Preimplantation genetic testing for a chr14q32 micro-deletion in a family with Kagami-Ogata syndrome and Temple syndrome.

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Abstract

Introduction:

Kagami-Ogata syndrome (KOS14) and Temple syndrome (TS14) are two disorders associated with reciprocal alterations within the chr14q32 imprinted domain. Here we present a work-up strategy for preimplantation genetic testing (PGT) to avoid the transmission of a causative micro-deletion.

Methods:

We analysed DNA from the KOS14 index case and parents using methylation-sensitive ligation mediated probe amplification and methylation pyrosequencing. The extent of the deletion was mapped using SNP arrays. PGT was performed in trophectoderm samples in order to identify unaffected embryos. Samples were amplified using multiple displacement amplification, followed by genome-wide SNP genotyping to determine the at-risk haplotype and next generation sequencing to determine aneuploidies.

Results:

A fully methylated pattern at the normally paternally methylated IG-DMR and *MEG3* DMR in the KOS14 proband, accompanied by an unmethylated profile in the TS14 mother was consistent with maternal and paternal transmission of a deletion, respectively. Further analysis revealed a 108 kb deletion in both cases. The inheritance of the deletion on different parental alleles was consistent with the opposing phenotypes. I*n vitro* fertilization with intracytoplasmatic sperm injection and PGT were used to screen for deletion status and to transfer an unaffected embryo in this couple. A single euploid-unaffected embryo was identified resulting in a healthy baby born.

Discussion:

We identify a microdeletion responsible for multi-generation KOS14 and TS14 within a single family where carriers have a 50% risk of transmitting the deletion to their offspring. We show that PGT can successfully be offered to couples with IDs caused by genetic anomalies.

Background

Disturbances of the chromosome 14q32 (chr14q32) imprinted domain are associated with both Kagami-Ogata syndrome (KOS14) (OMIM 608149) and Temple syndrome (TS14)(OMIM 616222), depending on the parental-origin of the aberration. Historically these two syndromes have been known as paternal uniparental disomy 14 (UPD(14)pat)-like syndrome and UPD(14)mat-like syndrome, respectively, as these are the most frequent genetic causes[1,2]. However, detailed molecular characterisation in patients without UPD has identified subtle deletions and imprinting defects in a subset of probands[3,4]. Therefore, both KOS14 and TS14 are recognised congenital diseases that belong to the group of imprinting disorders (IDs) that result from the abnormal dosage of imprinted genes. Imprinted genes are typified by their parent-of-origin monoallelic expression; in other words, they are only expressed from the maternally or paternally inherited copy, but not both. The control of this monoallelic expression is regulated by the interplay between different epigenetic mechanisms, but is largely dependent upon differentially methylated regions (DMRs) that orchestrate coordinated transcriptional repression[5].

The human chr14q32 locus harbours a cluster of imprinted genes within a 680 kb interval that includes the paternally expressed *DLK1* and *RTL1* transcripts, and the maternally expressed *MEG3/GTL2, RTL1*-antisense, *MEG8* and a large cluster of snoRNA (SNORD113-114) and micoRNAs (miR493-miR154)[6]. The parental origin-dependent expression is coordinated by the germline-derived *DLK1-MEG3* intergenic DMR (IG-DMR; *MEG3/DLK1*:IG-DMR)[7] and the somatically acquired secondary *MEG3* DMR (*MEG3*:TSS-DMR), both of which are methylated on the paternal allele[8]. Two additional intergenic DMRs have been identified within the locus associated with *MEG8* (*MEG8*:Int2-DMR) and *DLK1* (*DLK1*:Int1-DMR)[9,10], both of which are methylated on the maternal chromosome following fertilization, although their functions are not yet known.

Hypermethylation of the IG-DMR and *MEG3* DMR, maternal microdeletions and UPD(14)pat are associated with the KOS14 phenotype that includes facial abnormalities, small bell-shaped thorax with coat-hanger ribs, abdominal wall defects, placentomegaly, polyhydramnios and severe intellectual disability[11]. In contrast hypomethylation of IG-DMR and *MEG3* DMR, paternal microdeletions and UPD(14)mat cause TS14 which is characterised by prenatal and postnatal growth restriction, hypotonia, feeding difficulties, truncal obesity and precocious puberty[4, 12]. To date, ~60% of KOS14 and TS14 cases are caused by UPD14, while microdeletions involving IG-DMR and *MEG3* DMRs account for ~20% and ~10% of cases, respectively. Imprinting defects affecting the paternally methylated DMRs occurs in ~20% of cases for both diseases [12]. Because of the non-specific phenotypic features associated with TS14, a small proportion of idiopathic cases maybe associated with a differential diagnosis, such as Silver-Russell syndrome (SRS)[11, 13].

Preimplantation genetic testing, as defined in 2017 by Zegers-Hochschild *et. al.,* is a test performed to analyze the DNA from oocytes (polar bodies) or embryos (cleavage stage or blastocyst) for HLA typing or for determining genetic abnormalities[14]. Typically, single gene disorders and/or structural chromosome aberrations are detected via PCR methodologies or next generation techniques (NGS). Biopsy can be performed at different embryo developmental stages. Thus, polar body analysis can be performed on mature oocytes and/or the zygote, blastomere analysis can be accomplished at the cleavage stage and trophectoderm analysis at the blastocyst stage. In recent years a growing trend has been observed for laser trophectoderm biopsy in assisted reproduction techniques (ART) to the detriment of the other

two strategies[15]. For the detection of unaffected-euploid embryos whole genome amplification from trophectoderm samples can be performed. The amplification products are subjected to genome-wide single nucleotide polymorphisms (SNPs) array and linkage analysis, for example using *Karyomapping*[16]. This consists of analyzing hundreds to thousands of SNPs scattered throughout the genome of the parents, their embryos and a reference allowing the identification of the parental origin of the chromosomes. Analysis of results obtained from the parents and a reference (a relative with known genetic status, usually a sibling or a progenitor), allows for the identification of the at-risk chromosome. Knowing the haplotype at risk, it is possible to track which embryos have inherited the at-risk chromosome (or SNP combination at risk) or its normal counterpart. Thus, transfer of embryos carrying the condition can be avoided.

Testing for aneuploidy in the embryos of *in vitro* fertilization (IVF) patients and the subsequent transfer of those that are chromosomally normal has been shown to improve the ongoing pregnancy rate[17,18]. Genome-wide SNP analysis allows *Karyomapping* to support copy number variants (CNV) and, therefore, to detect some trisomies and/or monosomies. However, not all the aneuploidy events can be detected and comprehensively confirmation of euploidy requires NGS analysis.

Here, we report a family with a 108 Kb deletion spanning the IG-DMR and *MEG3* DMR interval that produces KOS14 on maternal transmission and TS14 when located on the paternally inherited chromosome. The exact size and location of this deletion indicates it is the smallest microdeletion associated with these IDs within a single family and our detailed description of its molecular and clinical consequences allowed for the design and successful implementation of PGT and the birth of an unaffected child without deletion.

Methods

Methylation pyrosequencing

Bisulphite treatment of 1ug of DNA was performed with the EZ DNA methylation-Gold spin columns (Zymo Research), following the manufacturer's protocol. Control DNAs from patients carrying UPD(14)pat and UPD(14)mat were used to detect extreme methylation values at the imprinted DMRs assessed. Pyrosequencing was selected for the quantitative assessment of DNA methylation at the IG-DMR, *MEG3* DMR, *DLK1* DMR and *MEG8* DMRs as previously described[9,10,19,20](see Supplementary Table 1 for primer sequences). Bisulphite PCR was performed with one primer being biotinylated. Immobilization of the PCR products for purification was achieved by streptavidin-coated sepharose beads (Qiagen) with the use of the PyroMark Q24 Vacuum Prep Workstation according to the manufacturer's instructions.

Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA)

Gene dosage and methylation analyses of the chromosomal region 14q32 including the IG-DMR and *MEG3* DMR were carried out using the SALSA MLPA Kit ME032-A1 (MRC Holland, Amsterdam, The Netherlands) according to the manufacturer's manual. Amplification products were analysed on an ABI3500 capillary sequencer (Applied Biosystems) followed by data analysis with Genemapper v5 Software (Applied Biosystems) and Coffalyser.net (MRC Holland).

SNP array

Saliva-derived genomic DNA from the index case was quantified using Nanodrop1000 spectrophotomer (Thermofisher) and Qubit (Thermofisher). Chromosomal microarray

analysis was performed with the genome-wide scan of 850,000 tag SNPs (Illumina Infinum CutoSNP-850k BeadChIP) following standard laboratory procedures. Copy-number variations were determined using the Chromosome Viewer tool contained in the Genome Studio package (Illumina). In Chromosome Viewer, gene call score <0.15 at a locus were considered "no calls". In addition, all allele frequency analysis was applied for all SNPs.

Long-range PCR

PCR products were generated from DNA samples of the KOS14 index case using Immolase DNA Taq polymerase (Bioline) and staggered PCR primers. An amplicon was obtained using C4F-ACCGGAGTTCCTTTCAGAGACA and TR3-GGTTCAGGCAGGGTACAGGACAT. The resulting PCR product was subject to Sanger sequencing in both directions using BigDye Terminator v3.1 kit (Thermofisher) and run on ABI 3730 DNA 48-capillary sequencer. The resulting electropherograms were analysed in Sequencher and subject to BLAST in the UCSC genome browser.

Embryo biopsy and tubing

Following standard assisted reproductive techniques, laser mediated trophectoderm biopsy was carried out and 4 to 7 cells on average were obtained from each embryo. After biopsy, embryos were vitrified for future transfer. Biopsied cells were washed and collected into sterile RNAse and DNAse free PCR tubes.

PGT-M for Imprinting disorder

Preimplantation Genetic Testing for monogenic disorders (PGT-M) was carried out using *Karyomapping*. This is a genome-wide SNP genotyping technique that allows for both the study of the imprinting disorder though linkage analysis and the identification of CNVs.

Pre-test analysis

Prior to IVF-Intracytoplasmic sperm injection (ICSI)-PGT cycle, 400 ng of genomic DNA samples from parents and the KOS14 index case were investigated in order to confirm the presence of informative SNPs inside the microdeletion and the flanking regions (2.0 Mb) through *Karyomapping* technique. This method involved the use of the Illumina Infinium HumanKaryomap-12 DNA analysis kit, according to the manufacturer's instructions.

Bead array datasets were then imported into dedicated software (BlueFuse Multi, Illumina), which assisted with the visualization and interpretation of the data obtained with *Karyomapping* cases. The outcome of the laboratory protocol is a set of genotype calls for each SNP on the array, depicted at four values: AA, AB, BB or NC (no call), where A represents the nucleotides adenine (A) and thymine (T) and B represents the nucleotides guanine (G) and cytosine (C) in the genetic sequence. We consider a SNP as informative when a genotype can be assigned to one of the chromosomes inherited from the mother or father (one parent must be heterozygous while the other is homozygous).

PGT-M analysis

Embryonic DNA was subjected to whole genome amplification using multiple displacement amplification (MDA) following manufacturer's protocols (Repli-G Single cell kit, Qiagen). Electrophoresis was carried out to confirm that all samples and positive controls amplified appropriately.

Afterwards, 8µl of MDA amplification product from each embryo sample was processed with the *Karyomapping* technique. After scanning of the bead chip, the data was imported into the BlueFuse Multi software in order to phase the SNPs in the embryo in relation to the alleles of the KOS14 index (which will be referred to as the reference) to determine the parental inheritance of the haploblock at-risk.

Embryo and reference were referred to be in phase when they inherited the same chromosome and to be out of phase when they inherited a different chromosome. A consequence of relative phasing is that recombination events in the reference cause a change of phase in all corresponding embryos, and as a result the position of recombination events can be determined. Since allele drop-out (ADO) can result in the loss of an informative allele, the software classifies the analysed SNPs in two groups: key SNPs and non-key SNPs. Key SNPs provide strong support of the predicted phase since they contain the informative allele so ADO could not have affected their phasing. In contrast, non-key SNPs provide weaker support of the predicted phase. This group does not contain the informative allele so it is not possible to distinguish between homozygous genotypes from the loss of the informative allele through ADO. Due to the large amount of phasing data available, *Karyomapping* results are not affected by ADO and also allows for the detection of meiotic and some mitotic CNVs, as well as tracing the parental origin of aneuploidies.

Preimplantation Genetic Testing for aneuploidies

Since *Karyomapping* has not been fully validated for the purpose of aneuploidy screening, embryos considered to be unaffected that did not show numerical chromosomal abnormalities by *Karyomapping* were analyzed by NGS. Aliquots of the same whole-genome amplified trophectoderm samples were used for NGS-based technology by means of VeriSeq PGS Kit (Illumina, San Diego, CA, USA) on Illumina MiSeq™ System according to the manufacturer instructions and were analyzed by the BlueFuse Multi software (Illumina).

The Veriseq PGS kit uses an engineered transposome for the preparation of sequencing-ready libraries to tagment the input DNA. Subsequently, a limited-cycle PCR uses the adapter sequences to amplify the insert DNA and to add index sequences to both ends of the DNA. Prepared VeriSeq PGS libraries are pooled and run on the MiSeq system, where secondary analysis of the data is performed, demultiplexing and aligning the reads to the reference genome. Data obtained are imported into BlueFuse Multi Analysis software, which process and display the data to provide genomic profiles of each sample in a run. Whole chromosome aneuploidy is called automatically. The effective resolution of the assay is 20 Mb. The number of reads after filtering, sample overall noise score and average quality alignment scores were assessed according to the VeriSeq quality control parameters.

Results

Clinical history

The index case was prenatally followed for omphalocele associated with severe polyhydramnios. At 26-weeks of gestation an amniodrainage was required to avoid premature delivery as the patient initiated uterine contractions and cervical shortening. The procedure was complicated by a placental abruption requiring an emergency caesarean section. Birth weight was 760 g and 5 minutes Apgar score was 8. Following birth, a silo was applied to contain the omphalocele and surgical correction was completed after 4 days. The newborn showed macroglossia with large filtrum, hypersialorrhea and a bell-shaped thorax with coat-

hanger ribs. Based on these clinical findings a diagnosis of KOS14 was suspected. The newborn presented respiratory and infectious complications dying at 74 days of life.

Molecular genetic findings

Methylation analysis in the KOS14 index case was performed by bisulphite PCR followed by pyrosequencing for the germline-derived paternally methylated IG-DMR. This revealed the presence of fully methylated sequences only, indicating that the patient has UPD(14)pat, a deletion or imprinting defects. Microsatellite analysis did not provide evidence of UPD. MS-MLPA was used to differentiate between a deletion and an imprinting defect, which identified a maternally-inherited deletion (Figure 1A). Subsequent pyrosequencing and MS-MLPA analysis in parental samples revealed that the mother also carried the deletion but on her paternal inherited chromosome (Figure 1A). Using Illumina genome-wide human SNP array analysis, the deletion was shown to include *DLK1* and the first 4 exons of *MEG3* (Figure 1B). Long-range PCR encompassing the last known intact SNPs was used to amplify across the deletion, which revealed it spanned 108,762 bp (hg19 chr14:g.101190852_101299552del)(Figure 2A, B). Sequence comparisons with the hg19 reference genome revealed a 1 bp thymine insertion between the centromeric and telomeric breakpoints.

Additional characterization of imprinted methylation

The 108 kb deletion removed not only the IG-DMR but the somatically acquired paternally methylated *MEG3* DMR and the intragenic maternally methylated *DLK1* DMR. These observations were confirmed using pyrosequencing in DNA from the KOS14 index case and the mother (Figure 3; Supplementary Table 2)[3]. In addition to these regions, an additional post zygotically-acquired imprinted DMR maps to the second intron of *MEG8*[10]. Although not contained within the deletion, this DMR presents with altered allelic methylation consistent with hierarchical acquisition dependent upon correct germline establishment of the IG-DMR (Figure 2) [21].

Paternal deletion is consistent with Temple syndrome

The methylation analysis of the paternally methylated IG-DMR and *MEG3* DMR was severely hypomethylated in the mother's DNA sample[3](Supplementary Table 2). Subsequent clinical investigations revealed that she presented with several main characteristics of TS14, including short stature with a final adult height of 152 cm, precocious puberty at 7 years (with Decapeptyl GNRH agonist therapy until 13 years) and had high fasting blood sugar levels consistent with diabetes. Diagnosis of TS14 in adulthood is difficult because of the debilitating non-specific clinical features, however the phenotype observed is consistent TS14 and a lack of *DLK1* expression[4, 12].

Pre-implantation genetic testing

The recurrence risk of KOS14 in subsequent pregnancies for the mother is 50% due to the dominant nature of inheritance. Since the couple wished to have a family, following genetic counselling, pre-implantation genetic testing was performed.

A total of 614 SNPs were assessed by the HumanKaryomap-12 BeadChip in the preliminary analysis of genomic DNA samples from the KOS14 index case and parents. The results obtained revealed the presence of 69 maternal informative SNPs (none in the microdeletion region, 37 in the left flanking region and 32 in the right one) and 143 paternal

informative SNPs (5 in the deleted region, 78 in the left flanking region and 60 in the right one).

Subsequently, three rounds of ovarian stimulation and ICSI resulted in 10 embryos (6 in the first cycle, 1 in the second and 3 in the third). Following trophectoderm biopsy, embryo testing was performed by *Karyomapping* to identify embryos that carried the deletion using SNP phasing and subsequent NGS for the analysis of numerical chromosomal abnormalities. Four embryos were diagnosed as affected, two as affected and aneuploid and 3 as unaffected with aneuploidy (Table 1). No recombination events affected the region of interest in any of the analyzed embryos. A single unaffected and euploid embryo was identified and transferred (Figure 4). The subsequent pregnancy was unremarkable and a healthy baby boy was born at term. The absence of the deletion was confirmed on saliva-derived DNA using MS-MLPA (Figure 1A).

Conclusions

Here we describe a child diagnosed with KOS14 due to a maternally inherited deletion of 14q32. The proband was noted to have several abnormalities consistent with the suspicion of KOS14, that was confirmed upon molecular investigations. Methylation and copy-number analyses determined that the deletion on the paternal chromosome was sufficient to cause TS14 phenotype in the proband's mother. KOS14 is frequently associated with prematurity due to polyhydramnios, with a mortality rate of ~30% that invariable occurs before 4 years of age due to postnatal respiratory insufficiency and infection. The cases that survive beyond this stage always have severe developmental delay[22]. This syndrome, unlike most other IDs, warrants PGT when the molecular mechanism indicates high reoccurrence, such as microdeletion or UPD associated with Robertsonian translocations[23].

Upon reviewing current medical literature, characterisation of KOS14 with deletions of different sizes has unveiled the regulatory mechanisms associated within the 14q32 imprinted domain. The deletion described here is one of smallest associated with both KOS14 and TS14 in the same family. A similar familial case resulting in both syndromes has been reported with deletion breakpoints in approximately similar locations (family A)[3] (Supplementary Figure 1), resulting in a deletion 6 bp larger. A maternally inherited 133 kb deletion has been described in KOS that left the *DLK1* gene and IG-DMR intact, which upon paternal deletion did not result in TS14[24]. Similarly, a 8.6 Kb deletion encompassing the IG-DMR was identified in a KOS14 case, with the mother showing some TS14-phenotypic features including short stature and obesity. However, the authors concluded that these clinical features were non-specific and appear to be irrelevant to the microdeletion[8]. This highlights the difficultly in diagnosing TS14 in adulthood and a consensus is required to whether TS14 is primarily a clinical diagnosis with confirmation using molecular testing similar to SRS[25]. Overall, these finding are consistent with KOS14 being caused by aberrant dosage of the imprinting *RTL1* gene[3, 26], whilst disruption of *DLK1* is responsible for TS14, as highlighted by a ~14 kb microdeletion that removed the *DLK1* promoter and first exon, leaving the remainder of the loci intact[27].

Underlying the mechanism associated with the KOS14 index case has allowed for the design of a PGT-M strategy to track the inheritance of the chromosomes involved. By using *Karyomapping*, we have been able to correctly diagnose which embryos have received the chromosome carrying the deletion, thus, avoiding the transfer of affected embryos. *Karyomapping* has allowed preimplantation genetic testing for monogenic diseases to be faster (by reducing the time for work-up), versatile (we can use the same platform for different

diseases) and powerful (much more markers are studied compared to conventional PCR techniques) as well as being applied in *de novo* cases[28].

Genome wide haplotyping analysis also allowed us to detect some aneuploidies in the embryo cohort. Aneuploidy is thought to be the main reason for the failure of IVF. Detection of anomalies, utilizing NGS technologies, and the confirmation of the correct chromosome complement increases the chance of pregnancy for couples undergoing ART. The combination of *Karyomapping* and NGS is a powerful method that allowed us to transfer one unaffected-euploid embryo that resulted in an unremarkable pregnancy and the birth of a healthy baby.

The use of trophectoderm biopsies allows for higher technical and biological robustness compared to other sampling strategies. Trophectoderm biopsies allowing for the analysis of a larger number of cells, thus reducing the technical error (lower ADO rates) and biological error, while reducing the impact of mosaicism on molecular analysis. Moreover, it has been reported that blastomere biopsy reduces implantation rates while blastocyst biopsies do not [29]. Interestingly, polar body (PB) biopsy has been proposed as an alternative to the other strategies especially in countries where embryo biopsy is prohibited for legal reasons[30,31]. However, although it should be potentially considered less invasive because it involves the removal of by-products of the meiotic division of the oocyte, this type of biopsy has many drawbacks. For instance, analysis of PBs provides an indirect approach, so that the genetic or chromosomal status of the oocyte is deduced from that of the PB. Both PBs from all mature oocytes and/or zygotes are needed regardless of their developmental potential and need to be analysed separately, increasing the workload and the cost of the procedure. Also, higher false-positive and negative error rates have been reported when PB biopsy is performed[32] and it is noted that mitotic aneuploidies and chromosomal abnormalities originating in the sperm cannot be detected.

Alternative PGT strategies, encompassing the recent advances in epigenetic technologies, include the quantification of DNA methylation in extremely low input samples. This has led to the suggestion that epigenetic profiling of embryo biopsies or PBs could complement PGT for IDs[23]. However, as described above, the accuracy using such methods would still be influenced by ADO and allelic methylation profiling at single-cell resolution is currently not feasible. Furthermore, despite the high methylation correlations between oocytes and PBs[33], it must be noted that biopsies from preimplantation embryos are obtained during a developmental time window when the epigenome is dynamic, and whilst methylation at imprinted DMRs is largely resistant to this epigenetic reprogramming, it remains to be determined if some disparity exists. In addition, for biopsies are taken before implantation, somatically acquired imprinted DMRs, including at *MEG3* DMR, are not established, so profiling such intervals could report erroneous results[34].

Although prenatal diagnosis of KOS14 has been performed before[35], to the best of our knowledge, this is the first report of the application of PGT-M in order to avoid an IDs in the progeny of a microdeletion carrier. When studying IDs, it is imperative that parental transmission is taken into consideration, as reciprocal inheritance of a genetic anomaly may result in syndromes with substantially different severity. The PGT-M strategy described here allows for the differentiation of parental chromosome complements, thus fulfilling the requirement in the detection of IDs to determine the transmission of the involved chromosome to the offspring. Preimplantation genetic testing is only warranted for the most severe disorders, such as KOS14, and not TS14. With the improvements in technology and growing knowledge of IDs, employing PGT-M for KOS14 is an attractive alternative to prenatal

testing and the subsequent choice to continue a pregnancy if ultrasound indicates an affected fetus.

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Contributors
Olga Gómez del Rincón, Africa Pertierra Cartada and Joan Sabria-Back were responsible for managing the clinical case and collecting samples. Ana Monteagudo-Sánchez, Marta Sánchez-Delgado, Jair Tenorio, Julian Nevado, Pablo Lapunzina, Arrate Pereda Aguirre and Guiomar Perez de Nanclares performed the molecular characterization of the family. Carles Giménez Sevilla and Estefanía Toro Toro were responsible for the PGT-M. Anne C. Ferguson-Smith supplied reagents for patient characterization. David Monk performed molecular characterization, coordinated the project and prepared the manuscript. All authors contributed to the final version of the manuscript.

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Competing interest
The authors declare no conflict of interest.

Patient consent for publication
Signed informed consent was obtained from the parents described in this study.

Ethical approval
Ethical approval was granted by the Bellvitge Institute for Biomedical Research (PR096/10).

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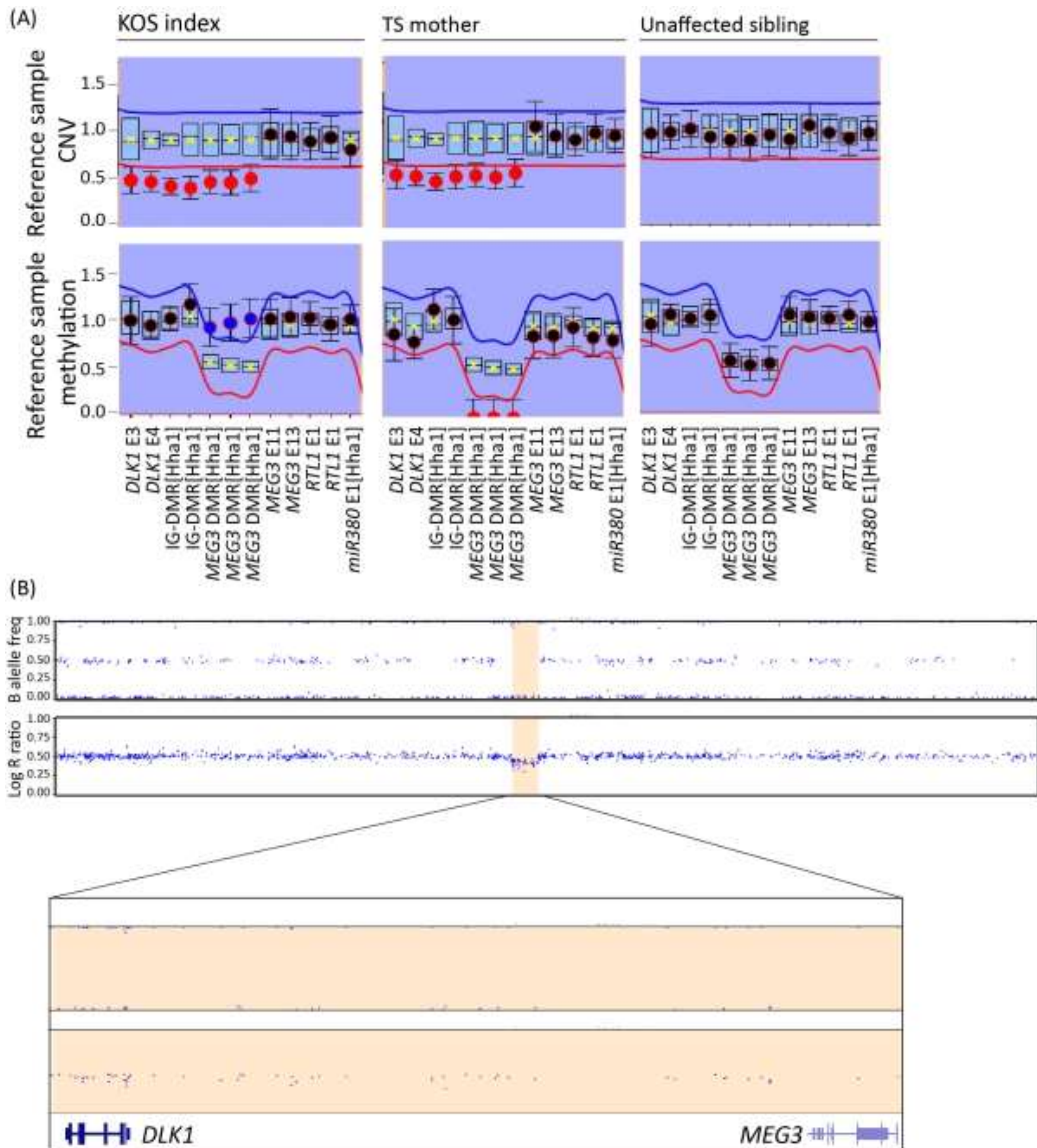

Figure legends

Figure 1. Molecular characterization of the chr14q32 deletion.

(A) MS-MLPA analysis in the KOS14 index case and maternal DNA-samples reveals a reciprocally inherited deletion encompassing the IG-DMR and *MEG3* DMR. The upper panels represent copy number, while the lower panels indicate methylation profile. Note the normal methylation at the *PLAGL1, GRB10* and *MEST* DMRs. (B) SNP array analysis determined the approximate size of the deletion in the KOS14 index case revealing loss of *DLK1* and the first 4 exons of *MEG3.*

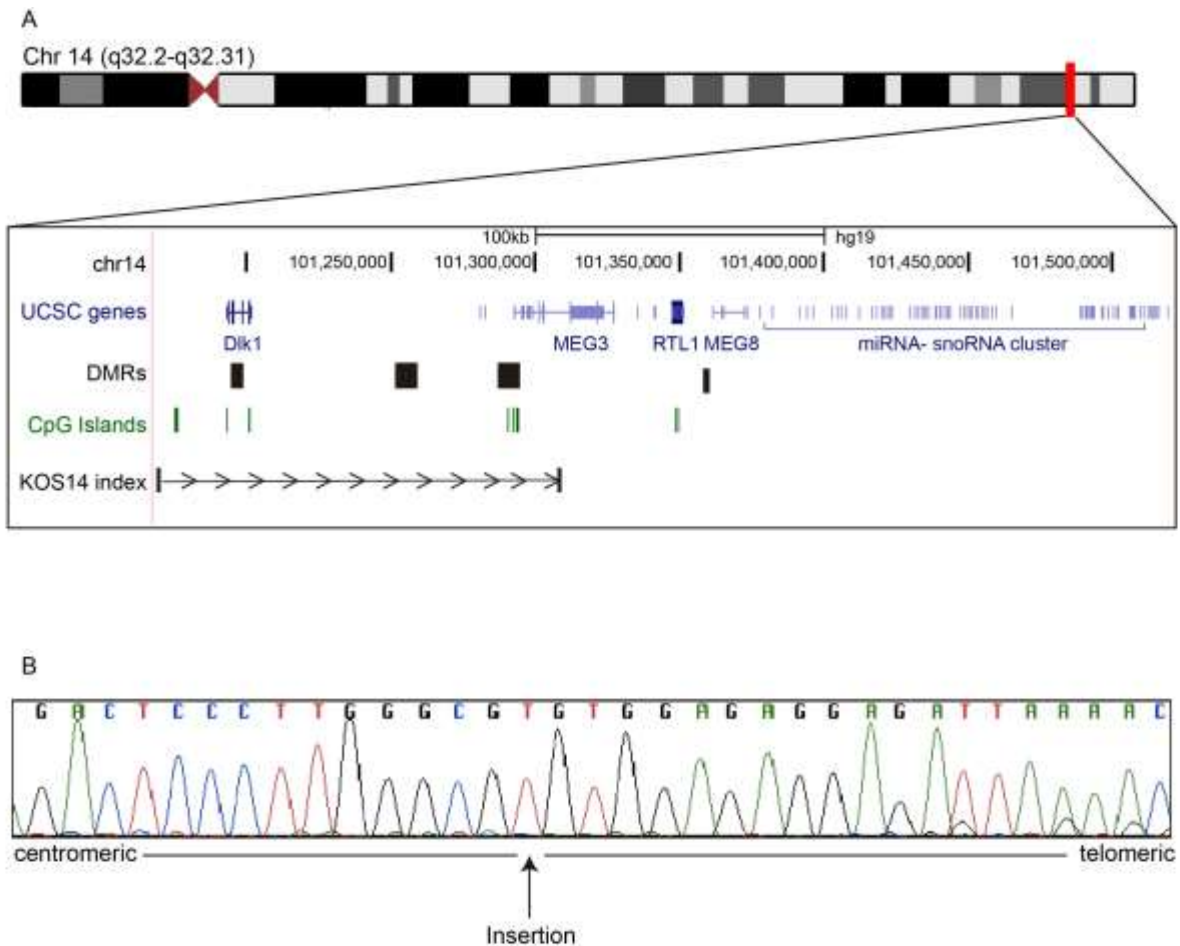

Figure 2. Defining the deletion breakpoints

(A) Schematic overview of the chr14q32 imprinted domain.(B) The position of the breakpoints were determined by long-range PCR followed by Sanger sequencing showing a single base insertion at the junction site and the resulting 108 kb deletion.

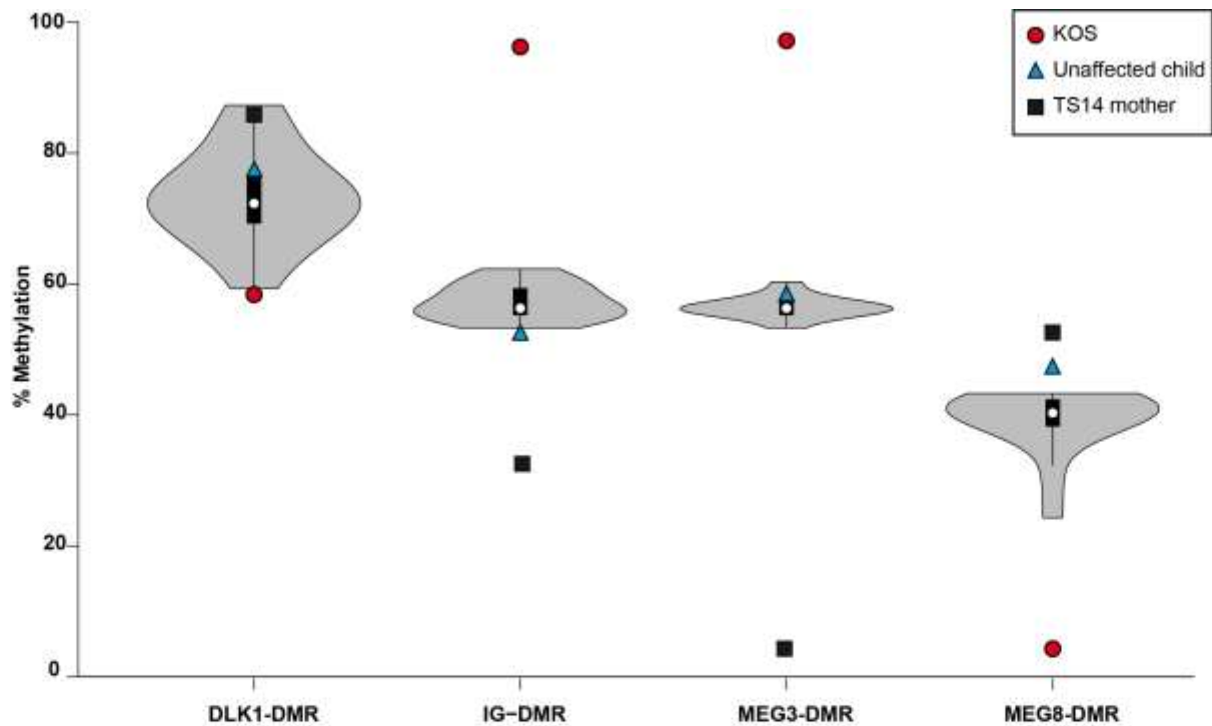

Figure 3. Methylation profiling of the KOS14 index case and parental DNA samples. Pyrosequencing was used to quantify methylation of CpG dinucleotides in the *DLK*-DMR (maternally methylated), IG-DMR (paternally methylated), *MEG*-DMR (paternally methylated) and the *MEG8*-DMR (maternally methylated). Violin plots represent the average methylation profiles of 15 control individuals, whereas data points are shown for the KOS14 index case (red circles), TS14 mother (black squares) and unaffected sibling (blue triangles).

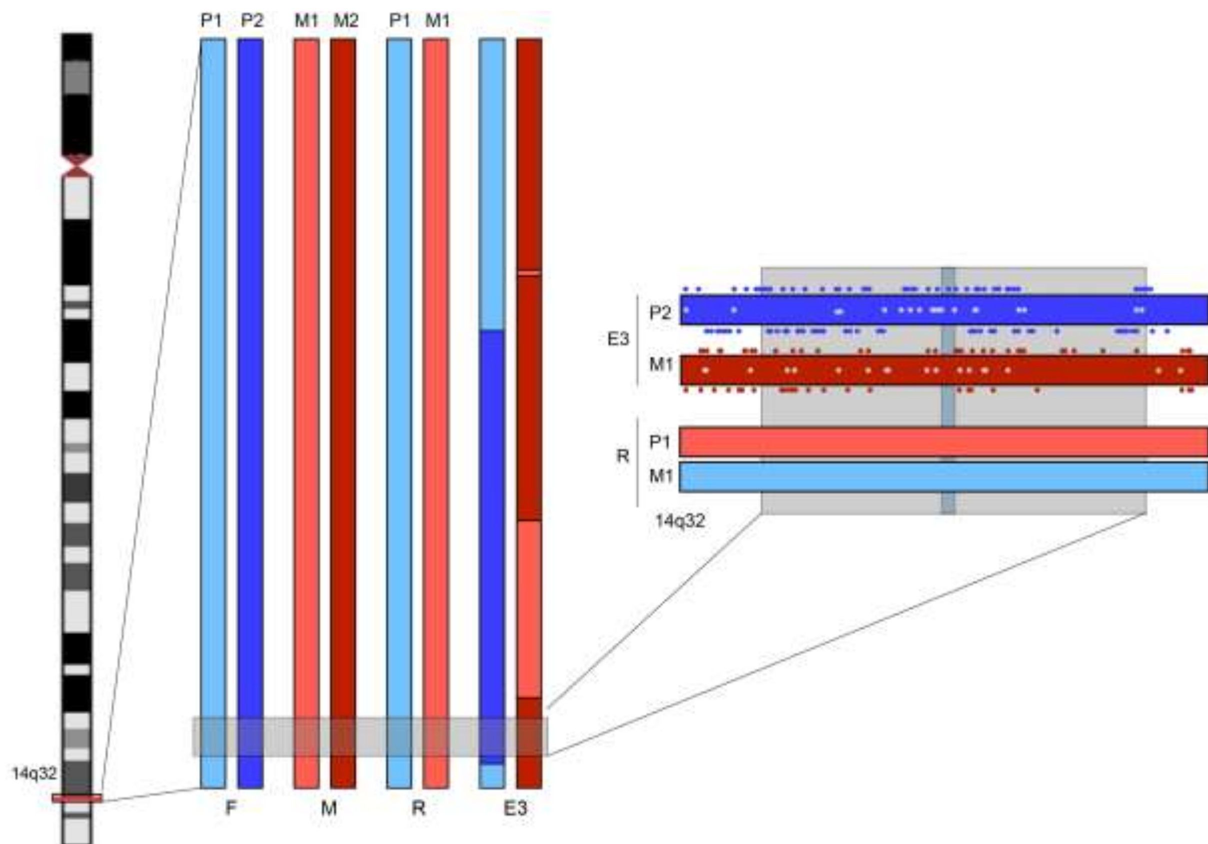

Figure 4. Haploblock analysis of the unaffected-euploid embryo as seen in BlueFuse software after analysis by *Karyomapping*. F refers to father genomic DNA sample, and P1 and P2 are the paternal chromosomes. M refers to mother genomic DNA, where M1 is the maternal chromosome associated to the mutant phase and M2 is the normal maternal chromosome. R is the reference, in this case, DNA from KOS14 index case. E3 refers to embryo 3, non-carrier of the maternal mutant phase. The 2 Mb region of interest is highlighted in grey. For E3, the paternal haplotype is represented in dark blue and the maternal haplotype in dark red. Dots above the corresponding red/green bars represent key SNP positions and those below are the non-key SNP positions. Dots inside the bar are no call SNPs.

Table 1. PGT results after analysis by *Karyomapping* and NGS.

Supplementary Figure 1. Mapping the breakpoints for the KOS14 proband and that responsible for family A in Kagami *et al*., 2008.

Supplementary Table 1. Primer sequences used in this study.

Supplementary Table 2. Pyrosequencing results for the affected family and controls.

TABLE 1.

CYCLE	EMBRYO	KARYOMAPPING ANALYSIS		NGS ANALYSIS	INTERPRETATION	EMBRYO TRANSFER
		IMPRINTING DISORDER	CNV	CNV		
1ST	1	AFFECTED	ND	NA	AFFECTED	NON SUITABLE
	2	AFFECTED	ND	NA	AFFECTED	NON SUITABLE
	3A	UNAFFECTED	MONOSOMY 9	NA	UNAFFECTED and ANEUPLOID	NON SUITABLE
	10A	AFFECTED	ND	NA	AFFECTED	NON SUITABLE
	11A	AFFECTED	TRISOMY 22	NA	AFFECTED and ANEUPLOID	NON SUITABLE
	13A	UNAFFECTED	ND	TRISOMY 14 (pter→q21.3)	UNAFFECTED and ANEUPLOID	NON SUITABLE
2ND	1	UNAFFECTED	MONOSOMY 22	NA	UNAFFECTED and ANEUPLOID	NON SUITABLE
3RD	2	AFFECTED	ND	MULTIPLE	AFFECTED and COMPLEX ABNORMAL	NON SUITABLE
	3	**UNAFFECTED**	**ND**	**NO CHROMOSOMES ABNORMALITIES OBSERVED**	**UNAFFECTED and EUPLOID**	**SUITABLE**
	4	AFFECTED	TRISOMY 16	NA	AFFECTED and ANEUPLOID	NON SUITABLE

ND: Not detected; NA: Not analyzed. Only unaffected embryos with no detectable aneuploidies by *Karyomapping* were analyzed by NGS.