WISKOTT-ALDRICH SYNDROME AT CHILDREN — DIAGNOSTIC PARTICULARITIES

SINDROMUL WISKOTT-ALDRICH LA COPII — PARTICULARITĂȚI DE DIAGNOSTIC

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Summary

Wiskott-Aldrich syndrome is a form of primary immune deficiency combined with thrombocytopenia and for a long time it was thought it is a single one with such symptoms, but now phenocopies are known. Another problem with this syndrome is a common problem of all primary immune deficiencies — majority of medical workers know very little about this group of diseases what leads to a very long times from the debute of disease to the specific diagnosis. Because of that, we shortly describe criteria for Wiskott-Aldrich and rekated disorders diagnostic and how we are performing molecular genetic diagnostic of it.

Rezumat

Sindromul Wiskott-Aldrich este o formă de imunodeficiență primară combinată cu trombocitopenie și multă vreme s-a crezut că este una singură cu astfel de simptome, dar acum se cunosc fenocopii. O altă problemă cu acest sindrom, comună tuturor deficiențelor imune primare - majoritatea lucrătorilor medicali știu foarte puțin despre acest grup de boli, ceea ce duce la un timp foarte lung de la debutul bolii până la diagnosticul specific. Din acest motiv, descriem pe scurt criteriile de diagnosticare a

tulburărilor Wiskott-Aldrich și rekated și modul în care efectuăm diagnosticul genetic molecular al acesteia.

Keywords: Wiskott-Aldrich syndrome, WAS, Sanger sequencing, SSCP.

Actuality

Wiskott-Aldrich Syndrome (WAS) is an X-linked primary immune deficiency, associated with thrombocytopenia [1]. It occurs with a frequency of 1 t0 10 at million of newborn boys [2,3], has phenocopy and hardly predictable course [1]. Even more of that, in Moldova primary immune deficiencies are in generally about unknon to medical community. Because of that in this article we describe criteria, scores and methods for WAS-related disorders diagnostic developed wordwide and in Laboratory of Human Molecular Genetics of Intitute of Mother and Child in Chishinau, Republic of Moldova.

Diagnostics worldwide

For a long time, WAS, X-linked thrombocytopenia (XLT) [4] and X-linked neutropenia (XLN) were considered different diseasess, but in 1995 Villa et al. [5] have established that all of these diseases can be caused by the same mutations in *WAS* gene. Despite of this, Wiskott-Aldrich syndrome as a heavier form of disease more fequently is caused by deletions or nonsense mutations (mutations causing formation of a stop-codon) and frameshift mutations, while other WS-related diseases are more frequently caused by missens-mutations (replacement of one aminoacid coding codon to another one aminoacid coding codon).

Taking in account all this information, European Society for Immunodeficiencies (ESID) released a list of Wiskott-Aldrich syndrome diagnostic criteria (tab. 1) [6].

Somewhat earlier Jin et al. Have developed a quantitative system for assaying WAS-related disorders severity [7] (tab. 2).

At 1992 Conley at al. [8] have reported an atypical form of Wiskott-Aldrich syndrome in a girl. At 2012 Lanzi et al. [9] have established this disease is caused by mutations in *WIPF1* gene. This disease is inheried in autosomal-recessive manner [10]. Now, this disease is officially called «Wiskott-Aldrich syndrome 2» [11].

Table 1. Diagnostic criteria for WAS.

Mandatory criteria	Thrombocytopenia (<70,000 platelets/mm^3) on two separate tests, **AND** Small platelets (platelet volume <7.5 fL)
Definitive criteria	Pathogenic *WAS* gene variant (SNP or deletion) detected on genetic testing, **OR** Absent or reduced WAS expression via Northern blot analysis of lymphocytes from a fresh blood sample, **OR** Absent or reduced WAS protein in lymphocytes, **OR** Maternal family history of WAS, XLT or XLN
Probable criteria	Eczema, OR Recurrent viral or bacterial infections, OR Autoimmune diseases, OR Cancer, OR Abnormal antibody response to polysaccharide antigens and/or low isohemagglutinins
Possible criteria	Same as above, but had spleen removal (a treatment for thrombocytopenia) so may no longer meet mandatory criteria, **OR** Mandatory criteria only

Taking in account all of these factors, definitive diagnosis of Wiskott-Aldrich syndrome is not so easy as it may appear at first sight and it required development of a special diagnostic protocol at Human Molecular Genetics Laboratory of Intutute of Mother and Child in Chisinau, Republic of Moldova.

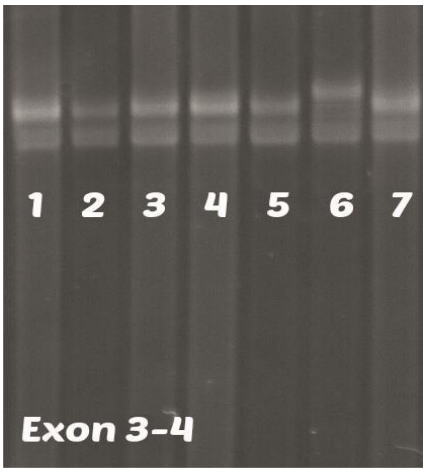
Table 2. WAS clinical score.

Score	Clinical signs	Disease
0	Neutropenia or mielodysplasia only.	XLN
0.5	Intermittent thrombocytopenia (low platelet counts sometimes but not always).	XLT
1	Thrombocytopenia and small platelets (microthrombocytopenia).	XLT
2	Microthrombocytopenia plus normally responsive eczema or occasional upper respiratory tract infections	XLT
2.5	Microthrombocytopenia plus therapy-responsive but severe eczema or airway infections requiring antibiotics	XLT/WAS
3	Microthrombocytopenia plus both eczema and airway infections requiring antibiotics	WAS
4	Microthrombocytopenia plus eczema continuously requiring therapy and/or severe or life-threatening infections	WAS
5	Microthrombocytopenia plus autoimmune disease or malignancy	XLT/WAS + autoimmune disease or cancer

Molecular-genetic diagnostics in Moldova

At 2021 Țurcan, D., Andrieș, L., Dorif, A., Sacară, V. [12] published work where they described a protocol for WAS molecular genetic diagnostics.

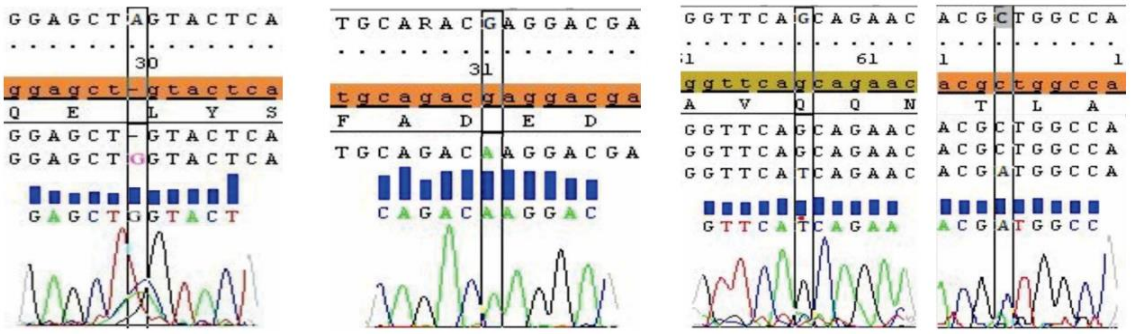
The first step of this protocol is to locate mutations using the SSCP method (pic. 1). This way it is possible to find out which exons contain mutations even before Sanger sequencing. It helps to save time and money performing necessary minimum of sequencing reactions.



Picture 1. Track number 6 shows abnormal SSCP pattern.

After mutations are located, those exons are sequenced using fluorescent dye-based Sanger sequencing and capillary elecrophoresis for sequencing products automated separation and detection. We should mention the fact Sanger sequencing is still considered the golden standard for sequencing quality.

Using this protocol were found several patients with WAS and XLT, includig one case of XLT with multiple mutations inside *WAS* gene (pic. 2).



Picture 2. Example of electrophoregrams from three patients: two with WAS caused by a single mutation and one with XLT caused by two different mutations.

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