Supplementary Data

Materials and Methods

Human subject recruitment

Blood samples were collected from the probands, their parents, their partners and other siblings. Tissue samples were collected from the products of conception III-12 and III-15 derived from miscarriage pregnancies of II-7, for histopathological examination and DNA analysis. DNA extraction was carried out from 5 to 10 ml of frozen EDTA blood samples by using the salting-out procedure[1]. DNA from frozen fetus tissues was extracted with the TRIZOL reagent (Sigma, USA), as described[2].

Karyotyping, array comparative genomic hybridization (aCGH) and SNP-array analysis.

Chromosome analysis of peripheral blood was performed for both male and female partners (II-5 and II-6, and II-7 and II-8). Metaphase spreads were made from phytohemagglutinin-stimulated lymphocytes according to the standard procedure to generate a resolution of 550 bands per haploid set[3]. Slides were processed for G-banding using trypsin–Giemsa (GTG)-banded chromosome preparations[3]. At least 25 metaphases were analyzed for each individual. Results were reported in accordance with the latest International System for Human Cytogenetic Nomenclature[4].

aCGH analysis of II-5, II-6 and III-12 was performed with the 24sure Microarray Pack version 3.0 (Illumina; cat. #: PR-10-408702-PK, USA). DNA labeling and hybridization were performed by using the Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis protocol (V 7.3, 2014). The array data was read by InnoScan 900 microarray scanner (INNOPSYS, France). The BlueFuse Multi v3.1 was used to analyze the 24sure experiments. We reported the median $\log_2$ ratio for each chromosome as the index of aneuploidy as analyzed by BlueFuse Multi software.

SNP array analysis of II-7 and II-8 was carried out with the CytoScan HD Array (Thermo Fisher Scientific) according to manufacturer's protocol. Data analysis was performed using Chromosome Analysis Suite Software version 4.0 (Thermo Fisher Scientific) following a standardized pipeline. Briefly: i) the raw data file (.CEL) was normalized using the default options; ii) an unpaired analysis was performed using 270 HapMap samples as a baseline in order to obtain copy numbers value and regions of homozygosity (ROHs) from .CEL files. The amplified and/or deleted regions were detected using a standard Hidden Markov Model (HMM) method. Size threshold for analysis was kept as 5 Kb for copy number variations (CNVs), and 1 Mb for ROHs. In order to identify clinical or functionally relevant genomic variants, we compared all chromosomal alterations identified to those collected in our internal database of ~5,000 patients studied by SNP Arrays since 2010 and public databases, including the Database of Genomic Variants (DGV; available online at: http://projects.tcag.ca/variation/), DECIPHER (available online at: https://decipher.sanger.ac.uk/) and ClinVar (available online at: https://www.ncbi.nlm.nih.gov/clinvar/).

Histological analysis

The product of conception III-12 was received in Bouin's solution at the department of pathology, Royan Reproductive Center. Fixed biopsies were embedded in paraffin block, cut into 5-µm-thick sections and stained with hematoxylin and eosin (H&E, Bahar Afshan, Iran) using standard procedures[5].

DNA methylation analysis

DNA methylation of the Differentially Methylated Regions (DMRs) of seven imprinted loci were investigated in the DNA extracted from tissues of the product of conceptus III-15 by sodium bisulfite conversion and pyrosequencing, as already described[6]. The control DNA for the analysis of imprinted DMR methylation derived from peripheral blood leukocytes of normal individuals. The level of methylation of the imprinted DMRs is maintained at 50% (one allele fully methylated) in somatic cells throughout pre- and post-natal development[7]. Primer sequences are reported in Supplementary Table 6.

Polymorphic sequence-tagged sites (STSs) analysis

The peri-centromeric and distal STS markers D1S498, TPOX, D4S405, D4S428, D5S1969, D5S630, D5S400, D5S818, D6S257, D6S460, D7S820, D8S532, D9S1874, D10S1790, D12S1663, D13S175, D13S317, D15S128, D16S539, D19S414, D19S566, D19S865 and DXS991 were genotyped by PCR and electrophoresis. Amplification was carried out on 50 ng of genomic DNA in a volume of 20 µl, with initial denaturation at 95°C for 4 min, followed by denaturation step at 94°C for 30 secs, annealing step at 60°C or 55°C for 30 secs, polymerization step at 72°C for 30 secs and final extension step at 72°C for 7 min. The amplification products were resolved by capillary electrophoresis by using an Applied Biosystems 3130 DNA Analyzer or by electrophoresis through polyacrylamide gel and silver staining. Allele size and peak height were determined by using GeneScan software (Applied Biosystems, Foster City, CA). Primer sequences are reported in Supplementary Table 6.

Whole exome sequencing (WES)

WES was performed on DNAs extracted from I-3, I-4, II-6 and II-7. Enrichment of coding regions and intron/exon boundaries were carried out using the 'all Exon V5 kit' (Agilent Technologies, Wokingham, UK). DNA sequencing was done at the Plateforme Biopuces et Séquençage IGBMC, Illkirch, France, on the HiSeq 2000 from Illumina®.

Exome data analysis

All steps from sequence mapping to variant selection, were performed using the ExSQLibur pipeline. Short reads were aligned to the human reference genome (hg18) using MAGIC (SEQC/MAQC-III Consortium, 2014). Duplicates and reads that mapped to multiple locations in the exome were removed from further analysis. Moreover, positions with sequence coverage <10 on either forward or reverse strand, were excluded. Single nucleotide variants (SNV) and small insertions/deletions (INDELs) were identified. We also compared these rare variants to an in-house database including 56 control exomes from subjects analyzed for unrelated pathologies and not described as having experienced RPL. All homozygous variants present in this control database were considered not to be linked with RPL and thus excluded as candidate. Variants with a minor allele frequency greater than 5% in the NHLBI ESP6500 or in 1000 Genomes Project Phase 1 data sets, or greater than 1% in ExAC and gnomAD, were excluded. Moreover, variants that scored as 'tolerated' by SIFT and as 'benign' by Polyphen-2 were excluded. The script of the pipeline used is reported as Supplementary Material.

Segregation analysis by Sanger sequencing

The selected candidate pathogenic variant was validated by Sanger sequencing in ten family members (I-3, I-4, II-5, II-6, II-7, II-8, II-9, II-10, III-12 and III-15). PCR primers (Supplementary

table 6) were designed using the Primer3 software (version 0.4.0; 29). Conventional PCR was performed using Taq DNA Polymerase Master Mix (Ampliqon, Odense, Denmark). PCR was performed at 94 °C for 4 min, followed by 30 cycles at 94 °C for 45 s, 60 °C for 45 s and 72 °C for 45 s. A final extension step is performed for 5 min at 72°C. The PCR products were assayed by using the 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Final data were analyzed by using the Sequencing Analysis version 5.2 (Applied Biosystems, Foster City, CA, USA) and FinchTV version 1.5.0 (Geospiza Inc.).

GnRH antagonist protocol and oocyte retrieval

The proband had an ultrasound scan (USS) performed on day 3 of menstrual cycle. Medication of the proband with recombinant FSH (rFSH) (300 IU Gonal-F, EMD Serono per day) was initiated at the day of USS and continued for 5 consecutive days, while follicular development was monitored by transvaginal ultrasound. The optimal rFSH dose was adjusted based on the size and number of developing follicles. Cetrotide (GnRH antagonist), was given daily by subcutaneous (SC) injection (0.25 mg/d) starting from day 7 of the stimulation cycle. Also, additional transvaginal ultrasounds were performed at days 8, 10 and 12 post-medications. Gonal-F and Cetrotide were administered continuously until at least two follicles reached ≥ 18 mm. GnRH agonist (Buserelin, Suprefact, Serono 0.5 ml SC) was given for triggering the final oocyte maturation. Serum concentrations of estradiol (E2), LH, and progestone (P) were tested in the proband on the day of HCG administration. The hormones were determined using the Immulite Automated Analyser System (ECL2012, Siemens, Germany) as instructed. Oocytes were retrieved 34-38 h after Buserelin injection and evaluated under an inverted microscope (Diaophot 300; Nikon, Japan) with an enlargement of 3400x.

In silico analyses

Template-based modeling was used to obtain a 3D structural model of cyclin-B3 protein, due to the lack of crystal structure for this protein. The isoform 1 of cyclin-B3 sequence was retrieved from the UniProt database (UniProt ID: Q8WWL7). Residues 1126-1388 of CCNB3 could be aligned to G2/mitotic-specific cyclin-B1 (CCNB1). A 3D-model of CCNB3 was built with SWISS-MODEL[8] using as templates 6gu2[9] or 2jgz[10] (chain B). The complexes of CCNB3 cyclin domain with CDK1 or CDK2 were built with the suite docking programs called pyDock[11] using as a receptor the structure of the kinase deposited in 6gu2 (chain A)[9] or 2jgz (chain A)[10]. No spatial or biological restrictions were used during simulations, which allowed a complete sampling of the docking landscape around the kinase. The interface of the complexes CCNB3 cyclin domain-CDK1 or CCNB3 cyclin domain-CDK2 with the lowest energy obtained with pyDOCK were analysed with the server PISA. The figure was prepared with UCSF Chimera[12].

The effect of V1251D substitution on CCNB3 was determined with DynaMut[13]. DynaMut carries out normal mode analysis with Bio3D[14] and ENCoM[15] and evaluates the effect of mutation on protein dynamics and stability due to vibrational entropy changes. DynaMut also provides the results obtained by structural methods such as mCSM[16], SDM[17], and DUET[18].

The alignment of orthologous cyclin B3 sequences was obtained by retrieving the sequences from the KEGG databank (ORTHOLOGY: K21771)[19] and aligning them using Clustal Omega[20].

URLS addresses for web resources used

1000 Genomes Project, http://www.1000genomes.org/

ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/

Clustal Omega, https://www.ebi.ac.uk/Tools/msa/clustalo/

ExAC Browser, http://exac.broadinstitute.org/

ExSQLibur pipeline, https://github.com/tkaraouzene/ExSQLibur

GenBank, https://www.ncbi.nlm.nih.gov/genbank/

GnomAD, http://gnomad.broadinstitute.org/

Mutation Taster, http://www.mutationtaster.org

NHLBI Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/

OMIM, http://www.omim.org/

PISA, http://www.ebi.ac.uk/pdbe/prot_int/pistart.html

PolyPhen-2, http://genetics.bwh.harvard.edu/pph2

PyDock, https://life.bsc.es/pid/pydock/

SIFT, http://sift.jcvi.org/

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Legends to Supplementary Figures

Supplementary Figure 1 Analysis of genomic integrity and histopathological features. (**A**) Karyotype of II-5. (**B**) Karyotype of II-6. (**C**) Karyotype of II-7. (**D**) Karyotype of II-8. (**E**) Log2 intensity ratios of all chromosomes of II-5 revealed by aCGH analysis. (**F**) Log2 intensity ratios of all chromosomes of II-6 revealed by aCGH analysis. (**G**) Log2 intensity ratios of all chromosomes of II-8 (upper profile) and II-7 (lower profile) revealed by SNP-array analysis. (**H**) Log2 intensity ratios of all chromosomes of III-12 revealed by aCGH analysis. (**J**) Histological analysis of tissue from III-12. Hematoxylin & Eosin x 40.

Supplementary Figure 2 Assessment of imprinted DNA methylation. Pyrosequencing quantification of the paternally methylated DMRs *H19/IGF2*:IG-DMR and *MEG3*:TSS-DMR (**A**) and the maternally methylated DMRs *KCNQ1OT1*:TSS-DMR, *MEST*:alt-TSS-DMR, *PLAGL1*:alt-TSS-DMR, *GNAS-AS1*:TSS-DMR and *GRB10*:alt-TSS-DMR (**B**). Reported data are the mean of at least two independent PCR and pyrosequencing experiments. P-values were calculated by two-tailed Student's T-test (* = $P \leq 0.05$; *** = $P \leq 0.005$). Statistics is reported in Supplementary Table 2.

Supplementary Figure 3 Alignment of orthologous cyclin B3 sequences in placental mammals around human CCNB3 Val1251. Protein sequences derived were retrieved from the KEGG databank (ORTHOLOGY: K21771) and aligned using Clustal Omega. Human Valine 1251 and its conservative replacement Threonin in orthologs are highlighted. MACFA, Macaca fascicularis; PHYMC, Physeter microcephalus; DELLE, Delphinapterus leucas; BALA, Balaenoptera acutorostrata scammoni;RAT, Rattus norvegicus; HORSE, Equus caballus;

MOUSE, Mus musculus; TRIMA, Trichechus manatus; URSMA, Ursus maritimus; URSAR, Ursus arctos; ENHLU, Enhydra lutris; LIPVE, Lipotes vexillifer; PIG, Sus scrofa; ODORO, Odobenus rosmarus divergens; VULVU, Vulpes vulpes; CANLF, Canis lupus familiaris.

Supplementary Figure 4 In silico docking of CCNB3 model onto CDK1 and CDK2. (**A**) Model of CCNB3-CDK1 complex. CDK1 is in cyan, the lowest energy pose of CCNB3 docked onto CDK1 is in yellow, two further low energy poses of CCNB3 docked onto CDK1 are in light and dark gray. As a control, the experimentally determined pose of CCNB1 on CDK1 (pdb entry 6gu2), is shown in green. (**B**) Model of CCNB3-CDK2 complex. CDK2 is in blue, the lowest energy pose of CCNB3 docked onto CDK2 is in pink, two further low energy poses of CCNB3 docked onto CDK2 are in light and dark gray. As a control, the experimentally determined pose of CCNB1 on CDK2 (pdb entry 2jgz) is shown in purple.

Supplementary Tables.

Supplementary Table 1 Summary of clinical data of II-6 and II-7.

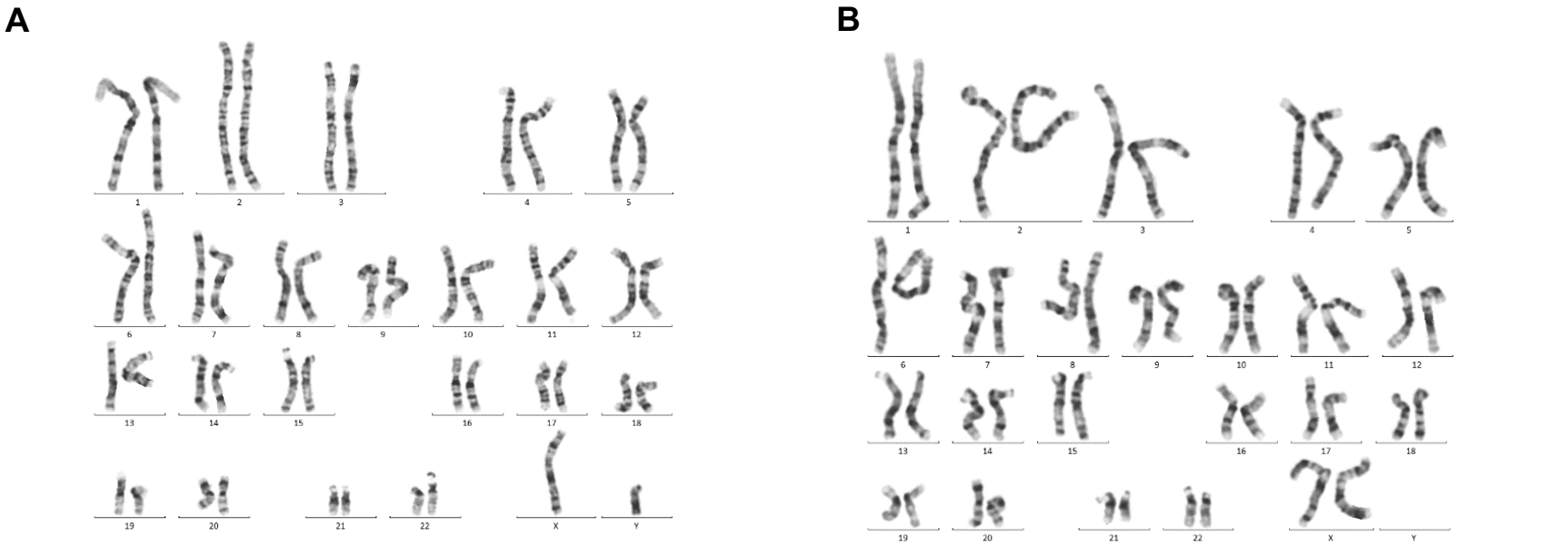
Supplementary Table 2 Statistics of methylation analysis.

Supplementary Table 3 Segregation of polymorphic STS markers.

Supplementary Table 4 Called homozygous variants in II-6 and II-7.

Supplementary Table 5 Primer sequences.

Supplementary Data Script of the bioinformatics pipeline used.



Supplementary Figure 1 A-D

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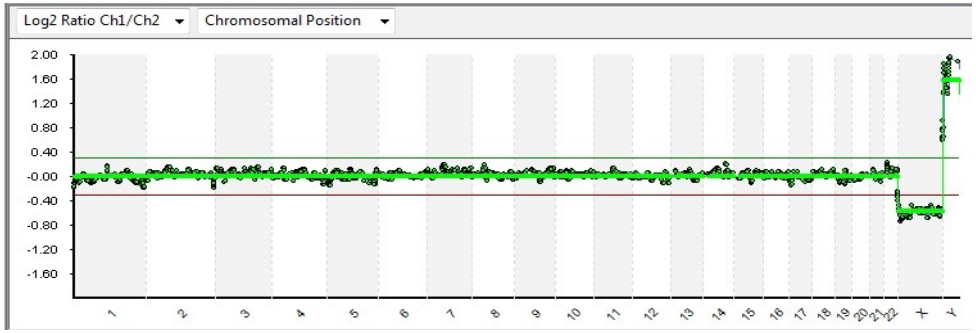

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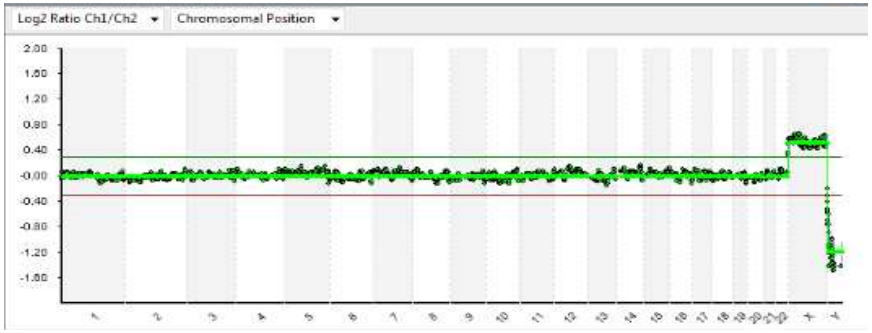

Supplementary Figure 1 E-F

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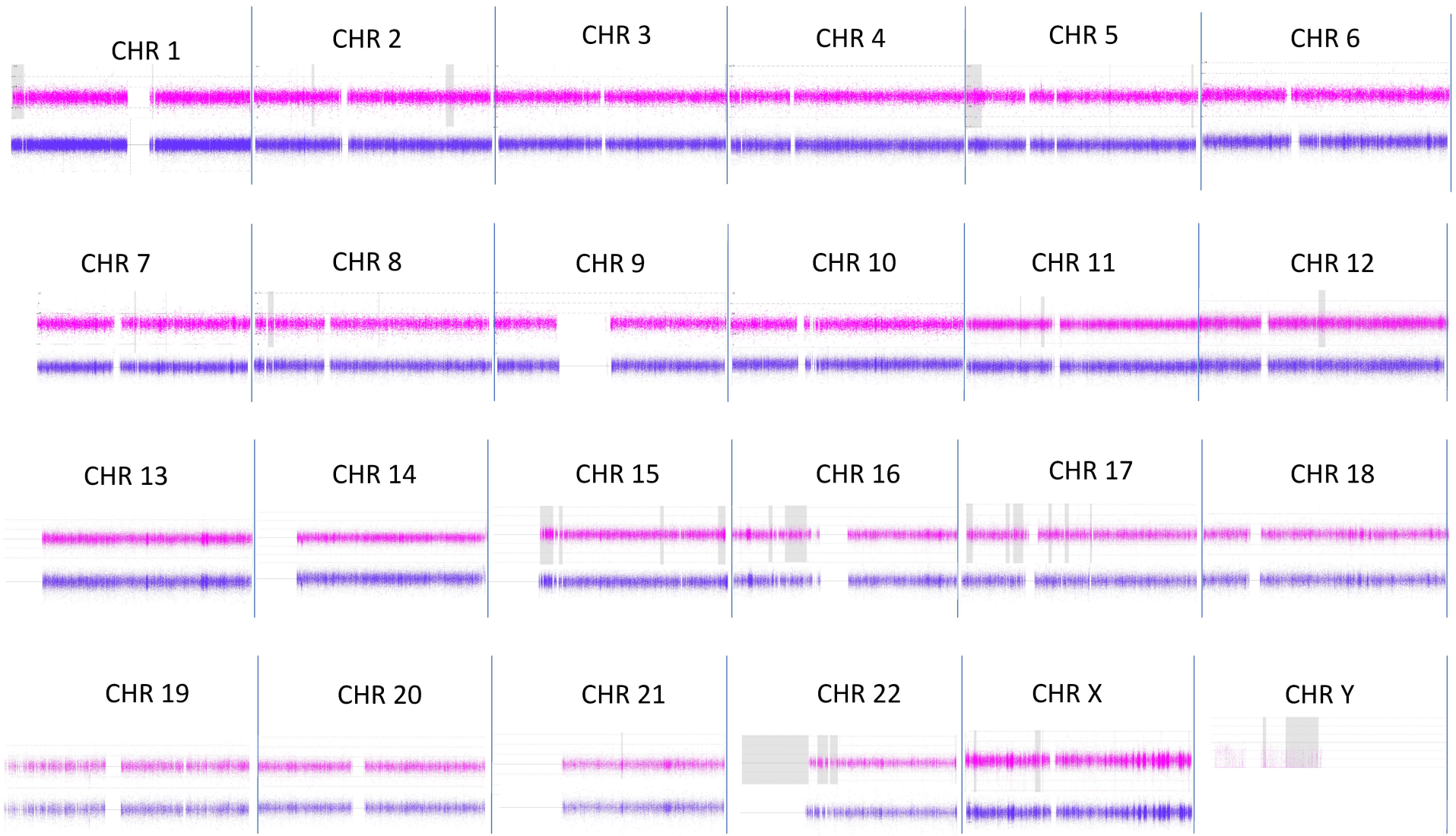
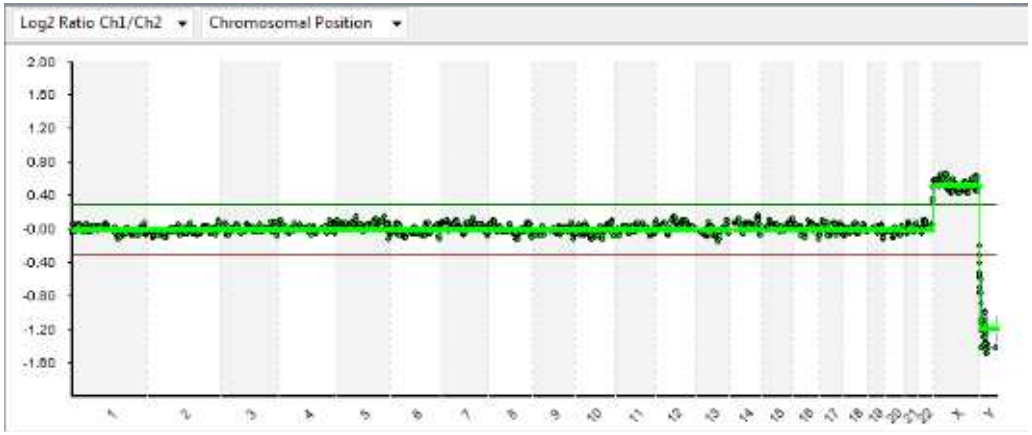


Supplementary Figure 1 G

H



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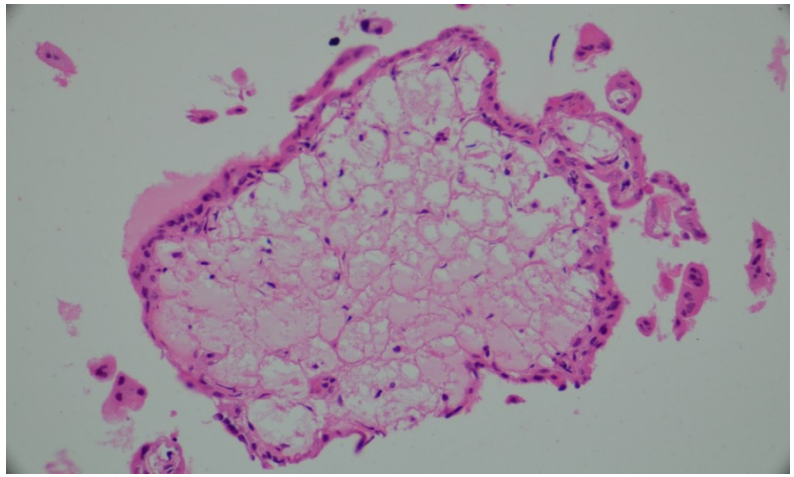
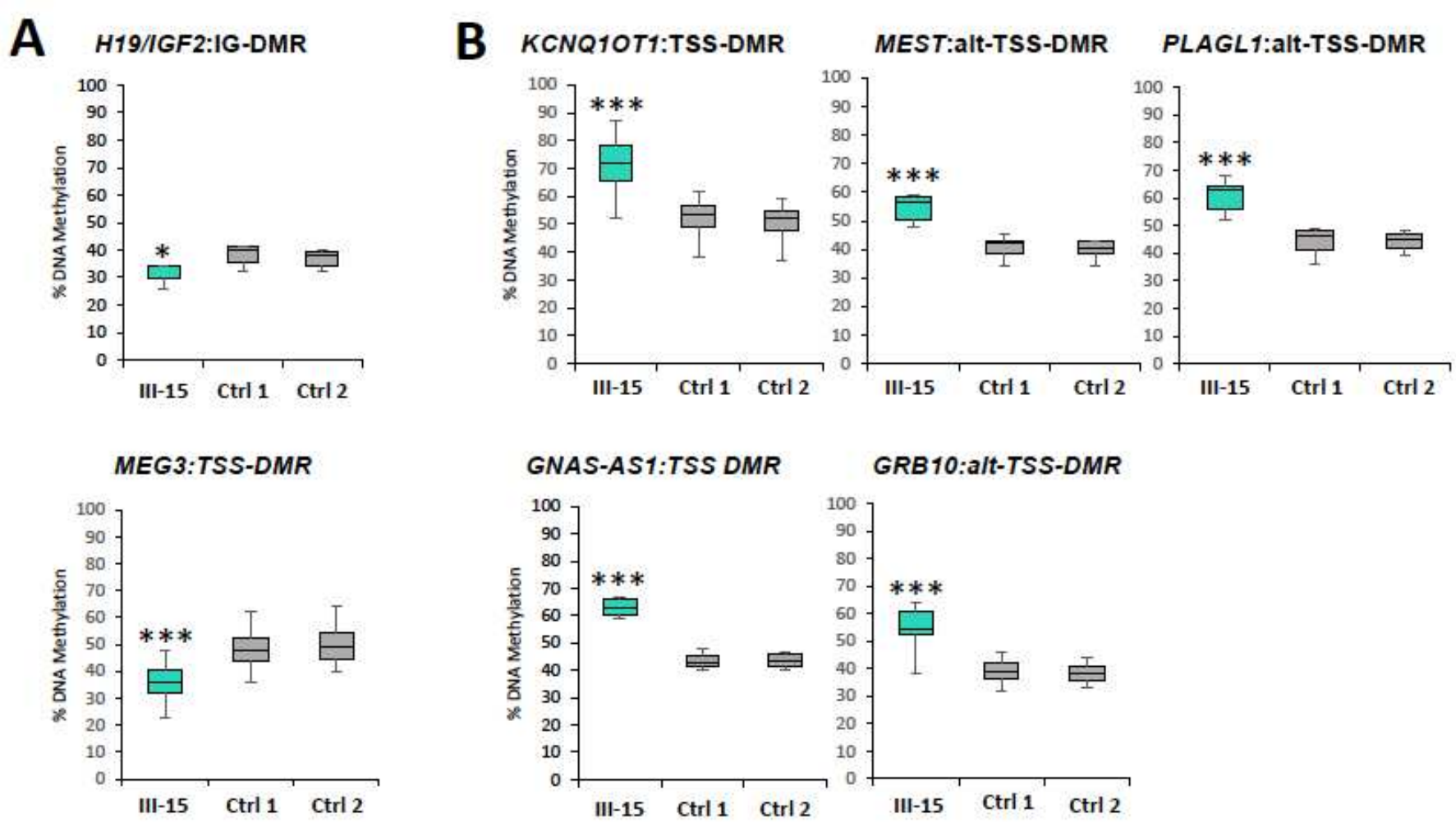


Supplementary Figure 1 H-J



Supplementary Figure 2

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CCNB3_HUMAN           EHNSPRVDDFVYICDDNYQRSEVLSMEINILNVLKCDINIPIAYHFLRRYARC
A0A2K5V9G9_MACFA      EHHPPCVDDFVYICDDNYQRYEMLNVEIDILNVLKFDINIPVAYHFLRRYARC
A0A2Y9S8S0_PHYMC      EPCPPCVDDFLYICDDIYKRDEMLAMEISILNTLKFDINIPIAYHFLRRYAKC
A0A2Y9MQS0_DELLE      EPSPPCVDDFLYICDDIYKRDEMLAMEISILKTLKFDINIPIAYHFLRRYAKC
A0A452CHG3_BALAS      EPCPPCVDDFLYICDDMYKRDEMLAMENSILKTLEFDINIPIAYHFLRRYAKC
F1LVT0_RAT            ESYPPSLTEFLYICEDLYPKSEMVSLERNILKTLNFDINIPIAYHFLRRYASC
K4Q4R0_HORSE          EPCPPCVDGFLYICDDIYQRNEMLTMEISILQTLKFDINIPIAYHFLRRYARC
A2AEP2_MOUSE          ESYPPSLSEFLFICEDMYEKSDMVSLESSILQTLNFDINIPTAYNFLRRYASC
A0A2Y9R2A8_TRIMA      ETCPPCVDDFLYICDDIYQRDEVLAMEISILKTLKFDINIPTAYHFLRRYARC
A0A384C062_URSMA      ESCPPCVDDFLYICDDIYQRDEMLTMEISILQTLKFDINIPIAYHFLRRYARC
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A0A4X1SQD0_PIG        EPCPPCVDDFLYICDDIYKRDEMLAMEIRILHTLEFDINIPIAYHFLRRYARC
A0A2U3ZC98_ODORO      ESYPPCVDDFLYICDDIYQRDEMLTMEISILQTLKFDINIPIAYHFLRRYARC
A0A3Q7QYB9_VULVU      EPCPPCVDDFLYICDDIYQRHEMLSMEISILQTLKFDINIPIAYHFLRRYARC
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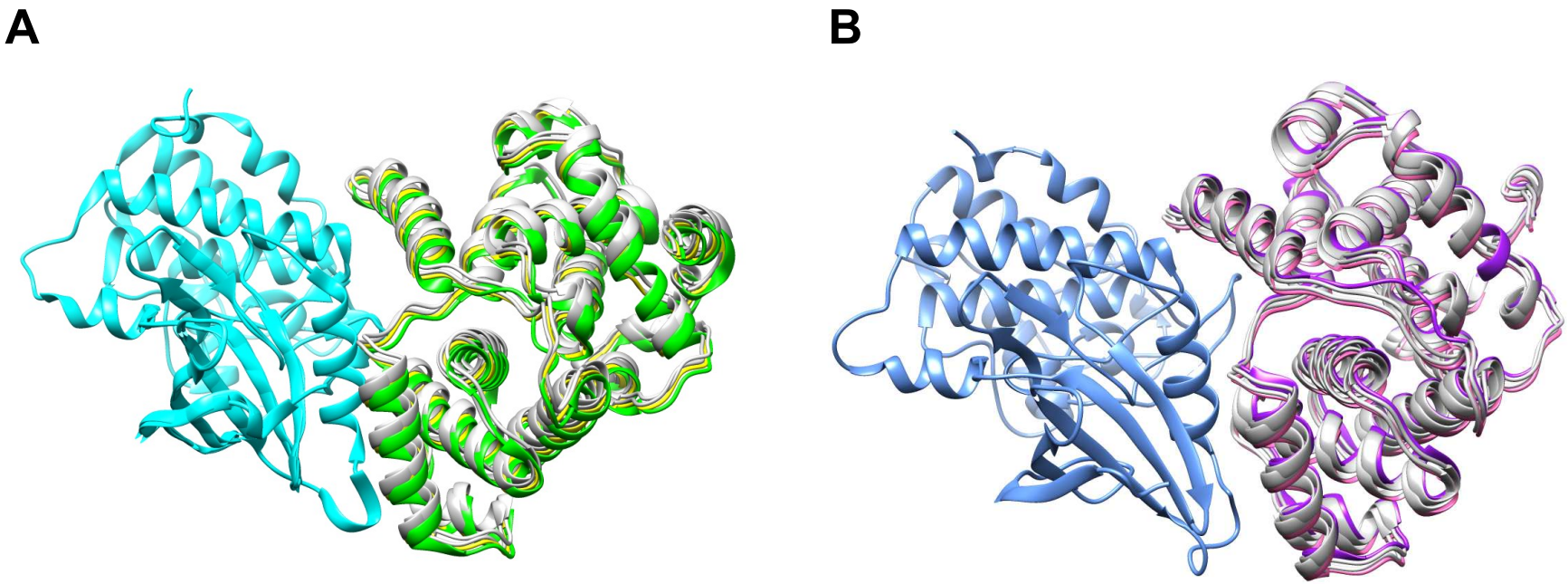
Supplementary Figure 3



Supplementary Figure 4

Supplementary Table 1 Summary of clinical data of II-6 and II-7

	Test	Result II-6	Result II-7	Normal Range
Coagulation Analysis	Activated Protein C Resistance	3.3	3.9	Negative: ≥2.9
	Prothrombin Time	12 (Sec)	13.5 (Sec)	11.0-14
	Homocystein	7	9	5-15
	Lupus Anti- Coagulant	Negative	Negative	Negative
	Anti-Thrombin III level	112	95	80-125
	Protein C	137	107	70- 150
	Protein S	145	95	60-150
Hormone Analysis	TSH	2.1 mlU/Lit	2.9 mlU/Lit	0.27-4.2
	Anti- Mullerian Hormone	1.5 ng/ml	2.1 ng/ml	0.6-7.7
Immunological Analysis	Anti-Thyroid Peroxidase	17	24	0-35.0
	Anti- Thyroglobulin Ab	81.9	100.7	0-115
	Anti Beta2 Glycoprotein	1.1	0.9	Negative: ≤10
	Anti- Cardiolipin Antibody	0.8	0.5	Normal: ≤12
Microbiological Analysis	Anti- HCV	Negative	-	Negative
	Anti-HIV I&II	Negative	Negative	Negative
	HBsAg	Negative	Negative	Negative
	Rubella IgG and IgM	Negative	-	Negative
	VDRL	Negative	-	Negative
Pelvic Ultrasound Analysis		Normal	-	Normal

Supplementary table 2 Statistics of methylation analysis

H19/IGF2:IG-DMR

t-test	Samples	1° CG Mean(r1:r2)	2° CG Mean(r1:r2)	3° CG Mean(r1:r2)	4° CG Mean(r1:r2)	5° CG Mean(r1:r2)
0,017068426	III-15	33	34	26	34	34
	Ctrl 1	39	41	32	41	40
	Ctrl 2	40	38	32	39	37

KCNQ1OT1:TSS-DMR

t-test	Samples	1° CG Mean(r1:r2)	2° CG Mean(r1:r2)	3° CG Mean(r1:r2)	4° CG Mean(r1:r2)	5° CG Mean(r1:r2)	6° CG Mean(r1:r2)	7° CG Mean(r1:r2)	8° CG Mean(r1:r2)	9° CG Mean(r1:r2)	10° CG Mean(r1:r2)	11° CG Mean(r1:r2)	12° CG Mean(r1:r2)
0,000277275	III-15	70	66	76	65	61	87	78	52	71	78	79	73
	Ctrl 1	53	50	54	49	46	62	57	38	51	56	59	55
	Ctrl 2	52	48	53	48	44	58	55	37	49	53	59	52

MEST:alt-TSS-DMR

t-test	Samples	1° CG Mean(r1:r2)	2° CG Mean(r1:r2)	3° CG Mean(r1:r2)	4° CG Mean(r1:r2)	5° CG Mean(r1:r2)	6° CG Mean(r1:r2)	7° CG Mean(r1:r2)	8° CG Mean(r1:r2)
4,66124E-06	III-15	59	56	59	48	51	57	57	50
	Ctrl 1	43	42	43	34	38	42	45	39
	Ctrl 2	43	41	43	34	38	40	42	39

MEG3:TSS-DMR

t-test	Samples	1° CG Mean(r1:r2)	2° CG Mean(r1:r2)	3° CG Mean(r1:r2)	4° CG Mean(r1:r2)	5° CG Mean(r1:r2)	6° CG Mean(r1:r2)	7° CG Mean(r1:r2)	8° CG Mean(r1:r2)
0,001263129	III-15	35	48	37	35	23	41	31	40
	Ctrl 1	46	62	50	46	36	50	43	53
	Ctrl 2	46	64	52	48	40	50	44	55

GRB10:alt-TSS-DMR

t-test	Samples	1° CG Mean(r1:r2)	2° CG Mean(r1:r2)	3° CG Mean(r1:r2)	4° CG Mean(r1:r2)	5° CG Mean(r1:r2)	6° CG Mean(r1:r2)	7° CG Mean(r1:r2)	8° CG Mean(r1:r2)	9° CG Mean(r1:r2)	10° CG Mean(r1:r2)	11° CG Mean(r1:r2)	12° CG Mean(r1:r2)	13° CG Mean(r1:r2)	14° CG Mean(r1:r2)
5,33651E-07	III-15	54	51	60	55	64	62	63	44	38	53	54	55	54	58
	Ctrl 1	37	34	41	37	46	45	45	35	32	39	39	39	39	40
	Ctrl 2	37	35	40	36	44	42	42	35	33	38	37	38	38	39

PLAGL1:alt-TSS-DMR

t-test	Samples	1° CG Mean(r1:r2)	2° CG Mean(r1:r2)	3° CG Mean(r1:r2)	4° CG Mean(r1:r2)	5° CG Mean(r1:r2)	6° CG Mean(r1:r2)	7° CG Mean(r1:r2)	8° CG Mean(r1:r2)	9° CG Mean(r1:r2)	10° CG Mean(r1:r2)	11° CG Mean(r1:r2)
3,25409E-07	III-15	62	64	64	52	68	63	58	56	52	63	67
	Ctrl 1	46	48	48	39	49	46	44	41	36	45	47
	Ctrl 2	45	46	47	39	48	47	44	42	39	45	48

GNAS-AS1:TSS-DMR

t-test	Samples	1° CG Mean(r1:r2)	2° CG Mean(r1:r2)	3° CG Mean(r1:r2)	4° CG Mean(r1:r2)	5° CG Mean(r1:r2)	6° CG Mean(r1:r2)	7° CG Mean(r1:r2)	8° CG Mean(r1:r2)
6,02372E-09	III-15	60	59	64	67	60	62	66	66
	Ctrl 1	41	40	43	46	42	43	48	44
	Ctrl 2	40	41	43	46	43	44	47	46

	Q1	MEDIAN	Q3	Q3-Q1	Min	Max	Upper whisker	II-I	III-II	Lower whisker
H19/IGF2:I	29,5	34	34	4,5	26	34	3,5	4,5	0	0
	35,5	40	41	5,5	32	41	3,5	4,5	1	0
	34,5	38	39,5	5	32	40	2,5	3,5	1,5	0,5
KCNQ1OT1	65,25	72	78	12,75	52	87	13,25	6,75	6	9
	49,25	53,5	56,75	7,5	38	62	11,25	4,25	3,25	5,25
	48	52	54,5	6,5	37	59	11	4	2,5	4,5
MEST:alt-T	50,25	56,5	58,5	8,25	48	59	2,25	6,25	2	0,5
	38,25	42	43	4,75	34	45	4,25	3,75	1	2
	38,25	40,5	42,75	4,5	34	43	4,25	2,25	2,25	0,25
MEG3:TSS-	32	36	40,75	8,75	23	48	9	4	4,75	7,25
	43,75	48	52,25	8,5	36	62	7,75	4,25	4,25	9,75
	44,5	49	54,25	9,75	40	64	4,5	4,5	5,25	9,75
GRB10:alt-	52,5	54,5	60,5	8	38	64	14,5	2	6	3,5
	36,5	39	42	5,5	32	46	4,5	2,5	3	4
	35,75	38	40,5	4,75	33	44	2,75	2,25	2,5	3,5
PLAGL1:alt	56	63	64	8	52	68	4	7	1	4
	41	46	48	7	36	49	5	5	2	1
	42	45	47	5	39	48	3	3	2	1
GNAS-AS1:	60	63	66	6	59	67	1	3	3	1
	41,25	43	45,5	4,25	40	48	1,25	1,75	2,5	2,5
	41,5	43,5	46	4,5	40	47	1,5	2	2,5	1

Supplementary Table 3 Segregation of polymorphic STSs

Marker	Chr	Cytoband	Distance from cen (bp)	II-7	III-12	III-15	II-8	Triploidy origin
D1S498	1	1q21.3	26766062	bb	abb	abb	ab	uninformative
TPOX	2	2p25.3	91806632	ab	aab	abc	bc	mat in III-12
D5S630	5	5p15.31	36844678	ad	acd	acd	bc	mat in III-12 and III-15
D5S818	5	5q23.2	73705541	ab	abb	abc	ac	mat in III-12
D5S400	5	5q34	119037229	ac	abc	aab	bb	mat in III-12 and III-15
D6S460	6	6q14.1	18520678	ab	bbc	aad	cd	mat in III-12 and III-15
D7S820	7	7q11.21-22	23789458	ab	abc	abc	bc	Uninformative
D9S1874	9	9p13.2	10145414	cd	NA	bdd	ab	mat in III-15
D10S1790	10	10q21.1	12950376	bc	NA	abb	aa	mat in III-15
D13S317	13	13q22-q31	63722123	ab	aab	bbb*	ab	Uninformative
D16S539	16	16q24.1	49786271	ac	abc	bcc	bb	mat in III-12 and III-15
D19S865	19	19p13,2	15503440	bd	cdd	bcd	ac	mat in III-12 and III-15

STS alleles are indicated for each individual by a letter with 'a' being the largest amplicon. Alleles are underlined if maternal origin is evident. mat, maternal; NA, not assessed. *only one type of allele is evident.

Supplementary Table 4 Called homozygous variants in II-6 and II-7.

POSITION (hg38)	REF	ALT	Consequence	IMPACT	SYMBOL	Feature	SIFT	PolyPhen	Exac-AF	gnomAD_AF
chrX:67545316	TGCA	T	inframe_deletion	MODERATE	AR	ENST00000374690	NA	NA	NA	NA
chrX:50346749	T	A	missense_variant	MODERATE	CCNB3	ENST00000376042.6	deleterious	probably damaging	NA	NA

Supplementary Table 5 Primer sequences

Primer	Sequence (5'-3')
CCNB3 (F)	GTGGTTCTCAGAGGGCAGAT
CCNB3 (R)	TGACCTCCCTTGTAACCAATAC
H19/IGF2:IG-DMR (F)	bio-GTGGTTTTTATGACTGTTTTATTTTTGATGA
H19/IGF2:IG-DMR (R)	ACTTCCCCTTCAATCTCACCA
H19/IGF2:IG-DMR (seq)	TACAAAATTAATTATAACTATAAAAT
MEG3:TSS-DMR (F)	bio-GTTTATTTAAGAGGGAATAGTTTTGAGAT
MEG3:TSS-DMR (R)	CCTCTCTCTCCATCCTACTCA
MEG3:TSS-DMR (seq)	AAAACCACTAAAAATCAACT
KCNQ1OT1:TSS-DMR (F)	GGAGAGTATTGTTTAGGTTAGGTTGTAT
KCNQ1OT1:TSS-DMR (R)	bio-CCTCCCCATCTCTCTAAAAAAATTTAA
KCNQ1OT1:TSS-DMR (seq)	GGTTAGGTTGTATTGTTG
MEST:alt-TSS DMR (F)	bio-AATAAAGGGGGTTTTGTTTTTTTAAT
MEST:alt-TSS DMR (R)	AACCCACCACCAAACTAAT
MEST:alt-TSS DMR (seq)	TAACCACTATAACCAAAATTAC
PLAGL1:TSS-DMR (F)	GTTAAGTGGTAGGAGGAGGTTT
PLAGL1:TSS-DMR (R)	bio-CTATACCTAAACCACCTTAACTTTACCC
PLAGL1:TSS-DMR (seq)	GGTAGGAGGAGGTTT
GNAS-AS1:TSS-DMR (F)	TAGGTTGTAGTGGGGTTAAAGGA
GNAS-AS1:TSS-DMR (R)	bio-CTATACCTAAACCACCTTAACTTTACCC
GNAS-AS1:TSS-DMR (seq)	GGTAGGAGGAGGTTT
GRB10:alt-TSS-DMR (F)	bio-GGTAGGGGTTTTTGTAGTTTG
GRB10:alt-TSS-DMR (R)	CTCTCCAAATACTCAAATAAACTC
GRB10:alt-TSS-DMR (seq)	CCAAATACTCAAATAAACTCC
D1S498 (F)	TTGCTGAAGGGACATAGTG
D1S498 (R)	TGCTGGGTTATATCCAATATC
TPOX (F)	CACTAGCACCCAGAACCGTC
TPOX (R)	CCTTGTCAGCGTTTATTTGCC
D4S405 (F)	ATCAGGAGATGTTGCCTTGC
D4S405 (R)	CAGGGCTATGATTGGATGTC
D4S428 (F)	TAAGAGGCTCGAACAACACTACT
D4S428 (R)	CCAGCATTTGGACTCTAAAGAA
D5S818 (F)	GGGTGATTTTCCTCTTTGGT
D5S818 (R)	TGATTCCAATCATAGCCACA

D5S1969 (F)	AGGGAACCTCACCTGG
D5S1969 (R)	GACAAGGGCTGGGATG
D5S630 (F)	CATGACGATGTGGGCAG
D5S630 (R)	CCTTTCAGTGTAGAAGTGTGTGTGT
D5S400 (F)	GCCTGGCTGATAGAATGAGA
D5S400 (R)	TTCCTAATTTGCTGGCTTCC
D6S257 (F)	GAGAACTCGTCCTTTGGTCC
D6S257 (R)	TGAGAAAATGTTCAGGCTAAAGATA
D6S460 (F)	AATTCCCATTTGAAGAAACC
D6S460 (R)	CAGTGGGCTCTCACCC
D7S820 (F)	CCAATATTTGGTGCAATTC
D7S820 (R)	CCTTAAAATCTGAGGTATC
D8S532 (F)	GCTCAAAGCCTCCAATGAC
D8S532 (R)	GACTTCGTGATCCACCTGC
D9S1874 (F)	GTATAGTATGGAGCAGAAATGTAAC
D9S1874 (R)	GGCCAAGGGATAAACAG
D10S1790 (F)	AGTGAAATGGCTACAACCAA
D10S1790 (R)	GCCTGAGATACATAAGGTGCT
D12S1663 (F)	GCCCATGATACTAAGTGAGAAATAC
D12S1663 (R)	GTAAAACGTGAAACAATCCTAAGA
D13S175 (F)	TATTGGATACTTGAATCTGCTG
D13S175 (R)	TGCATCACCTCACATAGGTTA
D13S317 (F)	ACAGAAGTCTGGGATGTGGA
D13S317 (R)	GCCCAAAAAGACAGACAGAA
D15S128 (F)	GCTGTGTGTAAGTGTGTTTTATATC
D15S128 (R)	GCAAGCCAGTGGAGAG
D16S539 (F)	GATCCCAAGCTCTTCCTCTT
D16S539 (R)	ACGTTTGTGTGTGCATCTGT
D19S414 (F)	CCAGACCTGTCCATCTTGTATGAAT
D19S414 (R)	TTAGAACAACGCTTGGGCATTT
D19S566 (F)	AGCTTCAGAGGCCATAGC
D19S566 (R)	CAGGTAGGGCTGGAATGTT
D19S865 (F)	GCTATTTGGGGTCTCTATCAATG
D19S865 (R)	GAAATCGCACAGTATTTGTCTCAC
DXS991 (F)	ACTTCAACCACAGAAGCCTC

DXS991 (R)	ATCATTTGAGCCAATTCTCC

Supplementary Data Script of the bioinformatics pipeline used.

```
bwa mem -t 24 -R '@RG\tID:1-3\tSM:1-3\tPL:Illumina\tPU:Hiseq2500' -M
~/98/hg18/ucsc.hg18.fasta 1-3_R1.fastq.gz     1-3_R2.fastq.gz > 1-3.sam


bwa mem -t 24 -R '@RG\tID:1-3\tSM:1-4\tPL:Illumina\tPU:Hiseq2500' -M
~/98/hg18/ucsc.hg18.fasta 1-4_R1.fastq.gz     1-4_R2.fastq.gz > 1-4.sam


bwa mem -t 24 -R '@RG\tID:2-6\tSM:2-6\tPL:Illumina\tPU:Hiseq2500' -M
~/98/hg18/ucsc.hg18.fasta 2-6_R1.fastq.gz     2-6_R2.fastq.gz > 2-6.sam


bwa mem -t 24 -R '@RG\tID:2-7\tSM:2-7\tPL:Illumina\tPU:Hiseq2500' -M
~/98/hg18/ucsc.hg18.fasta 2-7_R1.fastq.gz     2-7_R2.fastq.gz > 2-7.sam


for f in * .sam; do samtools flagstat $f > ${f/.sam/.stat} ;done
for f in *.sam ; do samtools sort -@ 24 -o ${f/.sam/_sorted.bam} $f; done

for f in *.bam; do java -jar /usr/local/bin/picard.jar MarkDuplicates  I=$f
O=${f/.bam/_1duplicates.bam}  M=${f/.bam/_dup_metrics.txt}; done

for f in *_1duplicates.bam; do samtools index $f; done


for f in *_1duplicates.bam; do java -jar -Xmx17G /home/gatk/gatk-package-4.0.6.0-local.jar
BaseRecalibrator  -I $f -R ~/98/hg18/ucsc.hg18.fasta --known-sites ~/98/hg18
/bundle/hg18/dbsnp_138.hg18.vcf -O ${f/.bam/.grp} ; done

for f in *_1duplicates.bam;  do java -jar -Xmx17G /home/gatk/gatk-package-4.0.6.0-local.jar
ApplyBQSR  -I $f -R ~/98 /hg18/ucsc.hg18.fasta --bqsr-recal-file ${f/.bam/.grp} -O
${f/.bam/_recal.bam} ; done

for f in *_recal.bam;  do java -jar /home/gatk-package-4.0.6.0-local.jar HaplotypeCaller -
R   ~/98/hg18/ucsc.hg18.fasta -I $f  -O ${f/.bam/.vcf}  -bamout ${f/.bam/_bamout.bam} ;
done
```