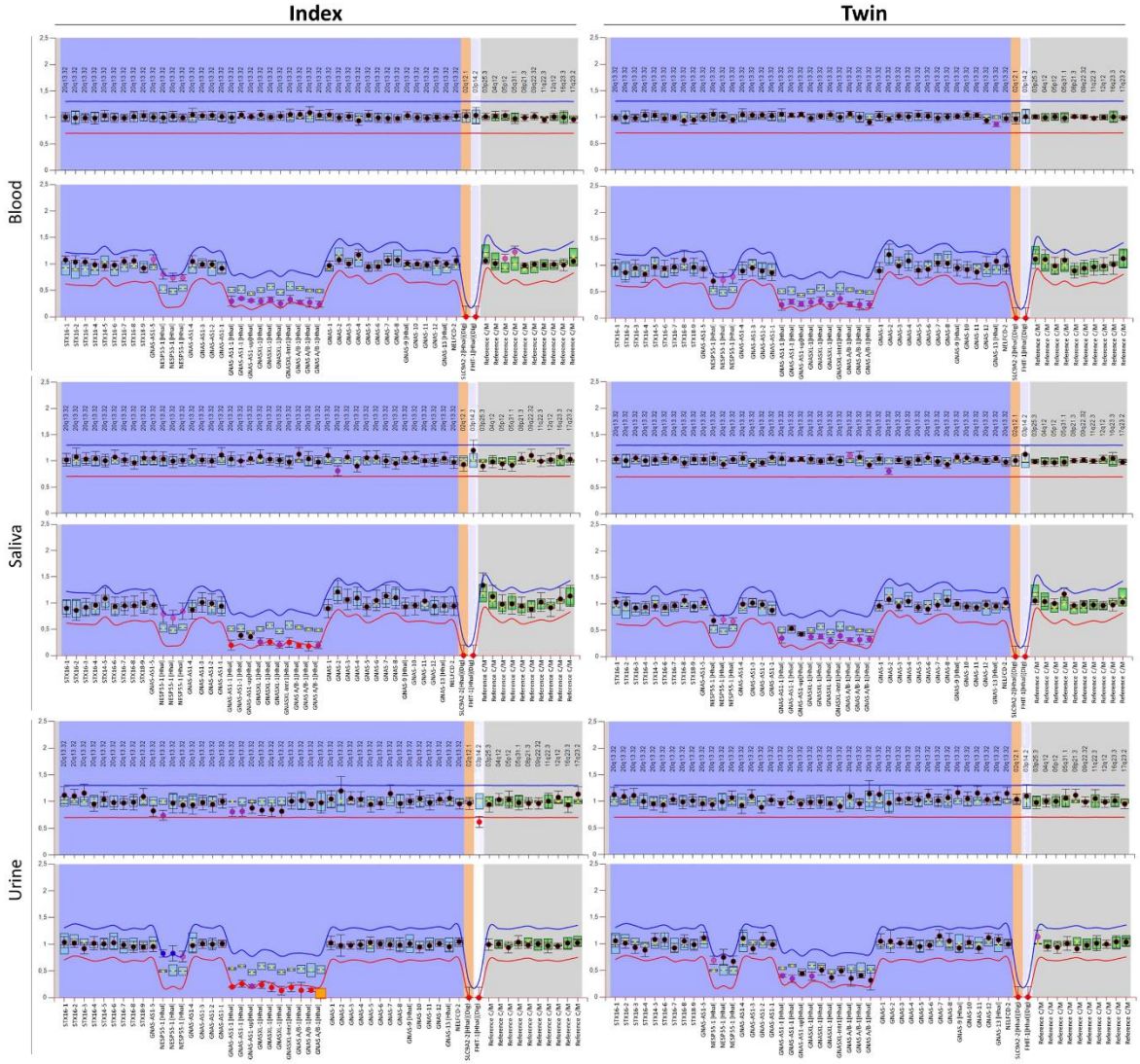
SUPPLEMENTARY MATERIAL

Supplementary Table 1: Genes included in the NGS panel "Imprinting Regulators v1". This panel captures genes involved in imprinting regulation, classified into distinct functional categories: MEG (Maternal Effect Genes), SCMC (Subcortical Maternal Complex), and HYDM (Hydatidiform mole). Mammalian MEG includes genes with currently unknown roles in humans but demonstrated maternal functions in embryonic development in other species, while Human MEG are those with documented functions in humans, some of which belong to the SCMC as indicated. The categories Embryonic and Gametogenesis refer to genes involved in embryonic expression/function and meiotic processes including genetic recombination and sister chromatid cohesion, respectively. Genes shown in bold harbor variants previously associated with Multi-Locus Imprinting Disturbance (MLID) in humans. Probe design was based on RefSeq 2020 transcript annotations (refseq-2020-02-09-gencodev33-2020-02-16-ccdsv22-2019-10-20).

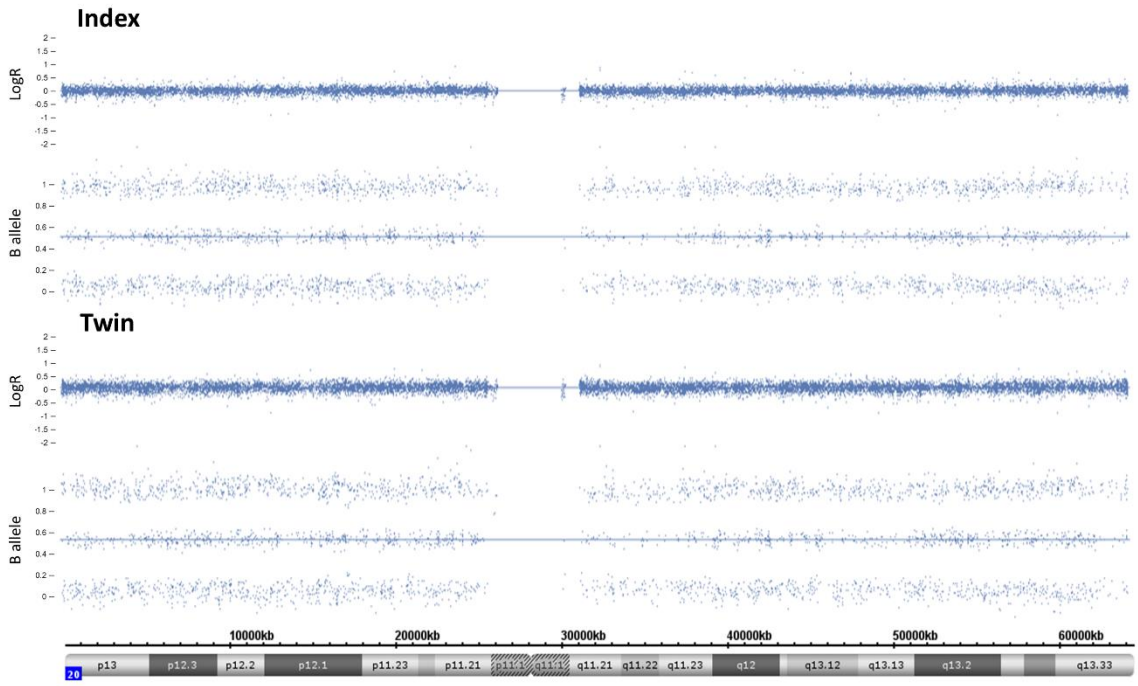
Genes included in IR panel v1	Gene type	Genes included in IR panel v1	Gene type
AGO2	Mammalian MEG	*PIK3R1*	Mammalian MEG
ATG5	Mammalian MEG	*PMS2*	Mammalian MEG
BCAR4	Mammalian MEG	*POU5F1/OCT4*	Mammalian MEG
BCAS2	Mammalian MEG	*RAD21*	Gametogenesis
BNC1	Mammalian MEG	*REC114*	Gametogenesis
BRWD1	Mammalian MEG	*RING1*	Mammalian MEG
BRME1/C19orf57	Gametogenesis	*RLIM*	Mammalian MEG
CTCF	Mammalian MEG	*RNF2*	Mammalian MEG
DICER1	Mammalian MEG	*SEBOX*	Mammalian MEG/ Regulador de MEG
DMAP1	Mammalian MEG	*SLBP*	Mammalian MEG
DNMT1	Mammalian MEG	*SMARCA4*	Mammalian MEG
DNMT3A	Mammalian MEG	*SOX2*	Mammalian MEG
DNMT3L	Mammalian MEG	*STAG3*	Gametogenesis
DPPA3	Mammalian MEG	*SYCE1*	Gametogenesis
ESR2	Gametogenesis	*SYCP2*	Gametogenesis
EZH2	Mammalian MEG	*TCL1A*	Mammalian MEG
FANCM	Gametogenesis	*TET3*	Mammalian MEG
FIGLA	Gametogenesis / MEG regulator	***TLE6***	**Human MEG (SCMC)**
FMN2	Mammalian MEG	*TOP6BL/C11orf80*	Gametogenesis
GAS6	Mammalian MEG	*TP73*	Mammalian MEG
GCLM	Mammalian MEG	*TRIM24*	Mammalian MEG
GJA4	Mammalian MEG	*TRIM28*	Mammalian MEG
HFM1	Gametogenesis	*UBE2A*	Mammalian MEG
HIRA	Mammalian MEG	*UCHL1*	Mammalian MEG

Genes included in IR panel v1	Gene type	Genes included in IR panel v1	Gene type
HIST1H2AA/H2AC1	Mammalian MEG	*XIST*	Mammalian MEG
HIST1H2BA/H2BC1	Mammalian MEG	*YY1*	Mammalian MEG
HIST1H3F/H3C7	Mammalian MEG	***ZAR1***	Mammalian MEG/ **Human MEG**
HIST1H4F/H4C6	Mammalian MEG	*ZBED3*	MEG humano (SCMC)
HSF1	Mammalian MEG	*ZFP36L2*	Mammalian MEG
HSF2BP	Gametogenesis	***ZFP57***	**Embryonic**
KDM1B	Mammalian MEG	*ZFP69B*	Embryonic
KHDC3L	**Human MEG (SCMC)**/ HYDM	*ZNF182*	Embryonic
KMT2D	Mammalian MEG	*ZNF2*	Embryonic
KPNA6	Mammalian MEG	*ZNF202*	Embryonic
MCM8	Gametogenesis	*ZNF257*	Embryonic
MCM9	Gametogenesis	*ZNF263*	Embryonic
MEI1	Gametogenesis	*ZNF273*	Embryonic
MEIOB	Gametogenesis	*ZNF28*	Embryonic
MIR181A1	MEG regulator	*ZNF30*	Embryonic
MIR196A1	MEG regulator	***ZNF445***	**Embryonic**
MIR212	MEG regulator	*ZNF468*	Embryonic
MSH4	Gametogenesis	*ZNF479*	Embryonic
MSH5	Gametogenesis	*ZNF506*	Embryonic
NLRP2	**Human MEG (SCMC)**	*ZNF519*	Embryonic
NLRP4	**Mammalian MEG**	*ZNF534*	Embryonic
NLRP5	**Human MEG (SCMC)**	*ZNF557*	Embryonic
NLRP7	**Human MEG (SCMC)/ HYDM**	*ZNF649*	Embryonic
NOBOX	Gametogenesis	*ZNF675*	Embryonic
NPM2	Mammalian MEG	*ZNF75D*	Embryonic
OOEP	**Human MEG (SCMC)**	*ZNF793*	Embryonic
PADI6	**Human MEG (SCMC)**	*ZNF90*	Embryonic
PDK1	Mammalian MEG	*ZNF695*	Embryonic

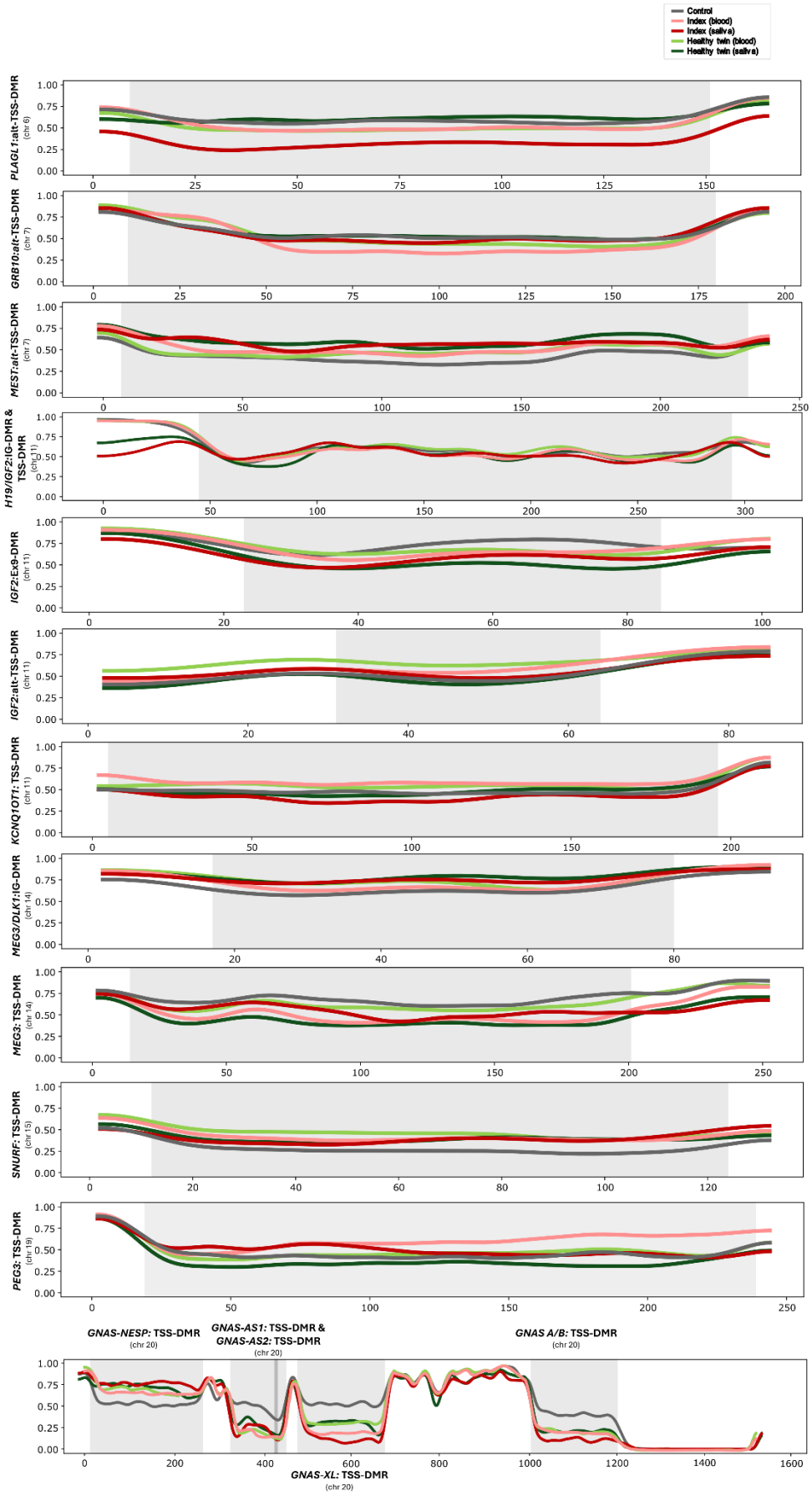
SUPPLEMENTARY FIGURES



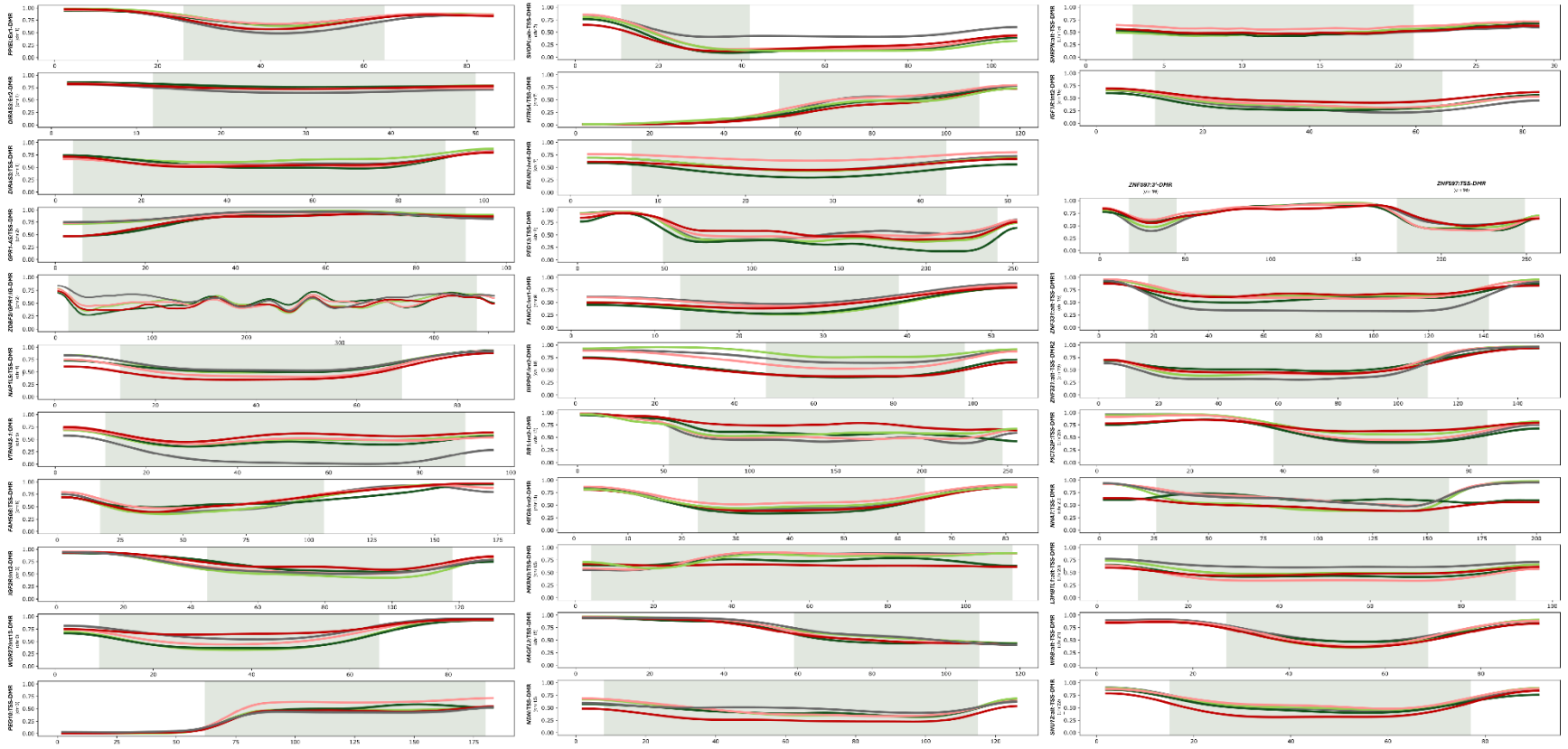
Supplementary Figure 1: Results of MS-MLPA studies in blood, saliva, and urine from both sisters. For each tissue, the upper panel displays the GNAS locus copy number, indicating no alterations in dosage. The lower panel for each tissue shows the methylation pattern in the different regions. It blood, both sisters have a very similar pattern, with slight hypermethylation in *GNAS-NESP:*TSS-DMR and hypomethylation in *GNAS-AS1*:TSS-DMR, *GNAS-XL:*Ex1-DMR and *GNAS A/B:*TSS-DMR. However, in saliva and urine, this methylation alteration is more pronounced in the patient, while in her healthy sister it is almost imperceptible.



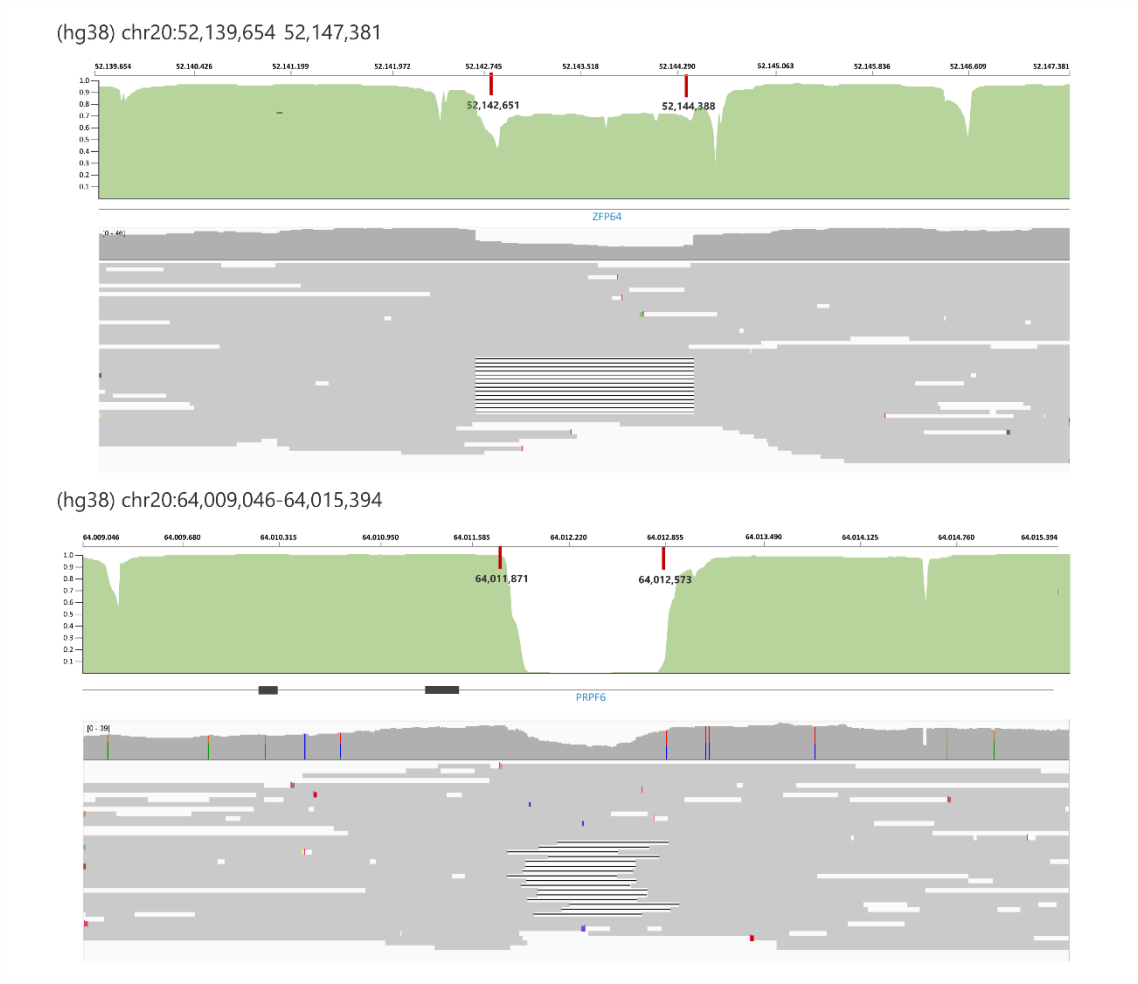
Supplementary Figure 2: Chromosome 20 was analyzed using SNP array technology. The results obtained in both sisters confirmed the absence of copy number variations affecting chromosome 20. Similarly, no regions of loss of heterozygosity were detected, ruling out the presence of uniparental isodisomy.


Control
Index (blood)
Index (saliva)
Healthy twin (blood)
Healthy twin (saliva)
PLAGL1:alt-TSS-DMR (chr 6)
GRB10:alt-TSS-DMR (chr 7)
MEST:alt-TSS-DMR (chr 7)
H19/IGF2:IG-DMR & TSS-DMR (chr 11)
IGF2:Ex9-DMR (chr 11)
IGF2:alt-TSS-DMR (chr 11)
KCNQ1OT1: TSS-DMR (chr 11)
MEG3/DLK1:IG-DMR (chr 14)
MEG3: TSS-DMR (chr 14)
SNURF: TSS-DMR (chr 15)
PEG3: TSS-DMR (chr 19)
GNAS-NESP: TSS-DMR (chr 20)
GNAS-AS1: TSS-DMR & GNAS-AS2: TSS-DMR (chr 20)
GNAS A/B: TSS-DMR (chr 20)
GNAS-XL: TSS-DMR (chr 20)

Supplementary Figure 3: Methylation analysis after long-read sequencing focused on DMRs described as clinical [1]. The panel depicts methylation distribution across the coordinates of these DMRs using DNA from saliva and blood samples of both sisters and a healthy control. The methylation pattern was comparable across samples, except at the *GNAS* locus.



Supplementary Figure 4: Methylation analysis after long-read sequencing focused on non-clinical DMRs [1]. The methylation pattern was similar for samples of both twins and a healthy control.



Supplementary Figure 5: Image comparing the genomic regions containing the two structural variants identified in the sisters. In both panels, the upper section corresponds to gnomAD data showing average read depth, whereas the lower section displays IGV coverage from the index patient's sequencing results. A decrease in read depth can be observed in the patient, called as a heterozygous variant. This reduction coincides in both regions with areas of lower read coverage in the general population, likely reflecting sequencing limitations or difficulties with short-read technologies.