Research Square

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A novel homozygous GINS2 variant with Meier–Gorlin syndrome with clinical and epigenetic characterisation

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Research Article

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Abstract

Background: Meier-Gorlin syndrome (MGS) is a rare disorder of DNA replication characterised by pre- and postnatal growth retardation, microcephaly, patellar hypoplasia, and microtia. Pathogenic variants in genes encoding components of the pre-replication and CMG helicase complexes cause MGS, with emerging genotype–phenotype correlations. To date, only one individual with biallelic GINS2 variants has been reported.

Methods and Results: We describe a second patient with GINS2-related MGS. The proband, a girl born to consanguineous parents, presented with prenatal growth retardation, complex craniosynostosis, postnatal growth failure, microcephaly, microtia, characteristic craniofacial features, and an anteriorly placed anus. Trio genome sequencing identified a homozygous GINS2 variant, NM_016095.3: c.333C>G p.(Asp111Glu), affecting a highly conserved residue and predicted to disrupt GINS complex stability. Immunological investigations revealed natural killer cell deficiency. Genome-wide DNA methylation analysis did not identify a known episignature but revealed differential methylation affecting genes involved in skeletal development, craniofacial morphogenesis, and cell proliferation, including pathways related to osteoclast differentiation and developmental transcriptional regulation.

Conclusions: This report confirms GINS2 as a cause of MGS and further delineates the associated phenotype. The recurrence of craniosynostosis in both reported individuals suggests that this feature may be characteristic of GINS2-related disease. In addition, our findings provide preliminary evidence of immune and epigenetic alterations associated with GINS2 deficiency and suggest disruption of developmental pathways involved in craniofacial and skeletal morphogenesis, expanding the molecular characterisation of this ultra-rare disorder.

1. Background

Meier-Gorlin syndrome (MGORS, ORPHA:2554) belongs to **primordial dwarfism (PD)**, a group of rare genetic recessive autosomal disorders characterised by severe pre- and postnatal **proportionate short stature** and severe microcephaly. Individuals with Meier-Gorlin syndrome also present with patellar hypo/aplasia, bilateral microtia and characteristic facial features (high forehead, micrognathism with full lips and small mouth, and accentuated nasolabial folds). Additional clinical features include sexual development abnormalities (cryptorchidism, scrotum malformation, clitoral hypoplasia, hypoplastic labia minora and majora), joint hyperlaxity, camptodactyly, and craniosynostosis. Intellectual ability is usually preserved [1].

In 2011, pathological bi-allelic variants were identified in *ORC1*, *ORC4*, *ORC6*, *CDC6*, and *CDT1* genes encoding proteins essential for the pre-replication complex of the nuclear genome [2, 3]. Further works identified additional genes (*CDC45*, *GMNN*, *GINS2*, *GINS3*, *DONSON* and some components of the minichromosome maintenance protein (MCM) complex, *MCM7*, *MCM5* and *MCM3*, encoding helicases and regulatory proteins of the pre-initiation complex) [4, 5]. Except for *GMNN* (a nuclear DNA replication associated with an autosomal dominant form of MGS), all these genes are associated with autosomal

recessive transmission. Today, genotype-phenotype correlation emerges in MGORS. For example, craniosynostosis is reported for pathogenic variants identified in the *CDC45* gene and not in genes coding for ORC complex proteins.

Among the DNA replication machinery, GINS complex (go-ichi-ni-san, standing for Sld5, Psf1, Psf2 and Psf3) plays a central role in DNA replication by stabilising and activating the MCM helicase within the CMG (CDC45–MCM–GINS) complex. It ensures proper initiation of replication by activating and stabilising DNA unwinding, and maintains the stability and progression of active replication forks, thereby supporting normal cell-cycle progression [6, 7]. Loss of GINS function particularly affects cell types with high proliferative demands (immune cells, bone and neural progenitors leading to tissue-specific vulnerabilities despite its ubiquitous cellular role [6]. Currently, pathogenic variants have been identified in the subunits of the GINS complex that exhibit a similar phenotype [5]. To date, only one individual has been reported with *GINS2*-related MGORS with a phenotype similar to CDC45-related MGORS, particularly characterised by craniosynostosis [8] (GINS2-R114, ST48 in our study).

In this study, we present a second case of MGORS associated with the *GINS2* gene (GINS2-D111, ST41), for which we have provided important clinical, genetic, immunologic and epigenetic data.

2. Methods

Informed consent for genetic analyses was obtained from the parents according to French ethical guidelines. Genomic DNA was isolated from peripheral blood using Qiasymphony instrument according to the manufacturer's procedure. The *GINS2* variant was identified by Trio whole-genome sequencing (WGS) (capture kit Illumina DNA PCR-free Prep, Tagmentation) on the Illumina NovaSeq 6000 platform (SeqOIA laboratory, Paris, France). Bioinformatic analyses were performed by SeqOIA bioinformatic platform, and the gLeaves application was used for the interpretation of DNA sequencing data (SeqOIA laboratory, Paris, France). The pathogenicity of variants was evaluated using Mobidetails (https://mobidetails.chu-montpellier.fr/api/variant/377797/browser/), including a link to DynaMut2 3D structure assay (https://biosig.lab.uq.edu.au/dynamut2/). Variant and familial segregation were confirmed by Sanger sequencing.

Residual DNA samples obtained from routine genetic testing were used for DNA methylation analysis. Samples from the ST41 patient and her parents, as well as from the ST48 patient and his parents, were bisulfite-converted using the EZ-96 DNA Methylation Kit (Zymo Research), following Illumina's recommended protocol, and subsequently hybridtomoised to Illumina Infinium MethylationEPIC v2 arrays (Hologic platform, Belgium).

The EVIAN pipeline (Université libre de Bruxelles, Belgium) was used for DMRs detection. Illumina EPICv2 IDAT files were processed using SeSAMe R package [9], and CpG beta values were then extracted. Each CpG site from ST41 sample was compared with the control cohort using a Z-score approach. CpGs with a p-value < 0.01 were kept for downstream analysis. DMRs were identified using 1

kb sliding windows containing at least four CpGs and showing a median methylation difference greater than 15% compared with controls. Overlapping windows were merged together.

Two control cohorts were used to generate the final results. The first one consisted of 50 age-matched controls from Erasme Hospital (17 females, 33 males). The second included only 17 age-matched female controls from the first cohort to account for a potential sex effect. The age of matched controls ranged from 1 month to 8 years. Gene ontology analyses were performed using Enrichr package (https://maayanlab.cloud/Enrichr/enrich).

3. Results

The one-year-old girl was referred for genetic evaluation because of complex craniosynostosis. She was the fifth child of related healthy parents with an unremarkable family history (Fig. 1A). Prenatal ultrasound had identified intrauterine growth retardation and frontal bossing, suggestive of bicoronal craniosynostosis. Prenatal tomodensitometry evidenced this diagnosis and short long bones. Prenatal genetic analyses by CGH-array and a panel of genes involved in craniosynostosis were normal.

She was born at term with a weight of 2400g (< 3rd percentile (P)), a height of 46cm (1st P) and an occipitofrontal circumference (OFC) of 32cm (3rd P). Auditory evoked potential (AEP) failed at 3 days of life while explorations revealed a normal audition at a few months of life.

Complex craniosynostosis was confirmed by postnatal tomodensitometry with bicoronal and bilambdoidal craniosynostosis responsible for turricephaly (Fig. 1F), surgically corrected at 12 months of age.

At one year, she presented with short stature (height: 62.5cm, -3.5 SD; weight 5.7kg, -3.5 SD) and microcephaly (OFC 40cm, -4 SD). Examination revealed craniofacial dysmorphic features with mid-face hypoplasia, prominent eyes with downslanting palpebral fissures, a small mouth, and a high, narrow palate. Ears were small, low set, and posteriorly rotated, with an atretic external auditory canal (Fig. 1D). She also had short, tapering fingers, short toes, a sacral dimple, and an anteriorly placed anus. Psychomotor evaluation showed normal development for her age.

At the last follow-up, aged 4 years, her height, weight, and OFC were at – 3 SD, measuring 91cm, 10kg, and 46cm, respectively (**Additional file 1**). Examination showed the previously described craniofacial features and cutis marmorata. The patella was palpable during clinical examination. She exhibited a language delay that required speech and language therapy. Motor development was normal. She attended a mainstream school. Cardiac, abdominal, and spinal ultrasound examinations, as well as an ophthalmological assessment including fundus examination, were normal. Endocrinological, hepatic, and malabsorption-related laboratory investigations were also unremarkable.

Genome sequencing (GS) was performed in the proband and her parents using a national high-throughput sequencing platform. The only putative pathogenic variation in the proband was a

homozygous variant, NM_016095.3: **c.333C > G** (p.Asp111Glu) in the *GINS2* gene (Fig. 1B). The other homozygous variants found in several genes were not related to OMIM disorders compatible with the observed phenotype. Interestingly, GINS2 is widely expressed with a higher expression in the thymus, small intestine and fetal brain (Fig. 1C).

Allelic frequency of NM_016095.3: c.333C > G (p.Asp111Glu) is 0.002946% in the database of populations (gnomad V4) with no homozygous variant. It has not been previously reported in individuals (HGMD database and Clinvar). The variant was considered to be probably pathogenic for the following: in silico prediction software (PolyPhen-2, CADD, ClinPred, AlphaMissense, REVEL). The first reported individual with similar clinical features carried a homozygous variant, c.341G > T, p.(Arg114Leu), located within the same functional region of the protein [8](**Additional file 2A**). The 111 aspartic acid is quite well conserved throughout evolution (Fig. 1B).

All family members were asymptomatic. Both parents were heterozygous for this variant. Familial segregation analysis showed that none of the four asymptomatic sisters was homozygous for this variant. According to the ACMG recommendations, this variant is classified as likely pathogenic (PM2, PM3, PP1, PP4). Due to the molecular characteristics of this variant, compatible familial segregation analysis, and specific clinical features aligned with the previously reported individual, we conclude that this variant is responsible for the phenotype in our patient.

Protein structure analysis

Structural analysis of GINS2 revealed that the p.(Asp111Glu) variant affects a residue within the highly ordered core of the GINS heterotetramer. Examination of available 3D structures of the human GINS complex (PDB: 2EHO, 2E9X) and the AlphaFold GINS2 model showed that Asp111 is embedded in a constrained secondary structure and participates in a local hydrogen-bond network that stabilises the fold of the GINS2 subunit (Fig. 1E). Aspartate substitution by glutamate preserves the negative charge but introduces a longer side chain, predicted to modify local geometry and disrupt hydrogen-bond interactions. Structural modelling indicated that this alteration may induce subtle conformational rearrangements in the surrounding region. Given the proximity of Asp111 to inter-subunit interfaces within the GINS complex, these structural changes are likely to affect tetramer stability or assembly. Together, these observations support a deleterious structural impact of the p.(Asp111Glu) variant in GINS2.

Additional phenotypic investigations: skeletal findings and immunophenotyping

As reported in MGORS, the patella was investigated. Skeletal survey revealed small proximal femoral epiphyses without vertebral malformation. Ultrasonography demonstrated a cartilaginous patella lacking an ossification center at 4 years of age (Fig. 1G).

The GINS complex, composed of GINS1–4, is an essential component of the CMG (CDC45–MCM–GINS). Pathological variants in GINS1 are associated with severe natural killer cell deficiency,

immunodeficiency, and growth retardation [10], whereas pathological variants in GINS4 are associated with NK deficiency combined with neutropenia [11]. To characterise this GINS2 new pathological variant, we assessed the patient's immunological status, given that other GINS genes present an immunodeficiency phenotype. Immune assays revealed decreased IgM (0.51 g/L, normal 0.53–1.62), a deficiency in natural killer (NK) lymphocytes (CD16+/CD56 + at 43.53/µL, normal 130–720/µL), without lymphopenia (lymphocytes at 3109/µL, normal 2300–5400/µL) and no T cell deficit (Fig. 2A-E). However, the patient did not present with recurrent diagnosed viral infection.

Methylation analysis

Further characterisation was performed using DNA methylation analysis of the GINS2-D111 patient, her two parents and two sisters (Fig. 1A). No previously known episignature could be correlated with the patient; the closest episignatures found are DEGCAGS (*ZNF699* gene) [12] and SPEN-related neurodevelopmental disorder [13], with respectively 52/200 and 52/122 positive probes (**Additional file 1B**). Analysis using the EVIAN pipeline, compared first with 50 unaffected controls, revealed methylation variations affecting 40 genes (**Additional file 2C**). Secondly, to avoid sex bias associated with the chrX, a similar analysis was performed on 17 young girls, identifying 32 genes with methylation differences involving certain genes that play a key role in osteoclast differentiation (Fig. 2E, **Additional file 1C,2D**). Gene ontology analysis showed that the 22 predicted downregulated genes (defined by promoter hypermethylation and gene-body hypomethylation) are involved in bone structure, formation and cell proliferation, as well as craniofacial bone morphology (*SH3BP2*, *CNOT2*, *FGF1*, *TNFRSF11A*, also known as *RANK*, *ANKRD11*). Gene enrichment analysis showed that RUNX1 may act as one of the most shared regulators, indicating an impact in very early development (**Additional file 2E**). Other genes, such as CLDN9 (implicated in tight junctions formation, homeostasis of the barrier of the inner ear and responsible for hearing loss OMIM#615799 [14]), show that heterozygous relatives have intermediate methylation profiles (Fig. 2F). Interestingly, two transcription factors, ZNF681 and NKX2-3, are found with hypermethylation at the promoter (Fig. 2G). The latter is involved in early developmental processes and exhibits marked promoter hypermethylation, suggesting that the molecular consequences of these alterations may occur at early developmental stages. ZNF681 is an understudied transcription factor, possibly involved in brain or cranial development [15, 16]. Similar results were obtained using two additional control cohorts. Conversely, only 9 genes were predicted to be upregulated.

A similar analysis was performed on DNA from the patient GINS2-R114 (NM_016095.2:c.341G > T, [8]). No known episignature was identified in this patient (**Additional file 3A**). The closest match corresponded to SPEN-related syndrome, with 23 of 122 probes classified as positive. Owing to the limited number of age-matched controls available in our reference cohort, differential methylation analyses could not be robustly performed for this 8-year-old patient. Additional analyses incorporating controls across a broader age range, together with a larger cohort of patients with MGORS, will be required to refine the methylation profile associated with GINS2-related disease.

4. Discussion

We report a second individual with MGORS due to *GINS2* variants, with a very similar phenotype to the first individual. Both individuals showed characteristic MGORS features, including pre- and post-natal growth retardation, microcephaly and microtia. Hypoplastic patella was described in the first individual, and a patellar ossification delay in our patient; however, her young age limits reliable assessment of overall skeletal growth and ossification. Craniosynostosis seems to be a common sign in GINS2-related MGORS, as in CDC45-related MGORS. The GINS2-R114 individual had a congenital cardiac defect, not seen in our case (**Additional file 1A**). Both individuals had an anteriorly placed anus. Anorectal malformations are common in CDC45-MGORS and range from anal stenosis to an anteriorly placed anus. Because of the small number of individuals with GINS2-MGORS, we cannot conclude whether anorectal malformations are common; thus, they must be assessed in individuals with GINS2 variants. Very few patients with CDC45 or GINS3-MGORS are described as having developmental delay and/or intellectual disability [17, 18]. Both individuals with GINS2 MGORS had normal motor development; our patient exhibited language delay. The previously reported individual had normal intelligence. Follow-up is needed to evaluate intellectual ability in our individual.

Defects in the DNA replication machinery, particularly the CMG helicase complex composed of CDC45, MCM proteins, and GINS proteins, have emerged as causes of selective NK-cell deficiency. Natural killer (NK) cells require rapid proliferation and clonal expansion during development and in response to infections. This strong dependence on cell cycle progression renders NK cell precursors particularly vulnerable to defects in DNA replication machinery. GINS1 and GINS4 deficiencies are responsible for chronic neutropenia and NK cell deficiency associated with growth retardation in GINS1 deficiency [10, 11]. MCM4 and MCM10 variants cause NK deficiency, with adrenal insufficiency and growth delay MCM4 variants. Because of the role of CMG complex in NK-cell development, we performed immunophenotyping in our individual. Interestingly, she had NK lymphocyte deficiency without neutropenia, or lymphocyte deficiency, and with no susceptibility to viral infections. These findings underscore the importance of the GINS complex in NK cell biology; nevertheless, the underlying pathophysiological mechanisms have yet to be elucidated.

Methylation analysis enables the identification of genomic regions associated with specific regulatory elements, such as gene promoters and enhancers. In this patient, the dysregulated pathways identified play an important role in craniofacial and skeletal development. Further RNA-seq analyses will be required to characterise the affected transcriptional networks better and clarify the underlying pathophysiological mechanisms.

5. Conclusions

We report a second individual with Meier–Gorlin syndrome caused by a homozygous GINS2 variant, providing further evidence that GINS2 is a disease-causing gene within the CMG helicase pathway. In addition to refining the clinical spectrum of GINS2-related disease, we identified NK-cell deficiency, supporting a role for GINS2 in immune-cell development. DNA methylation analyses highlighted alterations affecting genes involved in skeletal development and craniofacial morphogenesis,

suggesting that GINS2 deficiency may be associated with dysregulation of key developmental pathways. Together, these findings expand the phenotypic and molecular characterisation, providing a foundation for future genotype–phenotype and epigenomic studies of this ultra-rare disorder.

Declarations

Ethics approval and consent to participate

This research was conducted as part of the research programmes of this biobank: Imagine/INSERM U1163 (DC-2019-3504). The Erasme (ULB, Brussels) control samples used in this study were collected and used under ethics approvals B2017/001 and SRB2023107. This study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Written informed consent for the use of biological samples was obtained by the treating physicians at their respective institutions.

Availability of data and materials

ClinVar variant is submitted (SUB16248500).

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

PM, GP, KL, and VCD diagnosed and performed follow-up on GINS2-D111 patient. CC analysed genetic tests and performed protein structure analysis. CP analysed immune explorations. AB and MD set up the methylation analysis tool. AB, MD, ND and Md performed and analysed DNA methylation. MN provided the GINS2-R114 DNA sample. PM, CC, AB and Md wrote the initial manuscript. All authors participated in manuscript corrections and agreed to the final manuscript.

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Figures

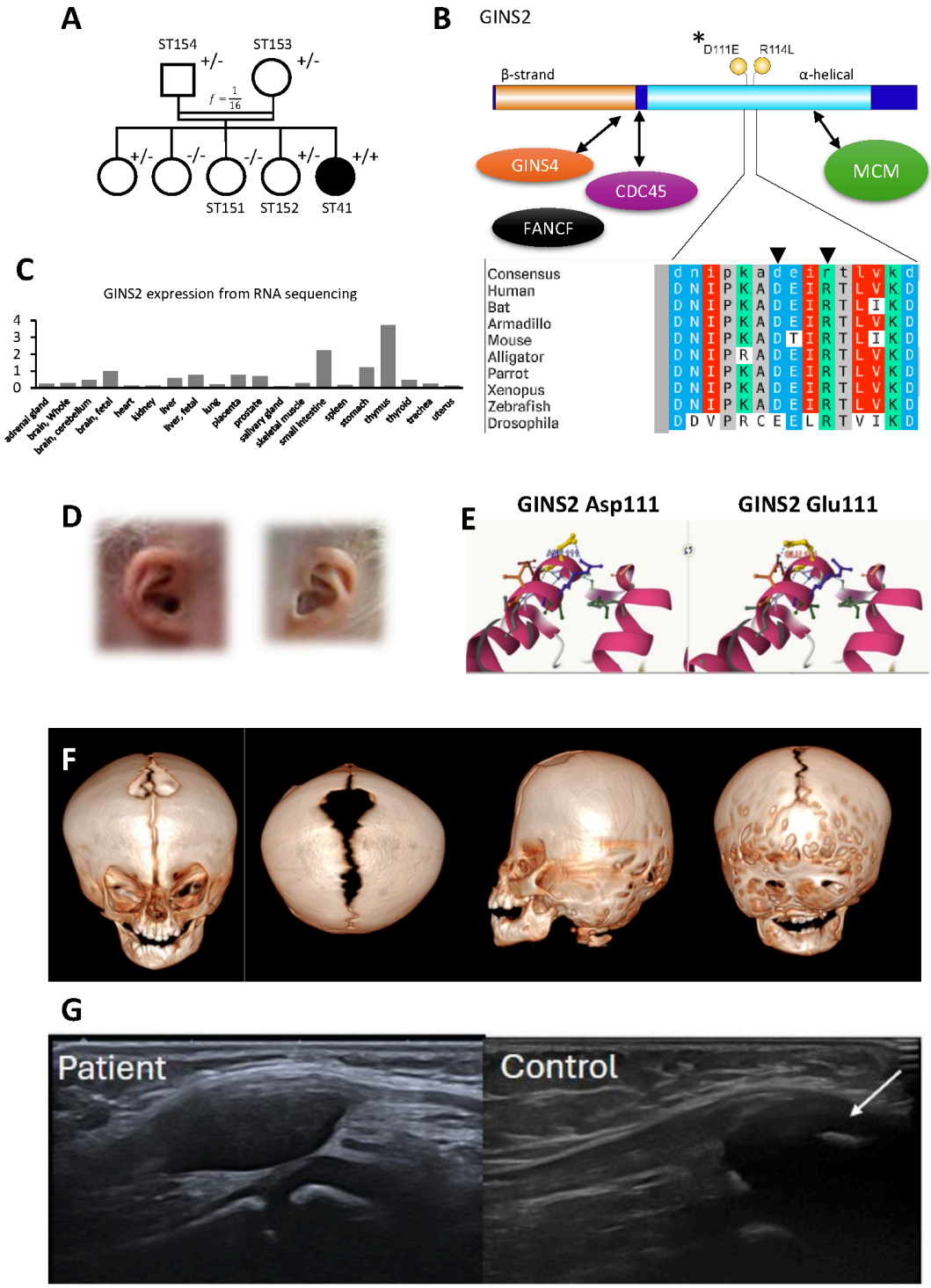


Figure 1

Figure 1

(A) Pedigree of the family. (B) Schematic representation of the GINS2 protein [19], * indicates the reported pathological variant, lollipops show variant position; interactions are documented in [20], conservation study was performed using the Mafft online tool. (C) GINS2 expression across different tissues from RNA sequencing of total RNA from the 20 human tissues project (NCBI). (D) Pictures of patients' ears. Note the small and dysplastic ears. (E) 3D protein representation of the variant GINS2-

D111E (F) Cranial tomodensitometry bicoronal and bilambdoidal craniosynostosis with digital impression and suture enlargement. (G) Patellar ultrasonography at 4 years of age; note the cartilaginous patella lacking an ossification center in the patient, whereas usual ossification in controls.

A

Ig	(g/L)	Ref. val.
IgG	11.7	(5.4-13.6)
IgA	0.81	(0.3-1.5)
IgM	0.51	(0.53-1.62)

B

T cells	(/µL)	Ref. val.
CD3+	2331.75	(1400-3700)
CD4+	1694.4	(700-2200)
CD8+	559.62	(490-1300)

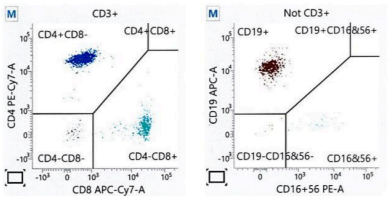
C

naive and memory T cells	%	Ref. val.
CD45RO+/CD3+CD4+	19.5	(14-47)
CD45RA+/CD3+	80.5	(73-86)
CD31+/CD4+	70.5	
CD31+CD45RA+/CD4+	68	(57-65)
CD45RA+CCR7+/CD3+CD8+	69.1	(45-75)
CD45RA-CCR7+/CD3+CD8+	0.6	(0.5-6)
CD45RA-CCR7-/CD3+CD8+	6.6	(4-38)
CD45RA+CCR7-/CD3+CD8+	23.7	(7-39)

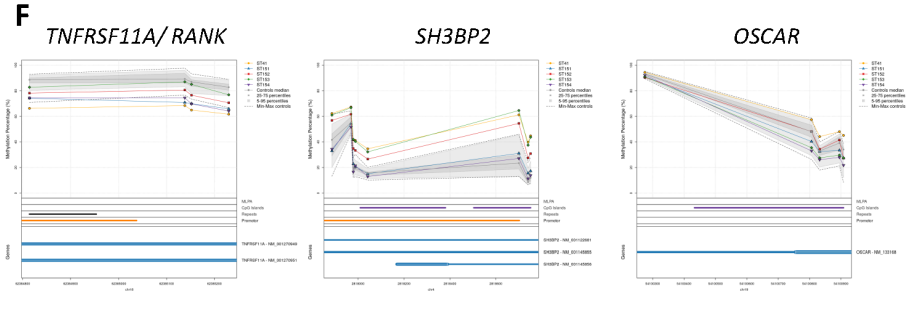
D

NK cells	(/µL)	Ref. val.
CD16+CD56+	43.53	(130-720)

E



F



G

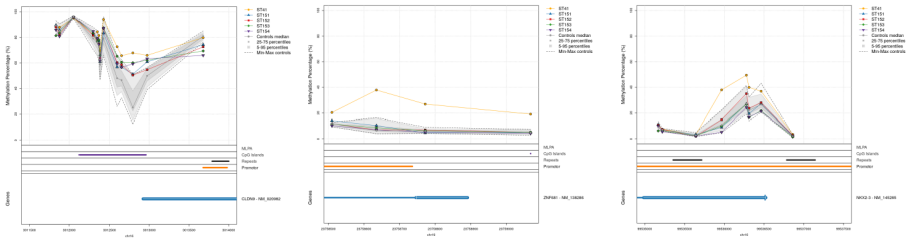


Figure 2

Figure 2

(A) Immunoglobulin phenotyping of the patient (units in g/L). (B) Immunophenotyping of T lymphocytes (units in g/L). (C) Immunophenotyping of naïve and memory T lymphocytes (units in %). (D) Immunophenotyping of Natural Killer lymphocytes (units in g/L). (E) Flow cytometry analysis of peripheral blood lymphocyte subsets. CD3+ lymphocytes were classified according to CD4 and CD8 expression (left panel). Non-CD3+ lymphocytes were analysed for CD19 and CD16/CD56 expression to identify B cells and natural killer (NK) cells, respectively (right panel). (F, G, H) Genome browser visualisation of DNA methylation levels (%) across differentially methylated loci identified by the EVIAN pipeline. The orange line represents the patient (ST41), whereas the grey line and shaded areas indicate the median and percentile ranges of the control cohort. Relatives are also indicated in blue (unaffected sister, ST151), red (heterozygous sister, ST152), green (heterozygous mother, ST153) and purple (heterozygous father, ST154).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.pdf
- Additionalfile2.xlsx
- Additionalfile3.xlsx