The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Deletion in the BCL11B gene and intellectual developmental disorder with speech delay, dysmorphic facies, and t-cell abnormalities – a case report

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ARTICLE INFO

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Key words:
BCL11B gene mutation, type 2 innate lymphoid cells, facial dysmorphia, immune disorder

ABSTRACT

Herein we described a retrospective analysis of a 13-year-old female patient with facial dysmorphia and immune disorder caused by BCL11B gene mutation. The patient upon physical examination presented a particular face (thin eyebrows, small mandible, and widened eye distance), delayed language and motor development. Supplementary examination showed expansion of CD8+, absence of type 2 Innate Lymphoid Cells, increased IgG and altered distribution of T cells. Genetic testing revealed a heterozygous frameshift variation in exon 4 of the BCL11B gene; c.1887_c.1893delCGGCGGG (p.Gly630Glyfs*91). Finally, a BCL11B gene mutation could lead to abnormal development of the nervous and immune systems, therefore, it is necessary to consider this syndrome in patients with the clinical and immunological phenotype described below.

INTRODUCTION

The Chromatin Remodeling Complex Subunit BCL11B is essential for the normal development of the immune and central nervous system, participating in processes of activation, differentiation, cell apoptosis and working as a modulator of early thymocyte development [1].

Currently, the scientific literature has few reports on BCL11B germline variants, Punwani et al. (2016) reported a male infant carrying a nonsense mutation in BCL11B with severe developmental delay, absent corpus callosum, craniofacial anomalies, and severe combined immunodeficiency (SCID)[2], while Lessel et al. (2018) reported 13 patients with distinct heterozygous mutations in BCL11B, all exhibit intellectual disability, developmental delay, and impaired T-cell development [3].

Finally, variants in BCL11B are associated with an autosomal dominant pattern of inheritance with immunodeficiency 49 (IMD49), describing a syndrome produced by a heterozygous mutation in this gene. IMD49 is related to severe combined immunodeficiency and intellectual disability. In the same manner is associated with Intellectual developmental disorder with dysmorphic facies, speech delay, and T-cell abnormalities [4].

CLINICAL CASE

A 13-year-old female patient, result of a twin pregnancy, with no paternal history of consanguinity or autoimmune diseases had a history of late independent ambulation (17 months), delay in language development (single words at 41 months), abnormal psychomotor development accompanied by dysmorphic facies, psoriasiform dermatitis with focal spongiosis, compatible with subacute eczema, left lacrimal obstruction and primary VZV infection during childhood.

She was admitted at nine months due to vomiting, liquid stools and significant paleness. A battery of laboratory tests was carried out where normochromic-normocytic anemia was observed, accompanied by consumption of haptoglobins, a positive direct Coombs test (IgG) and positive irregular antibodies. Eventually, autoimmune hemolytic anemia (AHA) was diagnosed. One month later, she was admitted to the emergency room due to symptoms similar to the one described previously, where a hemoglobin (Hb) of 5.3mg/dl was found, which later dropped to 3.7mg/dl. These AHA episodes later recurred at one year of life. Two years later, she was admitted due to ecchymosis secondary to a viral illness and was diagnosed with idiopathic thrombocytopenic purpura (ITP). Finally, the diagnosis of Evans syndrome was established, and the patient remained under closed control until she was five years old, when she again presented with ecchymosis and petechial accompanied by platelets $<10{,}000/mm^3$.

At six years of age, the patient was diagnosed with hypothyroidism due to autoimmune thyroid diseases (AITD), short stature and precocious puberty, for which replacement therapy was started with Levothyroxine 75 mcg/day and monthly administration of Decapeptyl 0.8 ml. AITD was diagnosed due to positivity to thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TgAb) and thyroid stimulating hormone receptor antibodies (TRAb).

At the age of nine, the patient was again admitted due to petechial on the chest and extremities, accompanied by intermittent abdominal pain, with the symptoms ceasing after administration of immunosuppressive treatment.

During that year, the patient debuted with vesicular and some bullous lesions grouped on an erythematous base compatible with herpes zoster, affecting several dermatomes from L2 to L5. Initially treated with oral and topical medication, but due to lack of response, she was

admitted for intravenous treatment with subsequent improvement.

Additionally, at 13, she was admitted with a platelet count of 6,000 per µl and symptoms similar to previous events. During this episode, the patient was referred to the clinical immunology team. The patient presented dysmorphic facial features on physical examination, including myopathic facies, thin eyebrows, small palpebral fissure, prominent nose, extended filter, thin upper lip, and abnormal dentition, with a visible slowing of the verbal processing of information and difficulty in the development of cognitive tasks (Table 1).

From an immunological point of view, an expansion of CD8+ with an anergic/senescent phenotype (CD8+CD62L-CD45RA+), absence of type 2 Innate Lymphoid Cells (ILC), increased IgG, altered distribution of T cells, low levels of NK cells (Figure 1, Table 2) and anti-nuclear antibodies (homogeneous pattern AC-1 ICAP 1/320) was observed. Extractable nuclear antibodies (ENA) test was performed obtaining positivity to anti-SSa/Ro, more specifically for Ro52 cytoplasmatic protein, anti dsDNA, anti-histone or anti nucleosome antibody tests were not performed. Even though the pathogenic role of the Ro antigen in autoimmune disorders is not fully dilucidated [5] it has been reported in few patients with autoimmune thyroiditis [6].

Previous cytogenetic analysis revealed normal female karyotype. Chromosomal array analysis by comparative genomic hybridization (CGH) was performed with negative result for pathogenic copy number variations. Genomic DNA of the proband, and both parents was extracted from peripheral blood for genetic analysis.

Clinical whole exome sequencing (WES) was achieved revealing a germline heterozygous deletion within exon 4 of the BCL11B gene. The variation involved a deletion which consequently generated a frameshift mutation in the DNA sequence and a premature stop codon, which is expected to results in a truncated protein

Table 1 Description of the clinical features

Neurological features	Skin disorders	Morphological changes	Autoimmune alteration	Immunological findings
Abnormal psychomotor development (Late independent ambulation)	Psoriasiform dermatitis	Short stature	Evan syndrome (Thrombocytopenia and hemolytic anemia)	Expansion of CD8+ with anergic/ senescent phenotype
Delay in language development	Left lacrimal duct obstruction	Myopathic facies	Autoimmune thyroid disease (AITD)	Deficiency of innate lymphoid cells type 2
Slowing of the verbal processing of information	Primary infection with varicella zoster virus with reactivation later in life	Thin eyebrows	Positive antinuclear antibodies (ANA) with Ro52 positive	Altered distribution of T cells
Difficulty in the development of cognitive tasks		Small palpebral fissure		Low NK cells
		Prominent nose		
		Thin upper lip		
		Abnormal dentition		

Table 2 Analytical results

Determination	Rank	Result
Expanded lymphocyte subpopulations		
CD3	[62.6-80.4%]	↑ 90.38
CD4	[32.6-51.5%]	¯ 30.65
CD8	[19-29%]	↑ 58.56
CD4/CD8	[1.21-2.64%]	¯ 0.52
CD19	[11.9-21%]	¯ 6.72
NK	[4.3-16.2%]	¯ 0.77
Absolute number of lymphocytes	[1340-3173 cells/ul]	↑ 4498
Absolute number CD3	[954-2332 cells/ul]	↑ 4042
Absolute CD4 number	[610-1446 cells/ul]	1379
Absolute number CD8	[282-749 cells/ul]	↑ 2634
Absolute number CD19	[173-685 cells/ul]	299
Absolute number NK	[87-504 cells/ul]	¯ 3. 4
Expanded memory T subpopulations		
T Recent Thymic Migrants Cells	[31-81%]	¯ 9.26
CD4 naïve	[37-97%]	¯ 21.32
CD4 TCM	[13-76%]	22.93
CD4 TEM	[0.49-25%]	↑ 54.71
CD4 TEMRA	[0-5.8%]	1.05

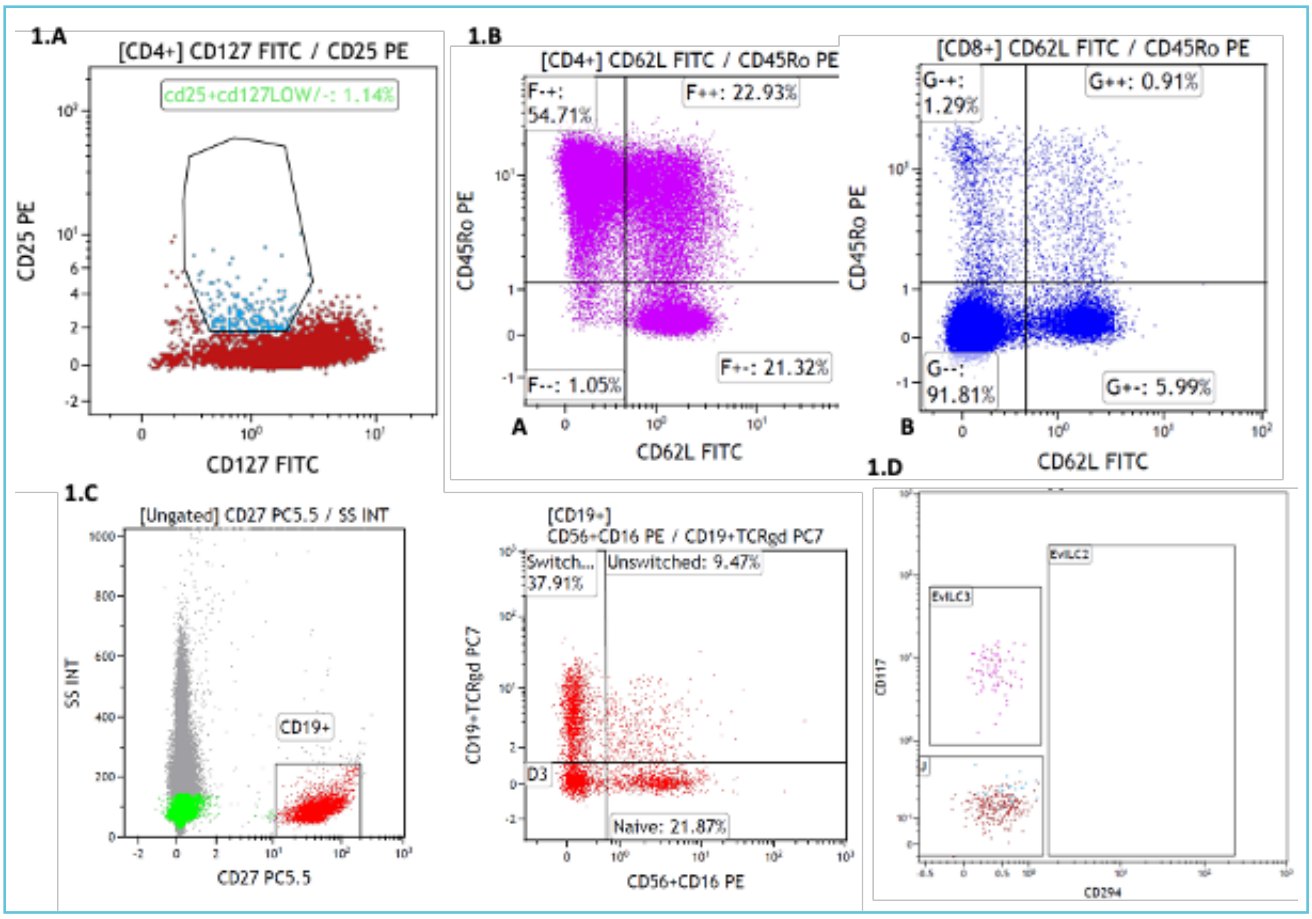
CD4 Regulatory T cells	[4-20%]	¯ 1.14
CD8 naïve	[20-95%]	¯ 5.99
CD8 TCM	[0.42-18%]	0.91
CD8 TEM	[4-100%]	¯ 1.29
CD8 TEMRA	[9-65%]	↑ 91.81
T Recent Thymic Migrants Cells Abs	[150-1500 cells/ul]	374
CD4 naïve Abs	[200-1700 cells/ul]	294
CD4 TCM Abs	[120-740 cells/ul]	316
CD4 TEM Abs	[5-210 cells/ul]	↑ 754
CD4 TEMRA Abs	[0-51 cells/ul]	14
CD4 Regulatory T cells Abs	[33-190 cells/ul]	¯ 16
CD8 naïve Abs	[78-640 cells/ul]	158
CD8 TCM Abs	[16-810 cells/ul]	24
CD8 TEM Abs	[16-810 cells/ul]	¯ 3. 4
CD8 TEMRA Abs	[35-420 cells/ul]	↑ 2418
Innate Lymphoid Cells (ILCs) subpopulation		
ILC 1	%	0.172
ILC 2	%	¯ 0
ILC 3	%	0.132

Absolute number, Abs. Terminally Differentiated Effector Memory T Cells, TEMRA. T helper cells, Th. Innate lymphoid cells, ILCs. Central Memory T Cells, TCM. Effector memory T cells, TEM.

¯,↑ Used to illustrate levels of the different determination, low and high respectively.

(NM_138576.3: c.1887_1893delCGGCGGG; p.(Gly630Thrfs*91). The variant was absent from the gnomAD databases and pathogenicity score prediction software analysis disclosed that the variant could be a disease causing variant. The variant has been previously reported as pathogenic in a 2-year-old boy and is described in the ClinVar database as a pathogenic variant (ID 1275761) diagnosing Intellectual Developmental Disorder with Speech Delay, Dysmorphic Facies, and T-Cell Abnormalities [1]. PCR and Sanger sequencing was finally applied to validate the mutation and the pedigree analysis (Figure 2.A). The results showed that the mutation in BCL11B arose de novo, which was not observed in both parents (Figure 2. B). This study was approved by the Ethics Committee of the Marques de Valdecilla University Hospital, and informed written consent was obtained from the parents of the patient.

Figure 1 **Flow cytometry panel of peripheral blood mononuclear cells (PBMCs) obtained from index case**



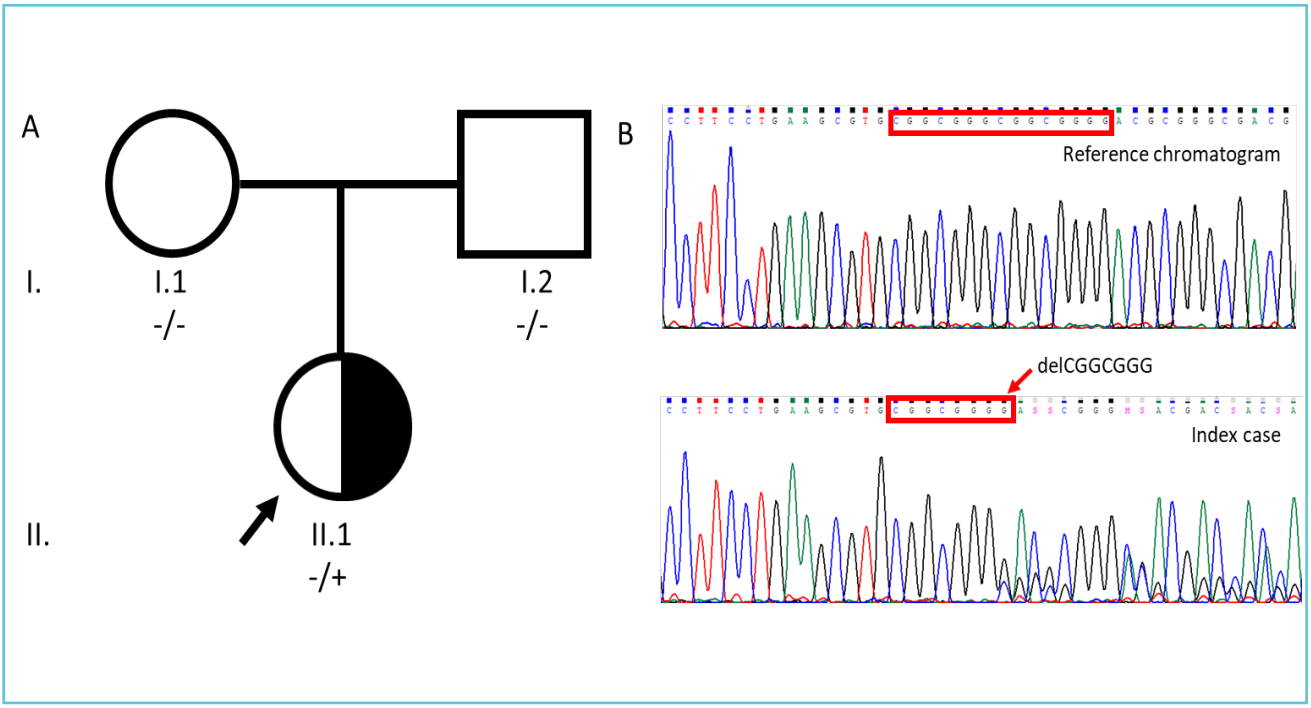
***Graph 1.A**, Panel for regulatory T cells (T reg), obtaining a total of 1.14% of CD4+CD25+CD27low cells.*

***Graph 1.B**, Panel for subtypes of memory T cells (T men), obtaining a total of CD4+ (A) lymphocytes (purple) of 21.32% naive T cells; 22.93% of central memory T cells (TCM); 54.71 of effector memory T cells (TEM) and 1.05% of recent thymic migrating T cells (TEMRA). For CD8 (B) lymphocytes (blue), 5.99% of naive T cells; 0.91% of TCM; 1.29% of TEM and 91.81% of TEMRA.*

***Graph 1.C**, Panel for B memory (B men), obtaining a total of 21.87% of naive B cells; 9.47% of unswitched B cells and 37.91 of switched B cells.*

***Graph 1.D**, panel for innate lymphoid cells (ILCs), obtaining an absence of type 2 ILCs (EvILC2).*

Figure 2 Genetic study of the index case



A. *Pedigree and mutation analysis of the family. Deletion arose de novo in heterozygosity in affected proband pointed with an arrow.*

B*. Sanger sequencing showing that the index case (II.1) carried a frameshift mutation (c.1887_1893delCGGCGGG), which was not found in reference chromatogram observed in both parents (I.1 and I.2).*

DISCUSSION

Variants in BCL11B are associated with Intellectual Developmental Disorder with Speech Delay, Dysmorphic Facies, and T-Cell Abnormalities. This syndrome is characterized by psychomotor developmental delay, intellectual disability, speech delay, autistic features, anxiety, and other behavioral abnormalities. Some of the cases described in the literature usually present slight delay in walking, with spasticity and abnormal movements [7][8].

Other peculiarities of this disorder are dysmorphic characteristics, which include thin eyebrows, small palpebral fissure, hypertelorism, epicanthal folds, prominent nose, and dental anomalies. Although this disorder is not an immunodeficiency, frequent infections, allergies or asthma have been observed [8].

From an immunological perspective, these patients may present an increase in eosinophils, alterations in the T cell compartment, a decrease in type 2 ILCs and, in some cases, exaggerated response of T helper 2 cells. Importantly, in our case, we described low counts of NK cells with a reshaping in the distribution of T cell. Alteration levels in NK cell has been previously discussed by Holmes TD et al (2021) [9]. This paper exposed the possible role of BCL11b in driving the differentiation of NK cells, finding that loss of BCL11b could substantially impact the generation and durability of mouse and human adaptive NK cells, suggesting the role of this gene in adaptive NK cell differentiation [9].

We also described variations in the percentage and total number of T cell lymphocyte, finding elevated absolute number of T cells lymphocyte, but more importantly, low levels of CD4+,

CD8+ naïve T cells, and regulatory T cells, resembling data described by Yang, Sai et al (2020) [10]. This discrepancy of our results, with few clinical cases reported in the literature, exposed the heterogeneity of this syndrome, and the function of the gene. Admittedly, BCL11b deficient mice show an arrest at the double negative stage of the thymocyte development [11]. Surprisingly, according to Lessel, Davor et al (2018), all analyzed individuals in their cohort (n=13) exhibited a severe reduction of peripheral ILC2 and impaired T cell development, but only two described low T cell percentage [3].

The variant identified in heterozygosis it is a loss-of-function variant that generates a premature stop codon that presumably causes the loss of 175 amino acids of the normal protein, thus being able to generate a non-functional truncated protein [12].

Our study of the patient with a mutation in BCL11B adds new data to the small group of subjects reported with this pathology, thus contributing to clinical advice and surveillance of this group of patients.

LEARNING POINTS

- Mutation on BCL11B must be considered when patients present a physical or immunological phenotype similar to the one we previously described.
- A heterozygous variant within exon 4, NM_138576.3: c.1887_1893delCGGCGGG p. (Gly630Thrfs*91) of the BCL11B gene has been currently described in the literature as a plausible cause of this syndrome.
- A multidisciplinary approach to patients with Intellectual Developmental Disorder with Speech Delay, Dysmorphic Facies, And T-Cell Abnormalities, including immunologists, hematologists, pediatricians, etc., is essential for a comprehensive approach to this syndrome.

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Disclosure

All authors contributed significantly to the analysis and interpretation of data, revising and approving the article. All authors declare no conflicts of interest.

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REFERENCES

1. Yan, S.; Wei, Y.S.; Yang, Q.Y.; Yang, L.; Zeng, T.; Tang, X.M.; Zhao, X.D.; An, Y.F. [A case report of BCL11B mutation induced neurodevelopmental disorder and literature review]. Zhonghua er ke za zhi = Chinese J. Pediatr. 2020, 58, 223–227, doi:10.3760/CMA.J.ISSN.0578-1310.2020.03.012.

2. Punwani, D.; Zhang, Y.; Yu, J.; Cowan, M.J.; Rana, S.; Kwan, A.; Adhikari, A.N.; Lizama, C.O.; Mendelsohn, B.A.; Fahl, S.P.; et al. Multisystem Anomalies in Severe Combined Immunodeficiency with Mutant BCL11B. N. Engl. J. Med. 2016, 375, 2165–2176, doi:10.1056/NEJMOA1509164.

3. Lessel, D.; Gehbauer, C.; Bramswig, N.C.; Schluth-Bolard, C.; Venkataramanappa, S.; van Gassen, K.L.I.; Hempel, M.; Haack, T.B.; Baresic, A.; Genetti, C.A.; et al. BCL11B mutations in patients affected by a neurodevelopmental disorder with reduced type 2 innate lymphoid cells. Brain 2018, 141, 2299–2311, doi:10.1093/BRAIN/AWY173.

4. Qiao, F.; Wang, C.; Luo, C.; Wang, Y.; Shao, B.; Tan, J.; Hu, P.; Xu, Z. A De Novo heterozygous frameshift mutation identified in BCL11B causes neurodevelopmental disorder by whole exome sequencing. Mol. Genet. genomic Med. 2019, 7, doi:10.1002/MGG3.897.

5. Yoshimi, R.; Ueda, A.; Ozato, K.; Ishigatsubo, Y. Clinical and pathological roles of Ro/SSA autoantibody system. Clin. Dev. Immunol. 2012, 2012, doi:10.1155/2012/606195.

6. Miyagawa, S.; Tanaka, M.; Okamoto, S.; Ishihara, T.; Nakajima, M.; Taira, K.; Yoshioka, A.; Asada, H. Autoimmune thyroid disease in anti-Ro/SS-A-positive children with annular erythema: report of two cases. Br. J. Dermatol. 2004, 150, 1005–1008, doi:10.1111/J.1365-2133.2004.05926.X.

7. Daher, M.T.; Bausero, P.; Agbulut, O.; Li, Z.; Parlakian, A. Bcl11b/Ctip2 in Skin, Tooth, and Craniofacial System. Front. cell Dev. Biol. 2020, 8, doi:10.3389/FCELL.2020.581674.

8. Prasad, M.; Balci, T.B.; Prasad, C.; Andrews, J.D.; Lee, R.; Jurkiewicz, M.T.; Napier, M.P.; Colaiacovo, S.; Guillen Sacoto, M.J.; Karp, N. BCL11B-related disorder in two canadian children: Expanding the clinical phenotype. Eur. J. Med. Genet. 2020, 63, doi:10.1016/J.EJMG.2020.104007.

9. Holmes, T.D.; Pandey, R.V.; Helm, E.Y.; Schlums, H.; Han, H.; Campbell, T.M.; Drashansky, T.T.; Chiang, S.; Wu, C.Y.; Tao, C.; et al. The transcription factor Bcl11b promotes both canonical and adaptive NK cell differentiation. Sci. Immunol. 2021, 6, doi:10.1126/SCIIMMUNOL.ABC9801.

10. Yang, S.; Kang, Q.; Hou, Y.; Wang, L.; Li, L.; Liu, S.; Liao, H.; Cao, Z.; Yang, L.; Xiao, Z. Mutant BCL11B in a Patient With a Neurodevelopmental Disorder and T-Cell Abnormalities. Front. Pediatr. 2020, 8, doi:10.3389/FPED.2020.544894.

11. Wakabayashi, Y.; Watanabe, H.; Inoue, J.; Takeda, N.; Sakata, J.; Mishima, Y.; Hitomi, J.; Yamamoto, T.; Utsuyama, M.; Niwa, O.; et al. Bcl11b is required for differentiation and survival of alphabeta T lymphocytes. Nat. Immunol. 2003, 4, 533–539, doi:10.1038/NI927.

12. Walker, J.A.; Oliphant, C.J.; Englezakis, A.; Yu, Y.; Clare, S.; Rodewald, H.R.; Belz, G.; Liu, P.; Fallon, P.G.; McKenzie, A.N.J. Bcl11b is essential for group 2 innate lymphoid cell development. J. Exp. Med. 2015, 212, 875–882, doi:10.1084/JEM.20142224.