Two Cases of B-Cell Deficiency Associated with *IGLL1* Variants Identified Through Newborn Screening in Ukraine

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INTRODUCTION

- The kappa-deleting recombination excision circles (KREC) assay in newborn screening (NBS) facilitates the identification of conditions associated with B-cell lymphopenia (Fig.1). The use of the KREC assay has been controversial and less commonly implemented compared to TREC assay. We present two cases of children with positive KREC screening results to highlight the importance of early detection and support the consideration of this assay for global indication
- **The aim of our study** was to present two additional cases of B-cell lymphopenia associated with *IGLL1* variants identified through NBS in Ukraine to highlight the importance of early detection and further support the consideration of the KREC assay for global implementation in newborn screening programs to identify early B-cell development defects.

CASE PRESENTATION

- **Case 1:** A full-term, healthy female newborn had a positive NBS result with undetectable KREC but normal TREC levels at birth.
- Follow-up revealed low B cell counts (70 cells/μL) but preserved T and NK cell subsets at 3 months. Immunoglobulin levels (IgA, IgM, IgG) remained within normal ranges at 3, 6, and 11 months of age (Table 1).
- No severe infections occurred until 1 year.
- Vaccine responses were notable for normal tetanus antibody titers but borderline diphtheria titers.
- Genetic testing identified variants of uncertain significance (VUS) in the *IGLL1* gene (Fig.2): the one allele with **c.425C>T** (p.Pro142Leu) and the other with c.368C>G (p.Ser123Cys) and **c.377T>C** (p.Leu126Pro).

Table 1. Immunologic parameters in case 1.

Parameter	20.03.24	25.06.24	23.10.24	Normal range
	3 mo	6 mo	10 mo	
CD3, %	85.7	88,8	83,1	50-76
CD3, cells/μL	3330	2620	2720	1800-6500
CD4, %	63.8	72,2	61,6	35-57
CD4,_cells/μL	2570	2010	2090	1200-4600
CD8, %	19.1	**14.6**	21.5	16-34
CD8, cells/μL	770	**410**	730	700-2400
CD19, %	**2**	**1.8**	**4.1**	17-32
CD19, cells/μL	**70**	**57**	**128**	500-2200
CD16/56, %	11.6	8.3	10.3	4-16
CD16/56 /mcl	440	258	325	100-900
IgA, g/l	0.15	0.17	0.25	0.02-0.83
IgM, g/l	0.64	0.64	1.27	0.03-1.45
IgG, g/l	3.9	2.5	4.2	2.32-14.11
IgE, IU/ml	<1.5	3.0	3.3	<8
Complement activity, CH50	67	65	80	19-65
Antibodies to diphtheria toxoid IgG, U/ml		**0.029**	0.020	<0.01 neg 0.01-0.099 doubtful
Antibodies to tetanus toxoid IgG, U/ml		0.28		<0.10 neg >0.11 positive

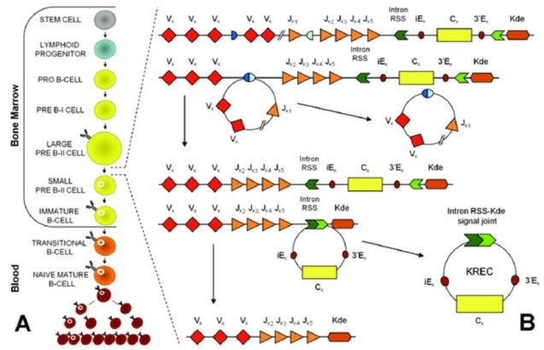

Figure 1. B-cell differentiation and K-deleting recombination excision circle formation (Chiarini M. et al., 2013)

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CASE PRESENTATION

- **Case 2:** A full-term healthy male newborn also presented with undetectable KREC but normal TREC.
- B-cell counts were low at one month (97 cells/μL) and decreased at seven months (68 cells/μL), with normal T and NK cell subsets.
- A transient IgG decline was noted at3.5 months and increased to low normal by 7 months (Table 2).
- Genetic testing revealed two IGLL1 variants on separate alleles: a missense VUS **c.425C>T** (p.Pro142Leu) and a likely pathogenic nonsense variant **c.258del** (p. Gln88Asnfs∗7). Immunoglobulin replacement therapy.
- Comparison of patients identified through NBS using the KREC assay, clinically diagnosed patients, and those identified as siblings or parents is shown in Table 3.

Table 2. Immunologic parameters in case 2.

Parameter	12.06.23	01.03.24	19.06.24	Normal range
	1 mo	3.5 mos	7 mos	
CD3, %	71.57		86.1	50-76
CD3, cells/μL	4798		4476	1800-6500
CD4, %	45.7		58.1	35-57
CD4, cells/μL	3065		3021	1200-4600
CD8, %	23		24.6	16-34
CD8, cells/μL	1542		1279	700-2400
CD19, %	**1.4**		**1.3**	17-32
CD19, cells/μL	**97**		**68**	500-2200
CD16/56, %	25		12,6	4-16
CD16/56, cells/μL	1720		655	100-900
IgA, g/l	0.06	0.07	0.84	0.02-0.83
IgM, g/l	0.03	0,46	0.41	0.03-1.45
IgG, g/l	4.3	**1.77**	**2.5**	2.32-14.11
IgE, IU/ml			5.4	<8

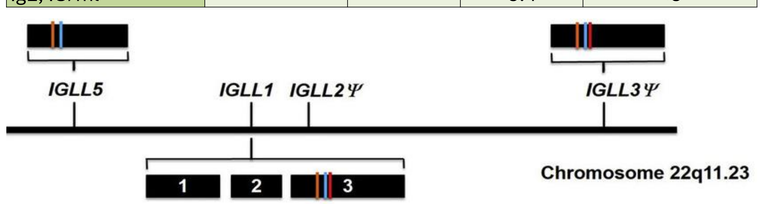

Figure 2. *IGLL1* gene structure (Gemayel et al. 2016.)

Table 3. Comparison of cases with *IGLL1* variants identified by NBS, clinically diagnosed, and diagnosed in siblings or parents

Characteristic	Patients identified by NBS n=15	Clinically diagnosed n=6	Diagnosed in siblings or parents n=4
Baseline characteristic		Me (range) or n (%)	
Age at immunologic diagnosis	3 (2-18) weeks	2.5 (0.2-8) years	8.5 (2-34) years
Sex, M/F	8/7	3/3	1/3
Clinical presentation			
Symptomatic	14 (93.3)	6 (100)	0
Mild URTI	12 (80.0)	3 (50.0)	No
LRTI	0	4 (66.7)	No
Mild GI infection	2 (13.3)	1 (16.7)	No
Other infections	No	2 (33.4) Meningitis, prolonged varicella, UTI, sepsis	1 (25.0) Conjunctivitis
Complications	No	2 (33.4) Bronchiectasis, conductive hearing loss, peritonitis	No
Atopy	5 (33.3)	No	1 (25.0)
Autoimmunity	No	No	No
Malignancy	1 (6.7)	No	No
Syndromic features	2 (13.3) duplex kidney, ASD	1 (16.7) pancreatic insufficiency, FTS, degenerative muscle disease, neuropathy	No
Transient neutropenia	11 (73.3)	1/2 (50.0)	No
Thrombocytosis at first year of life	14 (93.3)	1/2 (50.0)	NA
B-cells range, cells/μL	0-230	0-94	18-167
Normal IgG level at initial investigation	14 (93.3)	1 (16.6)	2/2 (100)
Low IgM level at initial investigation	13 (86.7)	6 (100)	0/2 (0)
Low IgA level	13 (86.7)	6 (100)	0/2 (0)
Genetic diagnosis			
Age at genetic diagnosis	2 (1-6) mos	3.5 (0,6-15) years	9.5 (2-34) years
Treatment			
IgRT	12 (92.3)	6 (100)	0 (0)
Age at IgRT, months	4.5 (1-6)	21.5 (3-96)	no
References	1	2-7	1, 4, 5

CONCLUSION

- Thus, this study highlights the potential underdiagnosis of B-cell lymphopenia secondary to *IGLL1* variants. Furthermore, the comparison between clinically diagnosed cases and those identified through neonatal screening underscores the importance of early diagnosis. Early detection allows for close monitoring of these patients from birth, timely initiation of IgRT, and prevention of complications and severe manifestations.
- Identification of ARA associated with *IGLL1* variants through neonatal screening, along with long-term monitoring of affected patients, will expand our understanding of the disease's course and improve care for these patients.

References: 1. PMID: 39147326; 2. PMID: 27576013; 3. PMID: 9419212; 4. PMID: 39549297; 5. PMID: 25502423; 6. PMID: 34619682; 7. PMID: 28769069.