Supplementary data

Title:
Lack of NKG2D in MAGT1-deficient patients is caused by hypoglycosylation

Authors

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Clinical synopsis of patient 1

Patient 1 (also referred to as patient 3 (P3) in Blommaert et al. 2019) is a 20-year-old Caucasian male, born from non-consanguineous parents (pedigree depicted in **Fig. S2**). The patient presented with an Epstein-Barr virus (EBV) primary infection at the age of 2, necessitating admission to the paediatric intensive care unit due to deterioration of his general condition, a petechial rash and pancytopenia with hypertriglyceridemia and mild coagulation abnormalities. Next, also a maculopapular rash and generalized oedema was noted. Bone marrow aspirate demonstrated absence of malignancy and mostly abundant granulocyte formation. Oxygen, antibiotics and IV fluid treatment was installed with a favourable evolution except for persistent hepatomegaly. A liver biopsy, performed after his stay at the paediatric intensive care unit, did not show hepatitis or hematophagocytosis. Serum ferritin, soluble CD25 was not determined. This episode was interpreted as a primary EBV infection, complicated with a macrophage activation syndrome. Afterwards, a persistent EBV viremia was observed (**Table S1** and **Fig. 3B**). Since the age of 8, recurrent herpes simplex virus (HSV) stomatitis episodes, treated with acyclovir, and HSV labialis (every 2-3 months) were noted. During childhood (age 10 years and onwards) he had recurrent warts (predominant on the hands and arms) that subsided later in adolescence. Recurrent furuncles were noted in childhood and adolescence, and post-appendectomy infection required IV antibiotics (age 10 years). Finally, he mentioned upper respiratory tract infections, sporadically leading to antibiotic treatment via his general practitioner (GP).

Clinical examination was normal at time point T0 (17 years, 10 months). Of note, no palpable glands were noted although recurrent enlarged cervical lymph nodes were mentioned and ultrasound revealed a spleen diameter at the upper limit of normal (cranial-caudal diameter of 12.4 cm, age 19, body height 1.77 m). Laboratory results demonstrated mild neutropenia, thrombocytopenia, lowered IgG (7.46 g/L, normal range (NR) 7.51-15.60), elevated transaminases, and persistent EBV-viremia (**Table S1** and **Fig. 3B**). Further immunological evaluation demonstrated normal total lymphocyte, T-, and NK-cell count, elevated $CD19^+$ B-cell count (1542/µL, NR 82 - 0.476), consisting predominantly of naive (94% $CD27^-IgM^+$) B-cells, next to lowered IgM-memory ($CD27^+IgM^-$, 2.2%) and switched memory ($CD27^+IgM^-$, 2.8%) B-cells. CD4, CD8 T-cell, regulatory T-cell (8.4% $CD4^+CD25^+CD127_{low}$) counts were normal. CD4:CD8 ratio was normal (0.89, NR 0.80-3.50). Double negative T-cells (DNT, alpha-beta T-cell receptor $CD3^+CD4^-CD8^-$) were elevated (2.8% of CD3), naive T-cell ($CD27^+CD45RA^+$) count was 62% of CD3 positive cells. Lymphocyte proliferation rate was normal using HSV, varicella zoster virus, or PHA (index for HSV was 14.03, for varicella zoster virus 159.41, for PHA 61.12; normal > 5.0). Stimulation with anti-CD3/CD28 on frozen or fresh PBMCs did not result in proliferation of patient lymphocytes.

The patient received standard vaccinations including vaccination with life attenuated measles, mumps, and rubella uneventfully and resulting in normal or near-normal serologic responses (anti-measles IgG 650 mIU/mL; anti-rubella IgG 8.4 IU/mL, normal >10.0 mIU/mL; anti-hepatitis B surface antigen IgG 11.5 mIU/mL, normal ≥ 10.0 mIU/mL; measured at the age of 19 years). Pneumococcal polysaccharide vaccination was refused. Varicella zoster virus and CMV serology demonstrated past infection, PCR for persistent CMV viremia was negative. PCR for polyoma virus (BK/JC virus) in urine was positive (> 8 log copies/mL).

Next generation sequencing revealed a pathogenic variant in the X-linked *MAGT1* gene (c.938T>G, p.Leu313*) at 17 years of age (Blommaert et al. 2019). Given the clinical features of persistent EBV viremia, macrophage activation syndrome during primary infection, decreased NKG2D expression (Blommaert et al. 2019) and the rare, previously unreported nor identified (ExAC database, gnomAD, 1000 genomes) variant in *MAGT1,* a diagnosis of X-linked immunodeficiency with magnesium defect, EBV infection, and neoplasia (XMEN) disease was made.

He was treated with oral magnesium supplementation from the age of 17 years and 10 months onwards using 3 g of Mg^{2+} gluconate (containing 162 mg Mg^{2+}, Ultra-Mg®, Melisana) per day. Increasing the frequency to twice or three times per day led to gastro-intestinal intolerance. Magnesium threonate, as was previously reported as a therapy for XMEN patients (Chaigne-Delalande et al. 2013), was not initiated because of unavailability via the local hospital pharmacy. Since magnesium supplementation, he noted a drop in upper respiratory tract infections and HSV stomatitis episodes (although from hindsight this was already absent the year before). EBV viremia was deemed to be non-responsive to magnesium supplementation (**Fig. 3B**). Of note, elevated transaminases were observed throughout the follow-up with a temporary flare-up coinciding with the start of magnesium supplementation. No changes in total serum magnesium were noted throughout the follow-up (**Table S1**). Subjectively, he felt his general condition was comparable to that of his peers. Although he was reluctant for a frequent clinical follow-up, adherence to magnesium supplementation was high, possibly suggesting (at least subjective) efficacy.

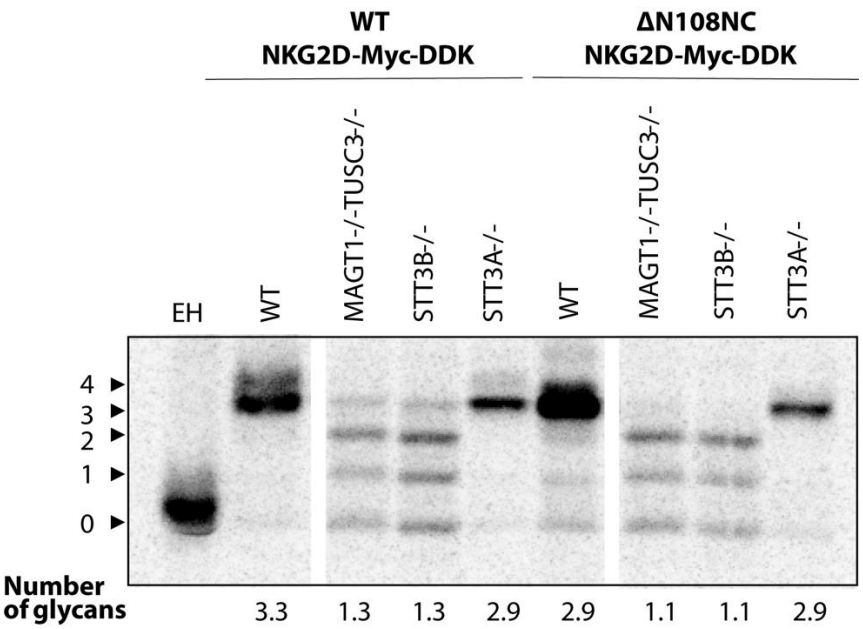

Figure S1: The N_{108}NC glycosylation site is suboptimal.

Metabolic pulse chase labelling of the WT NKG2D-Myc-DDK and the ΔN_{108}NC NKG2D-Myc-DDK construct, in different HEK293 engineered cell lines. Quantified values are shown below gel lanes and represent the number of glycans for the respective reporter. EH indicates Endoglycosidase H treatment, and serves as a mobility marker. White lanes between samples indicates where the figure was spliced.

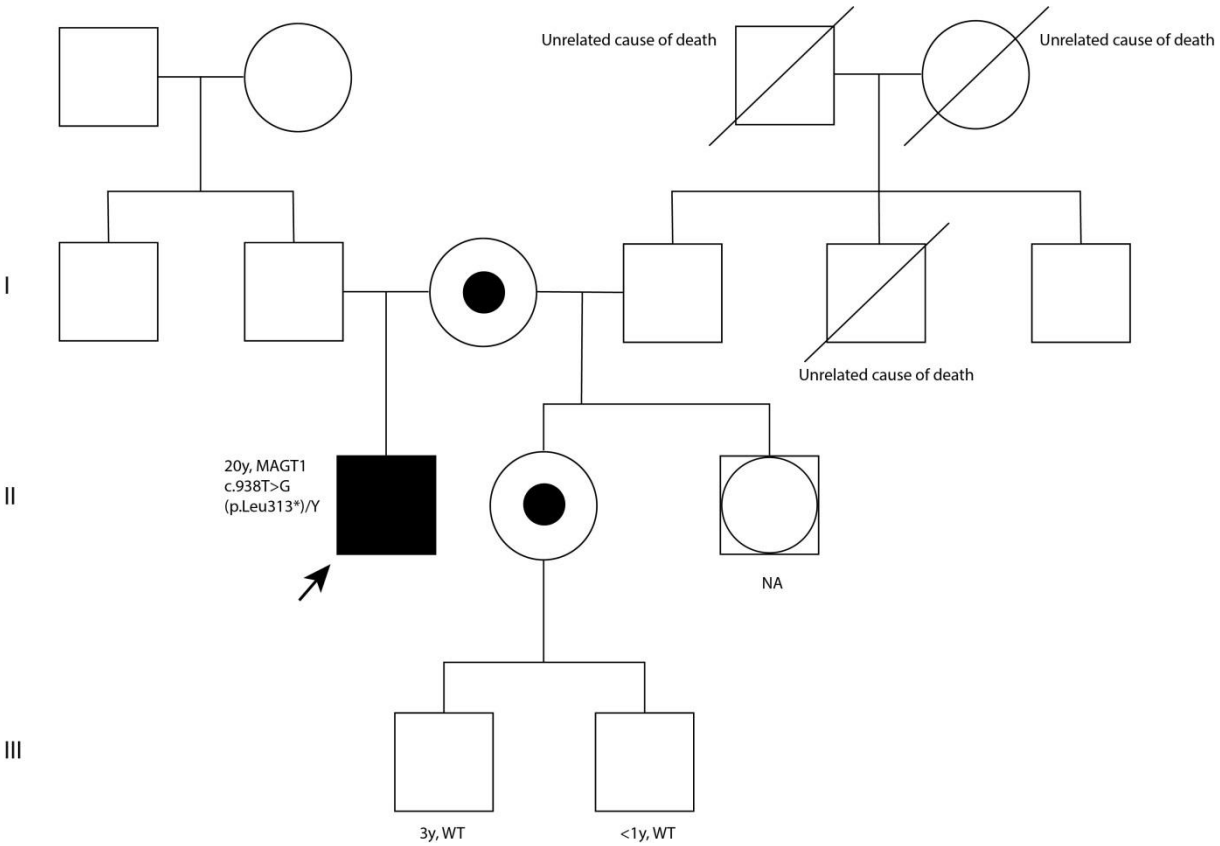

Figure S2: Pedigree of patient 1.

Index case (II.1) is from Caucasian, European descent. No consanguinity was noted and no other affected male family members were identified. Medical history of the other family members was negative except for recurrent nasal and labial herpes simplex virus infection in his half-sibling (II.2, carrier of *MAGT1* c.938T>G), which was regarded as non-exceptional.

Abbreviations: WT, wild type; NA, not available.

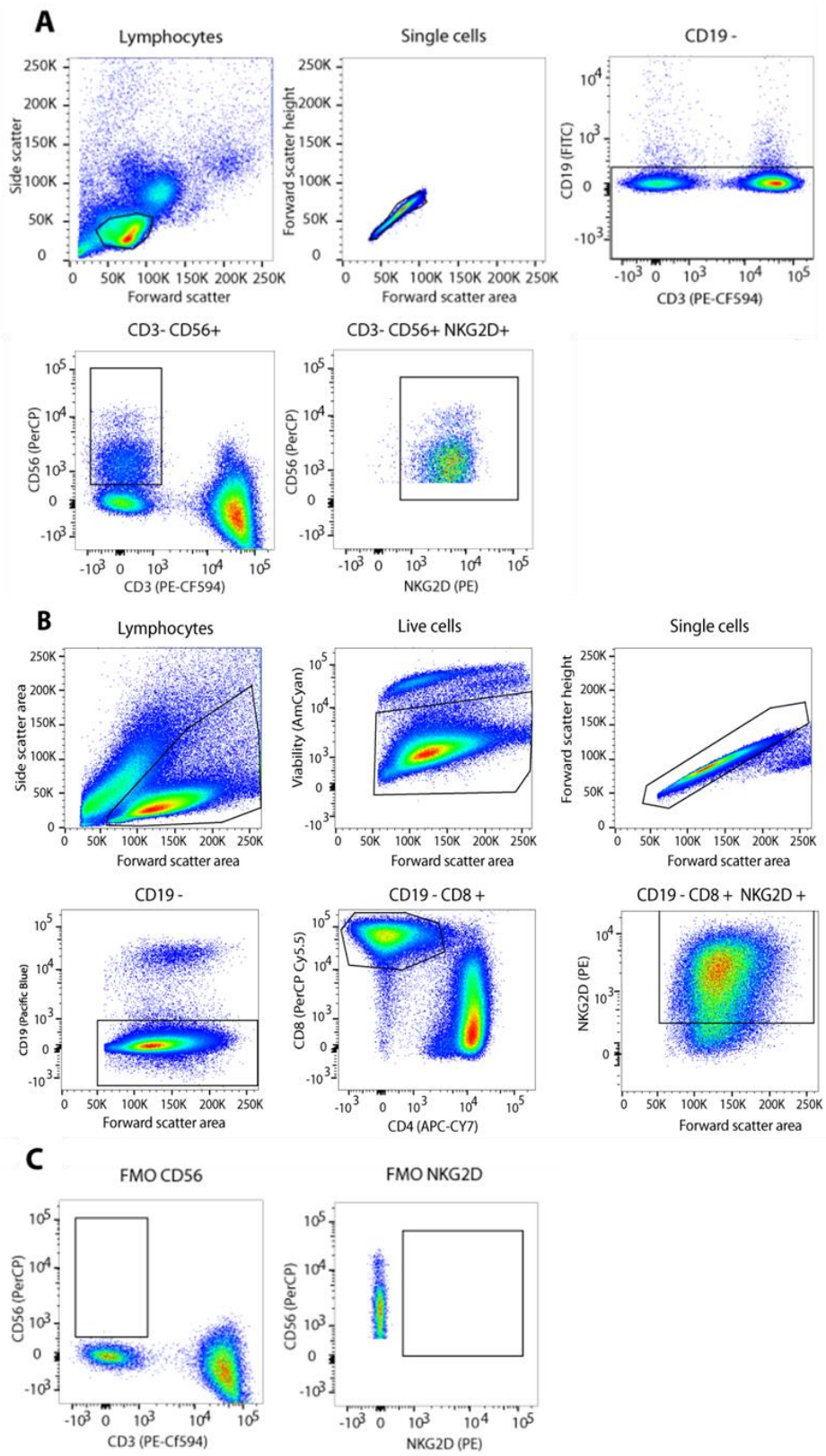

Figure S3: Gating strategy for NKG2D expression levels on NK cell surface.

(A) First, PBMC were selected on both size and singularity. Next, CD19 and CD3 negative cells were gated on expression of CD56 to identify NK cells. Finally, NK cells were gated for expression of NKG2D.

(B) First, PBMC were selected on size, living cells and singularity. Next, CD19 negative cells were gated on expression of CD8 to identify CD8+ cells. These cells were gated for expression of NKG2D;

(C) Fluorescence Minus One (FMO) for CD56 and NKGD2 to determine gates for both proteins.

Table S1: Summary of clinical values for P1 at different time points.

Values outside the normal range are marked in red. EBV = Epstein-Barr Virus, Treg = T regulatory cells

	Reference value	+644 days	+483 days	+287 days	+93 days	+63 days	T0 (17y 10months)
White blood cells	4-10 x10^9/L	4.46	6.43	5.17	4.58	4.59	4.92
Platelets	150-450x10^9/L	132	196	142	177	179	179
Hemoglobin	13.0 -16.0 g/dl	14.0	14.4	14.0	13.8	15.0	14.6
C-reactive protein	≤5 mg/L	0.9	1.0	0.5	1.9	0.8	0.3
Neutrophils	2.5-7.8 x10^9/L	1.5	2.6	1.8	1.4	1.2	1.6
Lymphocytes	20-50 %	58.7	51.6	55.7	59.2	68	59.6
CD19$^+$	0.082-0.476 x10^9/L	1.261	1.619	1.443			1.542
CD4$^+$	0.455-1.885 x10^9/L	0.572	0.646	0.554			0.531
CD8$^+$	0.219-1.124 x10^9/L	0.578	0.741	0.632			0.599
CD4/CD8 ratio	0.8-3.5	0.99	0.87	0.88			0.89
NK (CD3$^-$,CD56$^+$)	4-30 %	4.3	5.7	4.7			4.6
Treg (CD4$^+$, CD125$^+$CD127$_{low}$)	5-12 % of CD4+						8.4
IgG	7.51-15.6 g/l	8.72	8.39	7.46			8.44
IgA	0.82-4.53 g/L	0.46	0.60	0.48			0.52
IgM	0.46-3.04 g/L	0.52	0.48	0.41			0.41
Serum magnesium	0.63-1.05 mmol/L	0.78	0.82	0.78		0.82	
EBV viremia	EBV PCR IU/ml	1095	1667	1260		828	772
Aspartate transaminase	≤38 U/L	38		33	57	81	24
Alanine transaminase	≤41 U/L	76		57	95	100	42
Gamma glutamyl transferase	≤60 U/L	20		19	20	21	19
Alkaline phosphatase	40-130 U/L	112		121	133	149	145
Body mass index	18.5-25 kg/m^2	22.4	22.9	23.2	22.9		22.1