A CASE OF REFRACTORY CYTOPENIA AND INFLAMMATORY SYNDROME CAUSED BY HETEROZYGOUS MUTATION OF *AIRE* GENE: IS GENETICS ALWAYS RIGHT?

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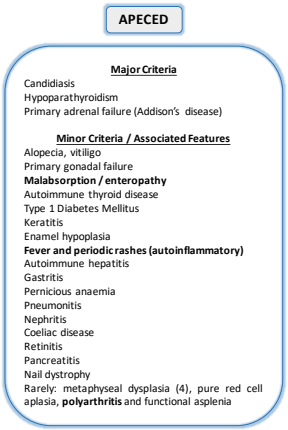
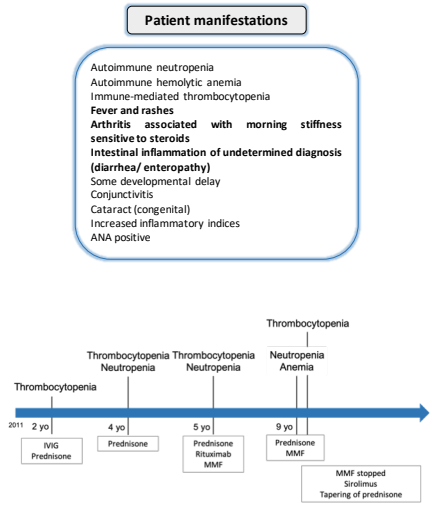
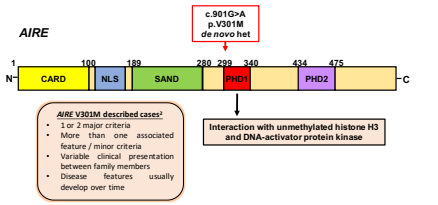
BACKGROUND AND AIMS. We report the case of a 9 years-old boy affected by autoimmune cytopenias and inflammatory symptoms. The patient received prednisone and IVIG, with good control of inflammation for two years. However, he developed severe ITP, refractory to first line therapy. Rituximab was administered with partial response, then mycophenolate mofetil and later sirolimus, with remission. Genetic analysis revealed a heterozygous variant in PHD1 domain of Autoimmune regulator (*AIRE*) gene. Also, the patient had two heterozygous variants of paternal inheritance in *PLCG2* and *UNC13D* genes. We aimed to investigate these results in relation to the patient's clinical, molecular and immunological phenotypes, and his response to treatment.

METHODS. Phenotypic analysis of T, B and NK cell subpopulations was carried out by flow cytometry (Miltenyi) and data analyzed with FlowLogic Software (Inivai). Genetic analysis of a panel of genes involved in immune dysregulation was carried out using a NimbleGen SeqCap EZ Choice Design (Roche) and the Illumina MiSeq platform for sequencing, using MiSeq Reagent Kit v3 and a 150 bp paired-end chemistry (Illumina). Data were analysed using an in-house pipeline and prioritization was made by selecting presence/absence in population databases (ExAc, ESP6500, 1000G, gnomAD), MAF, putative damaging effect (by SIFT, polyPhen, MutTaster, MutAssessor, CADD and FATHMM score). Putative causative variants were analysed by Sanger sequencing to confirm the NGS results and investigated in the parents to check their inheritance status.







IMMUNOPHENOTYPING

Population	Markers	2018	2020	HD (5)
Lymphocytes	FSC/SSC (%CD45)	16,49	26,2	43,3 (26-49,6)
T cells	CD3+ (% Lymphocytes)	78,33	72,72	73 (55-97)
CD4 helper T cells	CD3+ CD4+ (% Lymphocytes)	56,9	31,09	40 (26-61)
CD8 cytotoxic T cells	CD3+ CD8+ (% Lymphocytes)	12,66	18,85	25 (13-47)
TCRab double negative T	CD4- CD8- TCRab (%CD3)	0,48	0,55	<2%
Activated T cells	CD3+ HLA-DR+ (% Lymphocytes)	1,76	1,35	7 (3-14)
Naive helper T cells	CD4+ CD45RA+ CD27+ (% CD4)	78,95	44,85	67 (46-99)
Central memory helper T cells	CD4+ CD45RA- CD27+ (% CD4)	18,57	50,61	18 (0,35-100)
Effector memory helper T cells	CD4+CD45RA-CD27- (% CD4)	1,9	4,51	2 (0,27-18)
Terminally differentiated helper T cells	CD4+ CD45RA+ CD27- (% CD4)	0,57	0,03	0,1 (0,0031-1,8)
CD4 Recent thymic emigrants	CD4+ CD45RA+ CD31+ (% CD4)	41,81	14,59	58 (41-81)
Naive cytotoxic T cells	CD8+ CD45RA+ CD27+ (% CD8)	65,78	70,33	75,8 (57-83,7)
Central memory cytotoxic T cells	CD8+ CD45RA- CD27+ (% CD8)	13,47	22,67	16,6 (9,2-22,6)
Effector memory cytotoxic T cells	CD8+ CD45RA- CD27- (% CD8)	6,57	2,16	2,3 (0,7-14)
Terminally differentiated cytotoxic T cells	CD8+ CD45RA+ CD27- (% CD8)	14,18	4,84	3,8 (0,9-17,9)
B cells	CD19 (% Lymphocytes)	7,62	14,62	12 (4-33)
Class switch B cells	CD19+CD27+IgD- (%CD19)	1,94	9,3	6,5 (5,2-12,1)
Naive B cells	CD19+CD27-IgD+ (%CD19)	95,74	79,61	75,4 (69,4-80,4)
Marginal-zone like B	CD19+CD27+IgD+ (%CD19)	2,13	2,5	10 (7,5-12,4)
Transitional B cells	CD19+CD38+CD24+ (%CD19)	19,77	n.a	6 (4,5-9,2)
CD21low	CD19+CD38-CD21+ (%CD19)	0,78	n.a.	2,3 (0,9-3,5)
Plasmablasts	CD19+CD38++IgM- (%CD19)	1,36	n.a.	1,5 (0,7-3,5)
NK	CD56+ CD3- (% Lymphocytes)	4,54	6,44	8 (2-31)

RESULTS

- Genetic analysis revealed a *de novo AIRE* V301M heterozygous variant, as well as *PLCG2* P522R and *UNC13D* V781I heterozygous variants of paternal inheritance.
- PLCG2 expression and function was normal, and NK functional tests showed no defect; patient's father is healthy.
- Impaired B cell differentiation and very low activated T cells in PB
- APECED-associated auto-antibodies testing was negative

DISCUSSION

- *AIRE* defects cause APECED, a rare monogenic recessive disorder of immune dysregulation. Bi-allelic *AIRE* mutations are associated with complete phenotype, while heterozygous variants have been associated with milder phenotype[1].
- Clinical manifestations may develop over time[2,3] and are variable in family members carrying the same mutation, suggesting that other genes and environmental factors may affect the disease phenotype.
- Mutations in PHD1 domain cause different autoimmune phenotypes, including late onset APECED and isolated organ-specific autoimmunity, and act in a dominant negative manner, usually compound heterozygous[2].
- Acute onset of autoimmune cytopenias is not "typical" for APECED, as well as B cell impairment.
- *AIRE* V301M variant in our patient indicates that it is probably disease modifying than disease causing, and WES is ongoing to unravel other possible causative genes.

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