov B, Janus C, Cruz L, et al. (2002) Abnormal spine morphology and enhanced LTP in LIMK-1 knockout mice. Neuron 35: 121-132.
46. Nikitina EA, Medvedeva AV, Zakharov GA, Savvateeva-Popova EV (2014) Williams syndrome as a model for elucidation of the pathway genes - the brain - cognitive functions: genetics and epigenetics. Acta Naturae 6: 9-22.
47. Porter MA, Dobson-Stone C, Kwok JB, Schofield PR, Beckett W, et al. (2012) A role for transcription factor GTF2IRD2 in executive function in Williams-Beuren syndrome. PLoS One 7: e47457.
48. Capossela S, Muzio L, Bertolo A, Bianchi V, Dati G, et al. (2012) Growth defects and impaired cognitive-behavioral abilities in mice with knockout for Eif4h, a gene located in the mouse homolog of the Williams-Beuren syndrome critical region. Am J Pathol 180: 1121-1135.
49. Sakurai T, Dorr NP, Takahashi N, McLnnes LA, Elder GA, et al. (2011) Haploinsufficiency of Gtf2i, a gene deleted in Williams Syndrome, leads to increases in social interactions. Autism Res 4: 28-39.
50. Malenfant P, Liu X, Hudson ML, Qiao Y, Hrynchak M, et al. (2012) Association of GTF2I in the Williams-Beuren syndrome critical region with autism spectrum disorders. J Autism Dev Disord 42: 1459-1469.
51. Antonell A, Del Campo M, Magano LF, Kaufmann L, de la Iglesia JM, et al. (2010) Partial 7q11.23 deletions further implicate GTF2I and GTF2IRD1 as the main genes responsible for the Williams-Beuren syndrome neurocognitive profile. J Med Genet 47: 312-320.
52. Crespi BJ, Hurd PL (2014) Cognitive-behavioral phenotypes of Williams syndrome are associated with genetic variation in the GTF2I gene, in a healthy population. BMC Neurosci 15: 127.
53. Schubert C (2009) The genomic basis of the Williams-Beuren syndrome. Cell Mol Life Sci 66: 1178-1197.
54. Micale L, Fusco C, Augello B, Napolitano LM, Dermitzakis ET, et al. (2008) Williams-Beuren syndrome TRIM50 encodes an E3 ubiquitin ligase. Eur J Hum Genet 16: 1038-1049.
55. Imashuku S, Hayashi S, Kuriyama K, Hibi S, Tabata Y, et al. (2000) Sudden death of a 21-year-old female with Williams syndrome showing rare complications. Pediatr Int 42: 322-324.
56. Genuat D, Quentin S, Rizzari C, Lundin C, Coliva T, et al. (2014) Constitutional and somatic deletions of the Williams-Beuren syndrome critical region in non-Hodgkin lymphoma. J Hematol Oncol 7: 82.
57. Vanhapinha N, Knuutila S, Vettenranta K, Lohi O (2014) Burkitt lymphoma and Ewing sarcoma in a child with Williams syndrome. Pediatr Blood Cancer 61: 1877-1879.

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