



# Identification of novel candidate genes associated with meiotic aneuploidy in human embryos by whole-exome sequencing

B. Lledo<sup>1</sup> · A. Marco<sup>1</sup> · R. Morales<sup>1</sup> · J.A. Ortiz<sup>1</sup> · E. García-Hernández<sup>1</sup> · F.M. Lozano<sup>1</sup> · A. Cascales<sup>1</sup> · J. Guerrero<sup>2</sup> · A. Bernabeu<sup>2,3</sup> · R. Bernabeu<sup>2,3</sup>

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## Abstract

**Purpose** To identify novel genetic variants responsible for meiotic embryonic aneuploidy.

**Methods** A prospective observational cohort study that included 29 couples who underwent trophoctoderm biopsies from 127 embryos and performed whole-exome sequencing (WES) between November 2019 and March 2022. Patients were divided into two groups according to the expected embryo aneuploidy rate based on maternal age.

**Results** After variant filtering in the WES analysis of 58 patients/donors, five heterozygous variants were identified in female partners from the study group that had an impact on embryo aneuploidy. Additionally, a slowdown in embryo development and a decrease in the number of blastocysts available for biopsy were observed in the study group embryos.

**Conclusion** This study has identified new candidate genes and variants not previously associated with meiotic embryo aneuploidy, but which are involved in important biological processes related to cell division and chromosome segregation. WES may be an efficient tool to identify patients with a higher-than-expected risk of embryo aneuploidy based on maternal age and allow for individualized genetic counselling prior to treatment.

**Keywords** Embryo aneuploidy · Whole-exome sequencing · Candidate genetic variants · Chromosome segregation

## Introduction

Despite great medical advances, the average span of female fertility remains unchanged (22–25 years) [1]. A limited reserve of oocytes is formed before birth, but gradually declines with age, resulting in reduced fertility [2]. Errors in chromosome segregation during meiosis, such as chromosome synapsis, crossing over, and spindle building, occur frequently in human oocytes and cause aneuploidy in embryos. These errors increase dramatically in older women's oocytes [3]. However, the rate of producing aneuploid embryos varies among IVF patients within a given age group [4]. Recent studies have shown that maternal

genetic variants are associated with the risk of embryonic aneuploidy [5]. Understanding the molecular and genetic mechanisms of how meiosis is regulated is key to know the origins of human aneuploidy.

Aneuploidy is more frequently observed in oocytes than in spermatocytes [6]. All steps of male meiosis are constantly taking place in the adult, starting from germ cells (spermatogonia) that initiate meiosis. It takes about a month from the start of meiotic recombination to generate spermatids [7]. The cohesin complex and chiasmata must be maintained for a time frame that is rather short compared to female meiosis. Therefore, paternal age seems to be a less important factor for the fidelity of chromosome segregation [8]. On the other hand, numerous genes and proteins participate in meiosis, and mutations in these genes trigger sperm cell arrest, leading to male infertility [9].

Next-generation sequencing (NGS) technologies contribute to the rapid evolution of the infertility genetics field. NGS can assess many genes simultaneously at high resolution, which is ideal in cases with nonspecific phenotypes or genetic heterogeneity such as infertility [10]. The ACMG (American College of Medical Genetics and Genomics)

✉ B. Lledo  
blledo@institutobernabeu.com

<sup>1</sup> Instituto Bernabeu Biotech, 03016 Alicante, Spain

<sup>2</sup> Instituto Bernabeu of Fertility and Gynaecology, 03016 Alicante, Spain

<sup>3</sup> Cátedra de Medicina Comunitaria y Salud Reproductiva, Miguel Hernández University, Alicante, Spain

states that WES may be considered in the diagnostic assessment of an individual with a phenotype that suggests a genetic etiology and has a high degree of genetic heterogeneity [11]. Even in cases where medical management is not possible, the information itself (such as understanding the cause of the disorder, informing about a more accurate prognosis, understanding recurrence risks) may often be of great value. Additionally, it can eventually prove useful for patients and clinicians as novel disease gene relationships are being discovered. This approach is quickly evolving in male factor genetic diagnosis [12] and premature ovarian failure [13].

Little is known about the risk of embryo aneuploidy. Recent publications have identified genetic variants that are crucial for producing healthy oocytes in female IVF patients [14–17]. The association between maternal genetic variants and embryonic aneuploidy risk suggests the potential use of genomic data to predict embryonic aneuploidy risk in female IVF patients. These genetic variants can potentially be used to counsel patients regarding timing and adequate treatment.

In the present study, our aim was to identify genetic variants that could cause a genetic risk for meiotic embryonic aneuploidy. To our knowledge, this is the first time that WES has been performed on the couple in order to identify the paternal origin of the genetic risk. The information presented in this study could aid in molecular diagnosis and mechanisms of embryo aneuploidy.

## Materials and methods

### Study design and population

A prospective observational cohort study was conducted, which included a total of 29 couples who underwent PGT-A due to recurrent implantation failure, repeated pregnancy loss implantation, previous aneuploid pregnancy or severe male factor. Additionally, a clinical whole exome had been performed on the couple. In the case of egg or semen donor PGT-A cycles, WES had been performed in the gamete donors.

A total of 127 embryos from 29 couples were included in this study, which was carried out between November 2019 and March 2022. The inclusion criteria were couples whose female partner was 35 years or younger if they performed PGT-A with their own oocytes. Moreover, couples undergoing egg donation cycles were included. The exclusion criteria were abnormal karyotype and abnormal sperm FISH.

Our study population was divided into two groups based on the proportion of aneuploid blastocysts: low or high rates of aneuploid blastocysts, accordingly referred to as the control group (low-rate group) and the study group (high-rate group), respectively. To select the cut-off for the

aneuploidy rate to divide both groups, we used the data from 6851 embryos from 2221 couples using PGT-A in our clinic in the mentioned time span. The average aneuploidy rate for female patients younger than 36 years was 34.6%. We considered the 75th percentile of the aneuploidy rate in the female patients younger than 36 to establish the cut-off for the aneuploidy rate between groups, which corresponds to 50%. We defined couples with < 50% of aneuploid blastocysts as the control group ( $n = 14$ ), and couples with  $\geq 50\%$  of aneuploid blastocysts as the study group ( $n = 15$ ).

All work was conducted with formal approval of the Institutional Review Board and it follows the principles of the Declaration of Helsinki. Informed consent was obtained from all participants prior to the study.

### PGT-A

The controlled ovarian stimulation followed by the patients or the egg donors was an antagonist protocol customized by a specialized physician. The dose and the type of exogenous gonadotropin were adjusted according to their clinical history, BMI, and ovarian reserve markers (antral follicle count and anti-Mullerian hormone). In addition, some of the patients took oral contraceptive pills during the months prior to the start of the stimulation treatment. Final oocyte maturation was induced with 0.2 mg of a GnRH agonist (GnRHa) (Decapeptyl 0.1 mg®, Ipsen Pharma, Spain) when at least three follicles larger than 17 mm were detected by ultrasound. Oocyte aspiration was performed 36 h after induction by transvaginal ultrasound-guided needle aspiration. Mature oocytes were fertilized in the laboratory by intracytoplasmic sperm injection (ICSI) following IVF laboratory guidelines. After 16–18 h, those oocytes with two pronuclei (2PN) and two polar bodies (2PB) were considered correctly fertilized and were cultured until day 5–6 blastocyst stage. Assisted hatching was performed on day 3 and trophectoderm of day 5–6 hatching blastocysts were biopsied using a 200 mW diode laser (Hamilton Thorne, Beverly, USA). Biopsied blastocysts were individually vitrified and trophectoderm cells were processed for genetic analysis. The biopsied cells (5–10) were transferred to PCR tubes with 1  $\mu$ l of PBS.

As for genetic analysis, first, a whole genome amplification was performed (Picoplex kit, Rubicon Genomics®, Ann Arbor, MI, USA). The embryo biopsies were then analysed using NGS (Veriseq Illumina®, San Diego, CA, USA). Mosaicism diagnosis was inferred from the presence of an intermediate chromosome copy number in a next-generation sequencing NGS profile. Embryos with a copy number between 1.75 and 2.25 were considered euploid, while those with a copy number between 1.5–1.75 and 2.25–2.50 were classified as mosaic and those with a copy number below 1.5 and above 2.50 were considered aneuploid.

## Whole-exome sequencing

Peripheral blood sample was obtained from all participants including donors in egg or sperm donation cycles. Genomic DNA was extracted using the MagMAX DNA Multi-Sample Ultra 2.0 kit (Thermo Fisher Scientific, Colchester, UK) on a KingFisher™ Duo Prime system (Thermo Fisher Scientific, Colchester, UK), following the manufacturer's instructions. DNA was quantified using Qubit™ dsDNA HS Assay Kit with the Qubit Fluorometer (Thermo Fisher Scientific, Colchester, UK). Whole-exome sequencing (WES) of the genomic DNA was performed using Trusight One Expanded Sequencing Panel (Illumina®) which covers about 16.5 Mb genomic content (~ 6700 genes) on the NextSeq 550 (Illumina®), according to the manufacturer's protocol. The sequenced data were aligned to the human genome 19 (hg19) using the Burrows-Wheeler Alignment tool (BWA) and GATK algorithm was used for single nucleotide variations (SNVs)/InDel identification. Variant Call Format files (VCF) were recorded using Variant Interpreter software.

The following criteria were used for filtering and annotating of candidate variants: (1) minor allele frequency (MAF) < 0.05 in the gnomAD and 1000 genomes project for the European population, (2) variants in genes previously associated with aneuploidy, genes involved in chromosome segregation, chromatin cohesion, meiosis and cell division processes, (3) exonic/splicing boundaries ( $\pm 50$  bp) variants in genes, and (4) variants having potentially strong/moderate functional effects on the protein (nonsense, frameshift, inframe deletion, splice region, and missense variants). Within the variants of potentially moderate effect, the missense variants were evaluated using three in silico prediction algorithms (SIFT, PolyPhen-2 and MutationTaster). Other moderate variants, such as inframe deletions or variants in splice regions were considered separately. Moreover, the variants were assessed according to the guidelines from the American College of Medical Genetics and Genomics (ACMG) [18]. ACMG-based classification was performed first, and further evaluation was done using the ABC system for variant classification to ensure that relevant variants were not overlooked or inadequately appreciated [19]. The candidate variants identified by WES were confirmed by Sanger sequencing using the BigDye™ Terminator v3.1 Cycle Sequencing Kit and SeqStudio Genetic Analyzer (Applied Biosystems).

## Statistical analysis

Continuous variables were reported as mean value  $\pm$  SD and categorical variables as percentages. For quantitative variables, the *t*-student test was used as a parametric test or the Mann-Whitney test as a nonparametric test. Shapiro-Wilks

was used to assess normality and the chi-square test was used for categorical variables.

To perform the data statistical analysis, the R Statistical Software program, version 4.0.3, and Software Statistical Product and Service Solutions, version 20.0 (SPSS, Chicago, IL, EE.UU.) were used.

## Results

### Study population

A total of 127 embryos from 29 couples undergoing PGT-A were included. Fifteen couples were included in the study group and 14 in the control group. Table 1 shows the patient characteristics and the PGT-A data. Overall, the average female age was  $26.21 \pm 4.11$  years and the average male age was  $40.69 \pm 9.07$ . There were no significant differences between groups regarding male and female age. Also, no differences were reported in terms of percentage of recurrent implantation failure or pregnancy loss patients or normozoospermic patients. Regarding the PGT-A cycle, no differences were observed between the number of oocytes and MII retrieved and the number of cells biopsied per embryo. However, the average number of biopsied embryos per patient in the control and study group were  $5.36 \pm 2.50$  and  $3.47 \pm 1.36$  respectively ( $p < 0.05$ ). Also, the percentage of embryos biopsied on day 5 (85.33 vs 32.69;  $p < 0.05$ ) was higher in the control group. Finally, as expected, the percentage of aneuploidies was higher in the study group (18.64 vs 71.89;  $p < 0.05$ ).

### Variant detection

After sequencing the samples, the mean read depths ranged from 100–180 $\times$  in each sample, and over 98% of bases had a minimum coverage of 10 $\times$  depth in all samples.

The WES results from 58 patients/donors identified 45 variants in genes potentially associated with the phenotype mechanism. Of these, thirty-eight had a minor allele frequency (MAF) < 0.05, and the remaining 7 had frequencies not reported in databases. Exonic/splicing variants were manually registered, and 40 variants were discarded based on likely benign annotations from computational prediction tools.

Among the remaining 5 candidate variants, 4 were missense and 1 was non-sense. Within the missense variants, all of them were predicted to have deleterious effects by three in silico algorithms (SIFT, PolyPhen-2 and MutationTaster). According to the ACMG criteria, 2 variants were pathogenic and 3 were VUS (variant of unknown significance). A further classification using the ABC system showed that 1 variant was classified as pathogenic, 1 as a genetic

**Table 1** Patient characteristics and PGT-A data

	Total ( <i>n</i> = 29)	Control group—(< 50% aneuploidy) ( <i>n</i> = 14)	Study group—(≥ 50% aneuploidy) ( <i>n</i> = 15)	<i>p</i> -value*
Female age (mean ± SD)	26.21 ± 4.11	26.64 ± 3.95	25.81 ± 4.35	0.59
Male age (mean ± SD)	40.69 ± 9.07	41.71 ± 9.42	39.73 ± 8.95	0.57
PGT-A indications				
Couples with RIF (%)	2 (6.90)	1 (7.14)	1 (6.67)	0.459
Couples with RPL (%)	5 (17.24)	3 (21.43)	2 (13.33)	
Severe male factor (%)	10 (34.48)	6 (42.86)	4 (26.67)	
Previous aneuploidy pregnancy (%)	9 (31.03)	4 (28.57)	5 (33.33)	
Several indications	3 (10.34)	0 (0)	3 (20.0)	
Gamete donation cycles				
Oocyte donation cycles (%)	26 (89.66)	12 (86.67)	14 (92.86)	0.95
Sperm donation cycles (%)	4 (13.79)	2 (14.29)	2 (13.33)	0.87
PGT-A cycles data				
Number of oocytes retrieved (mean ± SD)	10.83 ± 1.56	11.2 ± 1.81	10.47 ± 1.25	0.20
Number of MII oocytes retrieved (mean ± SD)	9.59 ± 1.59	9.86 ± 1.79	9.33 ± 1.40	0.39
Number of fertilized oocytes (mean ± SD)	7.28 ± 2.00	7.79 ± 1.97	6.80 ± 1.97	0.26
Number of total embryos biopsied	127	75	52	-
Number of embryos biopsied per patient (mean ± SD)	4.38 ± 2.18	5.36 ± 2.50	3.47 ± 1.36	<b>0.02**</b>
% of embryos biopsied on day D5	58.91	85.33	32.69	<b>0.02**</b>
Number of biopsied cells (mean ± SD)	4.58 ± 1.41	5.00 ± 1.66	4.52 ± 1.60	0.45
Aneuploidy rate (%)	46.18	18.64	71.89	<b>&lt; 0.001**</b>
Mosaic embryos rate (%)	16.53	14.67	19.23	0.45

\*For quantitative variables, the *t*-student or the Mann-Whitney test and the chi-square test was used for categorical variables

\*\**p*-value < 0.05 are given in bold

variant that increases susceptibility for this phenotype, and 3 as genetic variants of potential interest. In summary, after all filtering process, the final number of candidate variants was 5 and all were likely pathogenic or with potential interest. The first variant identified was a missense mutation p.Leu466Pro detected in *TLE6* gene that encodes the transducin-like enhancer protein 6. The encoded protein is a component of the mammalian subcortical maternal complex, which is required for preimplantation development. Additionally, two missense variants: p.Glu57Lys in the *IKBK*G and p.Glu409Asp in the *BUB1*B genes were identified in the same patient. These genes encode the inhibitor of nuclear factor kappa B kinase regulatory subunit gamma and the mitotic checkpoint serine/threonine kinase B respectively. *IKBK*G is the founding member of an evolutionarily conserved family of kinases that function in numerous cell signalling pathways and interact with the PIDDosome, a multiprotein complex that controls the number of centrosomes. The *BUB1*B protein has been localized to the kinetochore and plays a role in the inhibition of the anaphase-promoting complex/cyclosome, delaying the onset of anaphase and ensuring proper chromosome segregation. The last missense mutation identified was a Valine substitution by a Methionine in the *TP73* gene encoding the tumor protein p73, a

member of the p53 family of transcription factors involved in genome stability. Finally, a non-sense mutation p.Tyr248Ter was found in the *AURKC* gene which encodes a Serine/threonine kinase a member of the chromosomal passenger complex (CPC) involved in events such as centrosome separation and chromosome segregation. Detailed characteristics of the variants identified are shown in Table 2. All variants were heterozygous and of maternal origin. The females carrying the candidate variants belonged to the study group, and two variants in different genes were identified in one patient. No potentially pathogenic variants were detected in patients from the control group. The 5 genetic variants were found in four patients of the study group. Therefore, the average detection rate of genetic variants with potential impact in embryo aneuploidy was 26.67 (4/15). Table 3 summarizes the clinical data of the couples in which the genetic variants were found.

## Discussion

Aneuploidy is common in early human development and is a leading cause of pregnancy loss, adverse obstetric outcomes and infertility associated to advanced maternal age [20]. In

**Table 2** Selected variants prioritised from the exome sequencing data in the study group

Individual	Gene	Origin	Transcript	dsSNP	Nucleotide change	Aminoacid change	Effect	GnomAD MAF	Zygoty	S/P/M	ACMG score	ACMG classification	ABC system	Variant classification
1	<i>TLE6</i>	Maternal	NM_001143986.1	-	1397T>C	(Leu466Pro)	Missense	-	Heterozygous	D/D/D	PM2/PP3	VUS	E/3+1	Genetic variant of potential interest
2	<i>IKBK</i>	Maternal	NM_003639.4	rs148695964	169G>A	(Glu57Lys)	Missense	0.0014	Heterozygous	D/D/D	PM1/PM2/PP3/PP5/PS3	VUS/Likely Benign / Conflicting interpretation	D/4+2	Genetic variant that increases susceptibility for the phenotype
2	<i>BUB1B</i>	Maternal	NM_001211.5	rs28989188	1227A>C	(Glu409Asp)	Missense	0.0004	Heterozygous	D/D/D	PM2/PP3	VUS	E/3+2	Genetic variant of potential interest
3	<i>TP73</i>	Maternal	NM_005427.3	rs757455947	277G>A	(Val93Met)	Missense	0.00044	Heterozygous	D/D/D	PM2/PP3	VUS	E/3+2	Genetic variant of potential interest
4	<i>AURKC</i>	Maternal	NM_001015878.1	rs55658999	744C>G	(Tyr248Ter)	Nonsense	0.0007	Heterozygous	D/D/D	PM2/PVS1/PP5	Pathogenic	A/5+5	Pathogenic

*GnomAD* MAF, minor allelic frequency in the *gnomAD* database for non-Finnish European population; *S/P/M*, the prediction of SIFT/Polyphen2/MutationTaster bioinformatics tools (SIFT *D*, deleterious; *T*, tolerated/Polyphen2; *D*, probably damaging; *B*, benign/MutationTaster; *D*, disease causing); *ACMG*, American College of Medical Genetics

**Table 3** Clinical information from the couples in which the variants were identified

	Couple 1	Couple 2	Couple 3	Couple 4
Female age (y)	28	21	25	35
Male age (y)	24	25	53	39
PGT-A indication	RIF	RIF and previous aneuploid pregnancy	Previous aneuploid pregnancy	RPL
Number of MII oocytes retrieved	10	9	12	9
Number of fertilized MII oocytes	7	8	8	7
Number of total embryos biopsied	3	4	6	3
Aneuploidy rate (%)	66.67	75	66.70	66.67
Mosaicism rate (%)	33.33	0	0	0
Number of embryo transferred	1	1	1	1
Pregnancy	No	Yes	Yes	Yes
Healthy baby at home	0	1	1	1
Genetic variant identified	TLE6: c.1397T>C	IKBKG:c.169G>A and BUB1B: c.1227A>C	TP73: c.277G>A	AURKC: c.744C>G

this study, we performed WES on genomic DNA from couples undergoing PGT-A to identify the risk of embryonic aneuploidies independent of maternal age. Due to the heterogeneity of the phenotype and the complex interactions between genes and genetic variants, a single variant or multiple variants in a single gene cannot be used to predict a greater risk of aneuploidies. WES has allowed us to simultaneously analyse genes related to chromosome segregation, chromatin cohesion, meiosis, and cell division processes. A model integrating multiple informative variants in a patient's genome could potentially provide a more accurate prediction. Recent studies suggest that some genetic variants in certain genes compromise the fidelity of chromosome segregation and predispose to embryo aneuploidy [17]. However, to our knowledge, this is the first study to consider WES of both paternal and maternal gametes. In this study, we report five potentially damaging genetic variants in female genomes related to meiotic processes and linked to a higher incidence of embryo aneuploidy.

In the present study, two patient groups were established based on the average aneuploidy rate in female patients under 36 years, with a 50% cut-off. The population of both groups was homogeneous, except for the total number of biopsied embryos per patient and the percentage of embryos biopsied on day 5. Although both groups had the same number of oocytes and MII oocytes retrieved, the study group showed a lower total number of biopsied embryos and a higher number of embryos biopsied on day 6. Previous research has shown that the transition to blastocyst stage is associated with a decrease in the aneuploidy rate. This leads to some abnormal embryos to arrest in development before reaching the blastocyst stage after activation of the embryonic genome at the cleavage stage [21]. This phenomenon could explain why patients in the study group with a higher

rate of embryo aneuploidies had lower number of embryos available for biopsy and their embryos were slower, needing more time to reach blastocyst stage.

Pathogenic genetic variants may dysregulate meiotic processes resulting in chromosomally abnormal gametes. Recent studies have shown that maternal genetic variants are associated with the risk of embryonic aneuploidy [5], such as variants in Aurora kinase B and C (*AURKB*, *AURKC*) [15], transducin-like enhancer (TLE) family member 6 (*TLE6*) [22], and centrosomal protein 120 (*CEP120*) [16] among others. Nonetheless, these studies were limited to analysing the maternal genomic variants. As we moved forward, we analyzed the paternal genomic variants too, in order to investigate in depth, the effect of a couple genetic variants on embryo aneuploidy. Even so, our results agreed with previous studies [23] showing that chromosomal abnormalities are mainly due to errors in maternal meiosis since all the variants classified as probably pathogenic identified in the study group were found in maternal genomes. This result together with the lower number of embryos available for biopsy in the study group suggests that females with high-risk variants contributed with fewer embryos for testing, suggesting that their embryos are less likely to blastulate.

Table 4 shows general information on the genes whose variants were identified in the present study, including gene description and biological process. The first gene associated with embryonic lethality in humans (*TLE6*) was recently identified [5]. This gene is a member of the subcortical maternal complex (SCMC), a multiprotein complex expressed uniquely in mammalian oocytes and early embryos, the components of which are encoded by maternal genes. It has been demonstrated that homozygous mutations in this gene lead to embryo arrest at cleavage stage and female sterility [24]. In one of our female patients from

**Table 4** Gene description of variants identified from the exome sequencing data in the study group

Individual	Gene	Cytogenetic location	Protein encoded	Gene function	Biological process	Total classified variants (P/LP/VUS/LB/B)	Disease model described (OMIM)	Infertility association
1	<i>TLE6</i>	19p13.3	Transducin-Like Enhancer Protein 6	Member of the subcortical maternal complex (SCMC)	Cell cycle progression	60 (7/4/32/10/7)	Preimplantation embryonic lethality (616814)	Embryo arrest and female sterility
2	<i>IKBK</i>	Xq28	Inhibitor of nuclear factor kappa B kinase regulatory subunit gamma	Member of the PID-Dosome	DNA damage, Transcription, Transcription regulation	135 (72/20/34/4/5)	Immunodeficiency 33 (300636)	Male infertility
2	<i>BUB1B</i>	15q15.1	Mitotic checkpoint serine/threonine kinase B	Serine/threonine kinase	Apoptosis, Cell cycle, Cell division, Mitosis	1215 (53/11/734/392/25)	Premature chromatid separation trait (176430)	Premature ovarian insufficiency
3	<i>TP73</i>	1p36.32	Tumor protein p73	Transcription regulatory factor	Apoptosis, Cell cycle, Host-virus interaction, Transcription, Transcription regulation	46 (6/12/18/2/8)	Ciliary dyskinesia, primary, 47 (619466)	Infertility, aging, and aneuploidy and/or cancer in mice
4	<i>AURKC</i>	19q13.43	Aurora kinase C	Serine/threonine kinase	Cell cycle, Cell division, Meiosis, Mitosis	40 (9/1/23/2/5)	Spermatogenic failure 5 (243060)	Male infertility and chromosome misalignment in mice

*P*, pathogenic; *LP*, likely pathogenic; *VUS*, uncertain significance; *LB*, likely benign; *B*, benign

the study group, a missense mutation (p.Leu466Pro) was detected in *TLE6* in the heterozygous state. The variant was a very low-frequency variant in the population as it had not been reported yet, and, together with the in silico predictions, it was classified as a genetic variant of potential interest. Considering its effect in the homozygous state, a heterozygous mutation in this gene could be causing an increase in aneuploidy, rather than embryonic lethality, as when is found in homozygosity. Nevertheless, more studies are needed to confirm it. Therefore, this variant could explain the unexpected increase in aneuploidies in this patient.

One of the youngest female patients (21 years) in the study group carried two missense variants: p.Glu57Lys in the *IKBK*G and p.Glu409Asp in the *BUB1*B genes. The *IKBK*G variant was classified as pathogenic, increasing susceptibility for the phenotype while the *BUB1*B variant was classified as a genetic variant of potential interest. *IKBK*G can interact with *PIDD1*, which forms part of the *PIDD*osome, a multiprotein complex that controls the number of centrosomes. *PIDD1* acts as an integrator for multiple types of stress that need instant attention, as various types of DNA lesion but also the presence of extra centrosomes that can foster aneuploidy [25]. *BUB1*B gene encodes a protein that plays a critical role in chromosome segregation by regulating the spindle-assembly checkpoint through three apparently independent mechanisms: acting as a diffuse inhibitor, facilitating catalysis at the kinetochore, and being required for chromosomal alignment [25]. In vitro experiments in which the reduced levels of the *BUBR1* protein (encoded by the *BUB1*B gene) resulted in premature separation of the chromatids [26]. Both of these findings in this patient could be the cause of the high rate of embryonic aneuploidies in this couple, which would not be expected based solely on the female age.

*TP73* gene may act as a regulator of mitotic checkpoint proteins and is therefore related to the chromosomal instability and cancer [27]. Studies in knockout mice revealed that partial or complete deletion, as well as specific mutations within mitotic checkpoint proteins, can induce several phenotypes, such as infertility, aging, and, most pertinently, aneuploidy and/or cancer [28]. A missense mutation (p.Val93Met) in the *TP73* gene was identified in a 25-year female partner from the study group. This variant has a very low frequency in the population and, along with in silico predictions it had been classified as genetic variant of potential interest. This mutation could explain why this couple has an increase in aneuploidy than expected.

Finally, and to end with the variants, a nonsense mutation (p.Tyr248Ter) classified as pathogenic was found in the *AURK*C gene. The Aurora kinases (*AURK*) are serine/threonine kinases critical in mitosis and meiosis. Mammals have three isoforms: A, B, and C. *AURK*B and *AURK*C are members of the chromosomal passenger complex (CPC),

essential for faithful chromosome transmission in meiosis by allowing correction of improperly attached kinetochore microtubules [29]. Abnormalities in Aurora kinase proteins result in genomic instability and aneuploidy. Female *Aurk*-knockout mice are subfertile and their oocytes frequently have chromosome misalignment [30]. A recent study in mothers of Down syndrome children suggests a possible relationship between *AURK*A/*AURK*C variants and increased risk of spontaneous abortion [31]. In our patient, the variant is a null variant (stop gained) where the protein is truncated causing loss of function. It is a known mechanism of pathogenicity producing an increase in the embryo aneuploidy rate.

The strength of this study is the inclusion of both male and female genomic data. On the other hand, our study has certain limitations. These include the challenges of any observational study, also functional studies to validate the pathogenicity of the variants are necessary. Therefore, more studies are needed to corroborate our findings.

Given our results and the apparent causal relationship between maternal genetic variants involved in meiotic processes and embryo aneuploidy, genetic testing to identify genetic biomarkers could complement existing clinical approaches to infertility and allow informed reproductive decision-making before a patient attempts to conceive or after an initial infertility diagnosis. This is particularly important for young patients with a high rate of embryonic aneuploidy is not typically expected. Our results show an adequate detection rate of WES in patients with a high percentage of embryonic aneuploidies. However, the clinical utility of WES in reproductive medicine requires evaluation through appropriate clinical studies. Future research could also aid in defining a panel of genes linked to this increased risk, which would lower test costs and improve cost-effectiveness.

## Conclusion

This work, like previous studies, confirms the association between maternal genetic variants and embryonic aneuploidy risk. Our results suggest that the maternal variants found in the *TLE6* (c.1397T>C), *IKBK*G (c.169G>A), *BUB1*B (c.1227A>C), *TP73* (c.277G>A), and *AURK*C (c.744C>G) genes could be genetic biomarkers that may be predisposing factors to incidence of embryonic aneuploidies. Moreover, we showed that embryonic aneuploidies may influence the embryo development, suggesting that females with high-risk variants contributed to fewer embryos for testing, suggesting that their embryos are less likely to blastulate. This proof of principle study should be validated by more research to recommend genetic testing to identify patients with a high risk of aneuploid embryos, which would allow us to provide an individualized preconceptional reproductive counselling.



## Declarations

**Ethical approval** This prospective study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The IRB of Instituto Bernabeu approved this study.

**Conflict of interest** The authors have no competing interests to declare that are relevant to the content of this article.

## References

- Nabhan AF, Mburu G, Elshafeey F, Magdi R, Kamel M, Elshebiny M, et al. Women's reproductive span: a systematic scoping review. *Hum Reprod Open*. 2022;11:1–19.
- Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences. *Endocr Rev*. 2009;30:465–93.
- Wartosch L, Schindler K, Schuh M, Gruhn JR, Hoffmann ER, McCoy RC, et al. Origins and mechanisms leading to aneuploidy in human eggs. *Prenat Diagn*. 2021;41(5):620–30.
- Cai Y, Ding M, Zhang Y, Sun Y, Lin F, Diao Z, et al. A mathematical model for predicting the number of transferable blastocysts in next-generation sequencing-based preimplantation genetic testing. *Arch Gynecol Obstet*. 2022;305:241–9.
- Biswas L, Tyc K, El Yakoubi W, Morgan K, Xing J, Schindler K. Meiosis interrupted: the genetics of female infertility via meiotic failure. *Reproduction*. 2021;161(2):R13–35.
- Bell AD, Mello CJ, Nemesh J, Brumbaugh SA, Wysoker A, McCarroll SA. Insights into variation in meiosis from 31,228 human sperm genomes. *Nature*. 2020;583(7815):259–64.
- Griswold MD. Spermatogenesis: the commitment to meiosis. *Physiol Rev*. 2016;96:1–17.
- El Yakoubi W, Wassmann K. Meiotic divisions: no place for gender equality. *Adv Exp Med Biol*. 2017;1002:1–17.
- Xie C, Wang W, Tu C, Meng L, Lu G, Lin G, et al. Meiotic recombination: insights into its mechanisms and its role in human reproduction with a special focus on non-obstructive azoospermia. *Hum Reprod Update*. 2022;28(6):763–97.
- Normand EA, Alaimo JT, Van den Veyver IB. Exome and genome sequencing in reproductive medicine. *Fertil Steril*. 2018;109(2):213–20.
- ACMG Board of Directors. Points to consider in the clinical application of genomic sequencing. *Genet Med*. 2012;14:759–61.
- Robay A, Abbasi S, Akil A, El-Bardisi H, Arafa M, Crystal RG, et al. A systematic review on the genetics of male infertility in the era of next-generation sequencing. *Arab J Urol*. 2018;16(1):53–64.
- Morales R, Lledo B, Ortiz JA, Lozano FM, Garcia EM, Bernabeu A, et al. Identification of new variants and candidate genes in women with familial premature ovarian insufficiency using whole-exome sequencing. *J Assist Reprod Genet*. 2022. <https://doi.org/10.1007/s10815-022-02629-3>.
- McCoy RC, Demko Z, Ryan A, Banjevic M, Hill M, Sigurjónsson S, et al. Common variants spanning PLK4 are associated with mitotic-origin aneuploidy in human embryos. *Science*. 2015;348(6231):235–8.
- Nguyen AL, Marin D, Zhou A, Gentilello AS, Smoak EM, Cao Z, et al. Identification and characterization of Aurora kinase B and C variants associated with maternal aneuploidy. *Mol Hum Reprod*. 2017;23(6):406–16.
- Tyc KM, El Yakoubi W, Bag A, Landis J, Zhan Y, Treff NR, Scott RT, et al. Exome sequencing links CEP120 mutation to maternally derived aneuploid conception risk. *Hum Reprod*. 2020;35(9):2134–48.
- Sun S, Miller M, Wang Y, Tyc KM, Cao X, Scott RT, et al. Predicting embryonic aneuploidy rate in IVF patients using whole-exome sequencing. *Hum Genet*. 2022;141(10):1615–27.
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405–24. <https://doi.org/10.1038/gim.2015.30>.
- Houge G, Laner A, Cirak S, de Leeuw N, Scheffer H, den JT. Stepwise ABC system for classification of any type of genetic variant. *Eur J Hum Genet*. 2022;30:150–9. <https://doi.org/10.1038/s41431-021-00903-z>.
- Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet*. 2001;2:280–91.
- Fragouli E, Alfarawati S, Spath K, Jaroudi S, Sarasa J, Enciso M, et al. The origin and impact of embryonic aneuploidy. *Hum Genet*. 2013;132(9):1001–13.
- Alazami AM, Awad SM, Coskun S, Al-Hassan S, Hijazi H, Abdulwahab FM, et al. TLE6 mutation causes the earliest known human embryonic lethality. *Genome Biol*. 2015;16:240.
- McCoy RC, Demko ZP, Ryan A, Banjevic M, Hill M, Sigurjónsson S, et al. Evidence of selection against complex mitotic-origin aneuploidy during preimplantation development. *PLoS Genet*. 2015;11(10):e1005601.
- Bebbere D, Masala L, Albertini DF, Ledda S. The subcortical maternal complex: multiple functions for one biological structure? *J Assist Reprod Genet*. 2016;33(11):1431–8.
- Sladky V, Schuler F, Fava LL, Villunger A. The resurrection of the PIDDosome - emerging roles in the DNA-damage response and centrosome surveillance. *J Cell Sci*. 2017;130(22):3779–87.
- Bohers E, Sarafan-Vasseur N, Drouet A, Paresy M, Latouche JB, Flaman JM, et al. Gradual reduction of BUBR1 protein levels results in premature sister-chromatid separation then in aneuploidy. *Hum Genet*. 2008;124(5):473–8.
- Tomasini R, Mak TW, Melino G. The impact of p53 and p73 on aneuploidy and cancer. *Trends Cell Biol*. 2008;18(5):244–52.
- Weaver BA, Silk AD, Montagna C, Verdier-Pinard P, Cleveland DW. Aneuploidy acts both oncogenically and as a tumor suppressor. *Cancer Cell*. 2007;11(1):25–36.
- Balboula AZ, Schindler K. Selective disruption of aurora C kinase reveals distinct functions from aurora B kinase during meiosis in mouse oocytes. *PLoS Genet*. 2014;10(2):e1004194.
- Schindler K, Davydenko O, Fram B, Lampson MA, Schultz RM. Maternally recruited Aurora C kinase is more stable than Aurora B to support mouse oocyte maturation and early development. *Proc Natl Acad Sci USA*. 2012;109(33):E2215–22.
- de Castro CML, Pereira COB, Aprigio J, Costa Lima MA, Ribeiro MG, Amorim MR. Aurora kinase genetic polymorphisms: an association study in Down syndrome and spontaneous abortion. *Hum Cell*. 2022;35(3):849–55.

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