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CASE REPORT

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SUPRAVALVULAR AORTIC STENOSIS AND PERIPHERAL PULMONARY STENOSIS IN FAMILY WITH BALANCED TRANSLOCATION T(7;14) AND BREAK POINT WITHIN THE ELASTIN GENE REGION

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We present a family form of balanced translocation t(7;14) found in a mother and her two sons. The mother had an aortic cardiac murmur, without hemo-dynamic repercussions and the children had almost identical clinical findings, significant supravalvular aortic stenosis, left ventricle intracavitary stenosis and multiple peripheral pulmonary stenosis but with no other clinical manifestations of Williams-Beuren syndrome, except, perhaps, a deep, metallic voice. The conventional chromosome analysis unexpectedly revealed a balanced translocation between chromosomes 7 and 14, the same translocation was found in the mother and both children. Subsequent fluorescent in situ hybridization analysis with WSCR probe showed that the break point was within the elastin gene region in the mother and both children. The proband karyotype was interpreted, according to ISCN (2005) as 46,XY,t(7;14)(q11.23;p12).ish t(7;14)(D7Z1+,ELNsp;D14Z1/D22Z1+,ELNsp+)mat, in other words, translocation had disrupted the elastin region and may have contributed to the developmental defects in Williams-Beuren syndrome. When going through the references on genetic examination of supravalvular aortic stenosis, Williams-Beuren syndrome and some other conditions that could not be placed in any of these two terminal categories because of the various phenotype characteristics, we found that such a result has not yet been published.

Descriptors: AORTIC STENOSIS, SUPRAVALVULAR - complications, genetics; PULMONARY VALVE STENOSIS - complications, gentics; CHROMOSOMES, HUMAN, PAIR 7 - genetics; CHROMOSOMES, HUMAN, PAIR 14 - genetics; TRANSLOCATION, GENETIC; ELASTIN - genetics

INTRODUCTION

Supravalvular aortic stenosis is an obstructive vascular disease, whether it appears as an isolated form (1, 2, 3, 4), or as a part of Williams-Beuren syndrome (5). In both cases, there are abnormalities of the elastin gene mapped on 7q11.23, with the difference that the isolated form of supravalvular aortic stenosis is a "single gene disorder" caused by various, well known, mutations of the elastin gene (6, 7, 8, 9, 10), while Williams-Beuren syndrome is a result of a larger deletion at 7q11.23 that includes disruption of the elastin region but other genes as well (11-17). It is argued that the size of deletion is about 2 Mb, but this is still being debated (11, 15, 18). Pathological findings include loss of normal orientation of elastic fibers and smooth-muscle cell hypertrophy, and usually elastic tissue hyperplasia in the media wall of the arteries is found (19). The subject of the phenotype characteristics of Williams-Beuren syndrome is covered in numerous publications (7).

Williams-Beuren syndrome is a deletion genomopathy (15, 20), contiguous gene deletion disorder caused by haplo insufficiency of genes at 7q11.23. (21), with autosomal dominant inheritance or sporadic cases in families (5). Differentiation between supravalvular aortic stenosis and Williams-Beuren syndrome is not always possible, considering the overlapping and great variability of phenotype characteristics. Also, there are atypical genetic findings which make a clear distinction between these two entities even harder to define (10, 22).

Here we present translocation t(7;14)(q11.23;p12), with breaking point within the elastin gene region, in a family where the mother and her two sons have identical genetic findings. The mother had only an aortic cardiac murmur, with no haemo-dynamic repercussions or other morphological findings, and both sons had almost identical clinical findings, supravalvular aortic stenosis and identical multiple peripheral pulmonary stenoses but no other phenotypic characteristics of Williams-Beuren syndrome, except, perhaps, deep metallic voice. According to the overview of the publications, this is the first case of balanced translocation t(7;14) in a family, with the breaking within the elastin gene region and vascu-

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lar findings of Williams-Beuren syndrome, with no other typical phenotype characteristics.

METHODS AND PATIENTS

The first patient came to our Department at the age of 3 for cardiological examination due to a systolic murmur, known since he was 10 moths old. Somatic, psychomotoric and intellectual development was normal. A deep, metallic voice was noticed. A harsh holosystolic murmur on the aortic area with transmission to the right clavicle and jugulum was audible, electrocardiogram showed biventricular hypertrophy with predominant left ventricle hypertrophy, echocardiography showed supravalvular aortic stenosis with a gradient of 50 mmHg, intracavitary stenosis in the left ventricle outflow tract with a gradient of 90 mmHg and mitral insufficiency grade I. Pressure in right ventricle (due to trivial tricuspid insufficiency) was 110 mmHg, without obvious pulmonary valvular stenosis or main pulmonary artery stenosis, but turbulence was noticeable in both pulmonary branches. Heart catheterization confirmed the echocardiographic findings with narrowed segments in the left ventricle outflow tract and supravalvular aortic region with a gradient of 100 mmHg (Figure 1), right ventriculography showed diffuse narrowing of both pulmonary branches (Figure 2). The child underwent surgery at the age of 4.5 due to supravalvular aortic stenosis and left ventricle outflow tract obstruction by the Doty procedure (residual gradient 20 mmHg) and resection of left ventricle outflow tract stenosis formed by the fibromuscular subaortic ring. However, during postoperative period, there was still aortic insufficiency grade II present, whilst the narrowing of both pulmonary branches was treated by balloon angioplasty.

The second patient was one year younger, the first patient's brother, who also came due to a systolic murmur, known since the newborn period. His somatic, psychomotoric and intellectual development was also normal. Electrocardiogram showed biventricular hypertrophy with predominant left ventricle hypertrophy, echocardiography showed supravalvular aortic stenosis with a gradient of 90 mmHg and mitral insufficiency grade I. Pressure in the right ventricle (due to trivial tricuspid insufficiency) was 70 mmHg, with no obvious pulmonary valvular stenosis or main pulmonary artery stenosis, but turbulence was noticeable in both pulmonary branches. Heart catheterization showed narrowed segments in the left ventricle outflow tract with gradient of 50 mmHg and supravalvular aortic stenosis with a gradient of 90 mmHg, right ventricle pressure was 80 mmHg due to multiple bilateral peripheral pulmonary stenosis, the pulmonal valve and main pulmonary trunk were normal. Cardiological status was very similar to his brother's. He underwent surgery at the age of 4,5 due to supravalvular and left ventricle outflow tract obstruction by the Doty procedure (residual gradient of 20 mmHg) and resection of left ventricle outflow tract stenosis (residual gradient of 30 mmHg). There is still mild aortic insufficiency and peripheral pulmonary stenosis present, which will be treated by balloon angioplasty.

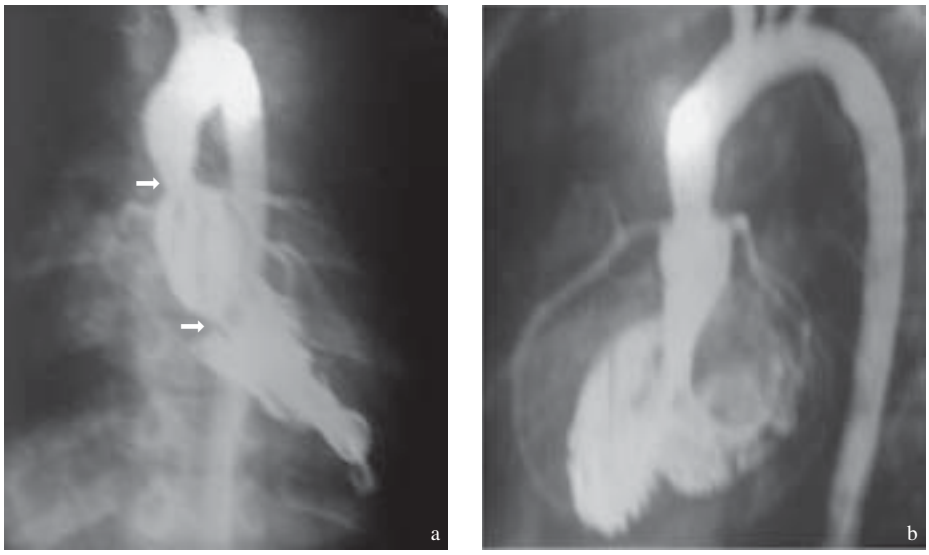

Figure 1 (a, b). *Angiographic findings (left ventriculography); A: PA projection, B: LAO 60^0) in our patients show severe stenosis in the cavity of left ventricle (gradient od 100 mmHg) (➞), supravalvular stenosis of ascending aorta (gradient of 60mmHg) (➞), enlargement of coronary arteries and mild mitral insufficiency*
Slika 1 (a, b). *Nalaz angiografije (ventrikulografija lijevog ventrikula; A: PA projekcija, B: LAO 60°) u naših pacijenata pokazala je tešku intrakavitarnu stenozu lijevog ventrikula (gradijent od 100 mmHg) (➞), supravalvularnu aortalnu stenozu (gradijent 60mmHg) (➞), proširenje koronarnih arterija i blagu mitralnu insuficijenciju*

Chromosome analysis

Chromosome analysis in both children and the mother was performed using high-resolution banding techniques on prometaphase chromosomes obtained from peripheral blood lymphocytes according to standard synchronization techniques (20).

Fluorescence in situ hybridization (FISH)

FISH studies were done in both children and the mother using the commercially available digoxigenin-labeled (Oncor) WSCR probe which maps to 7q11.23 in combination with a control probe mapping to 7q36, digoxigenin-labeled alpha satellite chromosome 7 probe (Oncor), which maps at the centromere of chromosome 7, and biotin-labeled alpha satellite chromosome 14/22 probe (Oncor), which maps at the centromeres of chromosomes 14 and 22. The procedure was performed as described previously with minor modifications (23, 24).

RESULTS

The conventional chromosome analysis unexpectedly revealed a balanced translocation between chromosomes 7 and 14, the same translocation being found in the mother and her sons (Figure 3).

Subsequent FISH analysis with WSCR probe showed the splitting of the elastin gene, break point being in its region (Figure 4).

The proband karyotype was interpreted, according to ISCN (2005) as 46,XY,t(7;14)(q11.23;p12). ish t(7;14)(D7Z1+,ELNsp;D14Z1/D22Z1+,ELNsp+)mat, in other words, translocation had disrupted the elastin region, which may

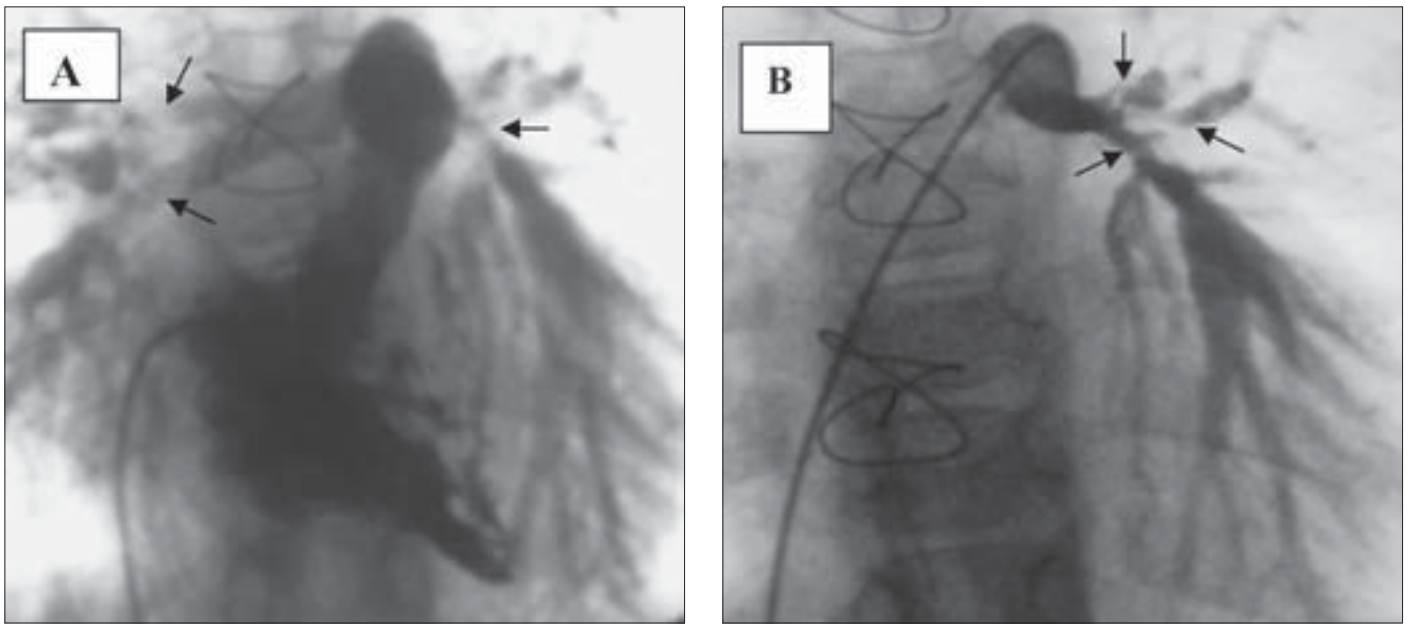

Figure 2 (a, b). *Angiographic presentation of multiple peripheral pulmonary stenosis in our patients (equal finding in both brothers)*
A: Right ventriculography (PA projection) shows hypertrophic trabeculation of right ventricle, free right ventricular outflow tract, normal pulmonary valve, multiple peripheral stenosis of pulmonary arteries and their branches
B: Selective angiography of right pulmonary artery with multiple stenoses of their branches. The gradient between right ventricle and peripheral pulmonary artery branches is 90 mmHg
Slika 2 (a, b). *Angiografski prikaz višestrukih perifernih pulmonalnih stenoza u naših pacijenata (jednak nalaz u oba brata)*
A: Desnostrana ventrikulografija (PA projekcija) pokazuje hipertrofičnu trabekulaciju desne klijetke, slobodan izlazni trakt desne klijetke, normalnu plućnu valvulu, višestruke periferne stenoze plućnih arterija i njihovih ogranaka
B: Selektivna angiografija desne plućne arterije s višestrukim stenozama njezinih ogranaka. Gradijent između desne klijetke i ogranaka plućnih arterija je 90 mmHg

have contributed to the developmental defects in Williams-Beuren syndrome (13).

DISCUSSION

Eisenberg et al. reported 22 cases of supravalvular aortic stenosis in two families, some of them also had an associated peripheral pulmonar stenosis, none had phenotype characteristics of Williams-Beuren syndrome. Other authors also reported cases with supravalvular aortic stenosis and peripheral pulmonary stenosis; Mc Kusick on the basis of personal communication (4), Chiarella (1), Ensing et al. found 22 members in one family, 13 of them had supravalvular aortic stenosis, 4 had peripheral pulmonary stenosis, none had unusual face or mental retardation (3). Most of the authors agree that supravalvular aortic stenosis is a result of mutation in the elastin gene 7 (7, 8). There are also reports, that deletions of the elastin gene at 7q11.23, recognized using FISH, occur in approximately 90% of patients with Williams-Beuren syndrome (25, 26).

Unlike, "single gene disorder", an isolated form of supravalvular aortic stenosis, some authors found that in Williams-Beuren syndrome at least 1.5-2.5 Mb DNA is commonly deleted (11, 18). Genetic findings as a reason for disruption of the elastin gene differ. There is a report of translocation t(6;7)(p21.1; q11.23) (6), a report of a patient with a *de novo* translocation 46,XX,t(6;7)(q27; q11.23) with severe supravalvular aortic stenosis and hydrops fetalis, who died shortly after delivery. The authors were unable to determine whether it was an isolated supravalvular aortic stenosis or Williams-Beuren syndrome in this patient (10).

There are reports about different genetic findings in patients with Williams-Beuren syndrome: reports on 15q12 duplication (27, 16), the deletion at the long arm of chromosome 15 (28), deletion 6(q22.2;q23) (29) and unbalanced 13;18 translocation (30). These kinds of reports open discussion about heterogenity. Duba et al. described disruption of the elastin gene in a family with balanced translocation t(7;16) (q11.23;q13) with various phenotype expressions of Williams-Beuren syndrome, ranging from a hoarse voice as the only feature to the full Williams-Beuren syndrome phenotype (22).

We, once more, underline the fact that our patients had a typical cardiovascular expression of Williams-Beuren syndrome, but also, only, deep, metallic voices as far as the phenotype characteristics of Williams-Beuren syndrome are concerned. The same translocation in the mother resulted in the normal phenotype. Further molecular cytogenetic evaluation is needed to determine whether there is additional DNA loss in their offspring eliciting a phenotypic effect. Our family confirms the hypotheses of heterogenity and various phenotype expression of the chromosome rearrangements in this region. There are reports that Williams-Beuren syndrome is a continuous gene deletion syndrome, a hemizygous deletion of a 1.5 Mb interval encompassing at least 17 genes at 7q11.23 (11). There are also hypotheses that, in addition to the ELN gene, the gene that encodes LIM kinase is deleted and responsible for human cognitive development (31, 14, 17). RFC2 gene is responsible for growth retardation (16), while, CYLN2 haplo-insufficiency is a reason for neurological alterations in Williams-Beuren syndrome (12). The results of this research bring us to the definition of gene map at 7q11.23 that includes ELN, LIMK1 and RFC2 gene (15).

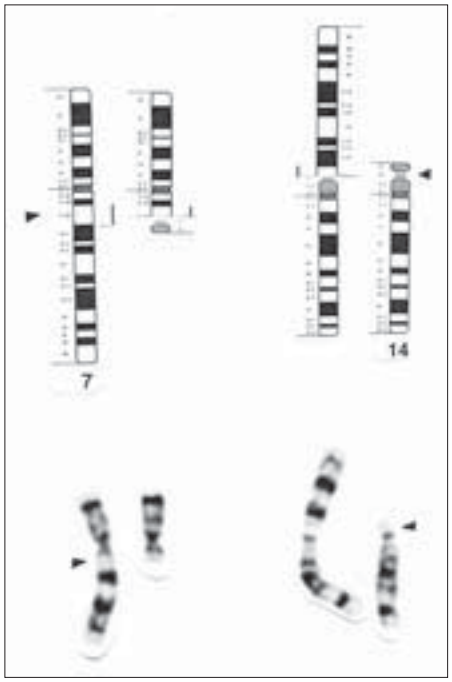

Figure 3. *Conventional cytogenetic analysis of patient (proband) U. M.*
Upper Partial idiogram illustrating the t(7;14) translocation. Arrows denote the 7q11.23 and 14p12
Lower Partial karyotype from the patient showing chromosome 7 and der(7) left and der(14) and 14 right. Arrows denote break points.
Slika 3. *Konvencionalna citogenetska analiza bolesnika (ispitanik) U. M.*
Gore: Djelomični ideogram prikazuje t(7;14) translokaciju. Strjelica označava 7q11.23 i 14p12
Dolje: Djelomični kariotip bolesnika prikazuje kromosom 7 i der(7) lijevo i der(14) i kromosom 14 desno. Strjelice označavaju mjesta loma

In conclusion, we wanted to show that the distinction between an isolated form of supravalvular aortic stenosis and Williams-Beuren syndrome is not always possible, considering that there are various forms for which the definition of supravalvular aortic stenosis as the "single gene disorder" and Williams-Beuren syndrome as continuous gene disorder and haplo-insufficiency at 7q11.23, is inadequate. Our patients had clear cardiovascular findings as part of Williams-Beuren syndrome, they also had deep metallic voices, but with normal face and normal cognitive and intellectual status. In addition, cytogenetic analysis unexpectedly revealed a balanced translocation t(7;14) with a break in the elastin region. Cytogenetic analysis of our patients, and unusual clinical expression could be a contribution to the understanding of the etiology of supravalvular aortic stenosis and Williams-Beuren syndrome.

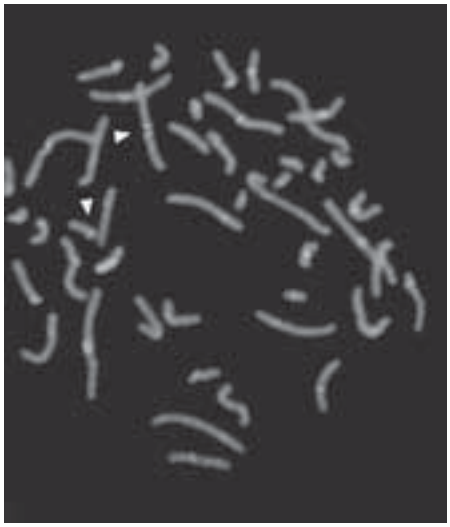

Figure 4. *Fluorescence in situ hybridization with WBS probe, arrows illustrate disruption of elastin locus by two separated signals one on der(7) and second on der(14)*
Slika 4. *Fluorescentna in situ hibridizacija s WBS probom pokazuje da je prijelomna točka pocijepala elastinsku regiju kao dva odvojena signala, jedan na der(7) a drugi na der(14)*

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S u m m a r y

SUPRAVALVULARNA AORTALNA STENOZA
I PERIFERNA PULMONALNA STENOZA S OBITELJSKOM BALANSIRANOM TRANSLOKACIJOM T(7;14)
I MJESTOM LOMA U ELASTINSKOJ REGIJI

I. Malčić, D. Dilber, H. Kniewald, R. Lasan, D. Begović, M. Jelušić

Prikazujemo obiteljski oblik balansirane translokacije t(7;14) koji je nađen u majke i dva njezina sina. Majka je imala samo šum nad aortnim ušćem bez hemodinamskog značenja, djeca su imala gotovo istovjetan klinički nalaz; značajnu supravalvularnu aortnu stenozu tipa pješčanog sata, intrakavitarnu stenozu u lijevoj klijetki i multiple periferne pulmonalne stenoze. Nisu imali niti jedan drugi klinički znak Williams-Beurenova sindroma, osim vjerojatno dubokog metaličnog glasa. Konvencionalna kromosomska analiza neočekivano je pokazala da je riječ o balansiranoj translokaciji t(7;14), identična translokacija pronađena je u majke i brata. Fluorescentna in situ hibridizacija s WSCR probama pokazala je da je prijelomna točka uzdužno pocijepala elastinsku regiju u sve troje ispitanika. Kariotip ispitanika interpretiran je prema ISCN-u kao 46,XY,t(7;14)(q11.23;p12).ish t(7;14)(D7Z1+, ELNsp;D14Z1/D22Z1+,ELNsp+)mat. Drugim riječima, translokacija je pocijepala elastinsku regiju, što može biti razlogom nastanka razvojnih anomalija karakterističnih za Williams-Beurenov sindrom. Pregledom kroz literaturu našli smo da ovakav nalaz nije dosad objavljen u genetičkoj obradi supravalvularne aortne stenoze, odnosno Williams-Beurenova sindroma ili stanja koja se ne mogu razvrstati u ove dvije krajnje kategorije zbog šarolikosti fenotipskih karakteristika.

Deskriptori: SUPRAVALVULARNA AORTNA STENOZA - komplikacije, genetika; PULMONALNA STENOZA - komplikacije, genetika; HUMANI KROMOSOMI, PAR 7 - genetika; HUMANI KROMOSOMI, PAR 14 - genetika; GENETSKA TRANSLOKACIJA; ELASTIN - genetika

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