Citation: Cell Death and Disease (2012) 3, e392; doi:10.1038/cddis.2012.116

www.nature.com/cddis

TP53 PIN3 and PEX4 polymorphisms and infertility associated with endometriosis or with post-*in vitro* fertilization implantation failure

DD Paskulin[1,3,6], JSL Cunha-Filho[2], CAB Souza[2], MC Bortolini[1,3], P Hainaut[4] and P Ashton-Prolla*[,1,3,5,6]

p53 has a crucial role in human fertility by regulating the expression of leukemia inhibitory factor (LIF), a secreted cytokine critical for blastocyst implantation. To examine whether *TP53* polymorphisms may be involved with *in vitro* fertilization (IVF) failure and endometriosis (END), we have assessed the associations between *TP53* polymorphism in intron 2 (PIN2; G/C, intron 2), PIN3 (one (N, non-duplicated) or two (D, duplicated) repeats of a 16-bp motif, intron 3) and polymorphism in exon 4 (PEX4; C/G, p.P72R, exon 4) in 98 women with END and 115 women with post-IVF failure. In addition, 134 fertile women and 300 women unselected with respect to fertility-related features were assessed. *TP53* polymorphisms and haplotypes were identified by amplification refractory mutation system polymerase chain reaction. *TP53* PIN3 and PEX4 were associated with both END ($P = 0.042$ and $P = 0.007$, respectively) and IVF ($P = 0.004$ and $P = 0.009$, respectively) when compared with women both selected and unselected for fertility-related features. Haplotypes D-C and N-C were related to higher risk for END ($P = 0.002$, $P = 0.001$, respectively) and failure of IVF ($P = 0.018$ and $P = 0.002$, respectively) when compared with the Fertile group. These results support that specific *TP53* haplotypes are associated with an increased risk of END-associated infertility and with post-IVF failure.

Cell Death and Disease (2012) **3,** e392; doi:10.1038/cddis.2012.116; published online 27 September 2012
Subject Category: Cancer

TP53 encodes the multi-functional tumor suppressor transcription factor p53 which has a crucial role in maintaining genomic stability in somatic cells exposed to oncogenic or genotoxic stress, thus preventing tumor formation.[1] In response to a wide range of stress signals, p53 accumulates in the nucleus and regulates the expression of a large panel of genes involved in the control of cell cycle arrest, apoptosis, cell senescence, DNA repair and energy metabolism. One of the transcriptional targets of p53 is leukemia inhibitory factor (*LIF*), the gene encoding LIF. LIF is a secreted cytokine with broad roles in the control of lymphocyte proliferation and differentiation. It has also been identified as a critical factor for blastocyst implantation.[2] Control of p53 over LIF expression is operated through a p53-response element located in intron 1 and conserved in both mouse and human *LIF* genes.[3]

Recent studies have demonstrated that p53 regulates female reproduction and blastocyst implantation through LIF. Implantation is a critical step in mammalian embryonic development during which the blastocyst establishes close interactions with the uterus, leading to the formation of the placenta supporting fetal development.[4] Hu *et al.*[4] have demonstrated that p53 regulates LIF expression in the uterus of female mice. p53-deficient mice express lower levels of LIF than their p53-competent counterparts and show impaired blastocyst implantation and consequently, impaired fertility.

There is strong evidence that genes at critical regulatory nodes in the p53 pathway are under evolutionary selection[5,6] and that SNPs in the p53 pathway influence human fertility.[7] Of these, one of the most studied is *TP53* polymorphism in exon 4 (PEX4 of the *TP53* gene), widely known as p.P72R (C/G, rs1042522). This single-nucleotide polymorphism (SNP) located at the second position of the codon 72 consist in either an ancestral C allele whose frequency in African populations is around 0.70 or a derived G allele whose frequency in European and Asian populations varies from around 0.50 to 0.80. Presence of the C allele results in a proline in codon 72, and presence of the G allele, in an arginine. These polymorphic protein variants significantly differ in their biological properties and there is evidence that R72p53 has higher transcriptional activity toward a particular subset of p53 target genes, including *LIF*, than P72p53.[8] Previous studies have identified an association between *TP53* PEX4 and infertility[7] or endometriosis (END).[9–11] It has been suggested that the effect of PEX4 on *LIF* expression and fertility may account for population differences in the distribution of PEX4 alleles in different parts of the world.

[1]Department of Genetics and Molecular Biology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; [2]Department of Obstetrics and Gynecology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; [3]Post-Graduate Program in Genetics and Molecular Biology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; [4]International Prevention Research Institute, Lyon, France; [5]Medical Genetics Service, Hospital de Clínicas de Porto Alegre, and National Institute of Science and Technology in Populational Medical Genetics (INAGEMP), Porto Alegre, Brazil and [6]Genomic Medicine Laboratory, Experimental Research Center, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil
*Corresponding author: P Ashton-Prolla, Departamento de Genética, UFRGS e Serviço de Genética Médica e Centro de Pesquisa Experimental, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350, Porto Alegre 90035-903, Brazil. Tel: +55 51 3359 8010; Fax: +55 51 3359 8760; E-mail: pprolla@hcpa.ufrgs.br
Keywords: *TP53*; single-nucleotide polymorphisms; IVF; endometriosis
Abbreviations: TP53, tumor protein p53; LIF, leukemia inhibitory factor; PIN2, polymorphism in intron 2; PIN3, polymorphism in intron 3; PEX4, polymorphism in exon 4; SNP, single-nucleotide polymorphism; END, endometriosis; IVF, *in vitro* fertilization; D, duplicated allele; N, non-duplicated allele
Received 30.4.12; revised 21.6.12; accepted 02.7.12; Edited by Y Shi

These differences may reflect subtle adaptation to environmental constraints affecting fertility. However, the magnitude of the PEX4 effect on infertility associated with different pathological causes remains controversial.[12,13]

PEX4 is in strong linkage disequilibrium with another common polymorphism located in its close vicinity, PIN2 (polymorphism in intron 2; rs1642785; G/C). The PIN2 G allele has been associated with human papillomavirus persistence[14] and individuals with two copies of the PIN2 G allele have been reported as having an increased risk of osteosarcoma.[15] Recently, it has been shown that another polymorphism in intron 3 of the *TP53* gene, PIN3 (Polymorphism in Intron 3, rs17878362, 16 bp duplication, N = non-duplicated, D = duplicated) overlaps with a G-quadruplex motif, which regulates p53 mRNA splicing generating an alternatively spliced form, which supports the synthesis of an isoform of p53 lacking the N-terminal transactivation domain (Delta40p53).[16] PIN3 D allele is associated with increased risk of colorectal,[17] lung[18] and breast cancer,[19] whereas the N allele has been reported in association with an average acceleration of 19 years in the mean age at first cancer diagnosis in a Brazilian cohort of *TP53* germline mutation carriers.[20] The effects of this polymorphism in END or infertility have not been investigated so far.

Although the association between END and infertility is well known (END affects up to 50% of women with infertility),[21] the cause of infertility in the disease is not fully understood but is thought to involve hormonal,[22] immunological,[23] genetic,[24] proliferative (endometrial) and uterine alterations.[25] We hypothesized that *TP53* polymorphisms that alter p53 function may be associated with *in vitro* fertilization (IVF) failure and with END-associated infertility.

Results

Patients and healthy study subjects did not differ significantly regarding self-attributed skin color (Supplementary Table S1). Overall, a self-denomination of 'white' color predominated in all study subgroups (END, FIV, Unselected and Fertile). In terms of reproductive history, the mean number of pregnancies in women of the fertile and unselected for fertility groups was 3.62 ± 1.9 and 3.22 ± 2.1, respectively. In the later, nulliparity was observed in 2.6%.

Women in the fertile and unselected for fertility groups presented higher mean age at recruitment (42.68 ± 12.8 years and 43.2 ± 12.7 years, respectively) as compared with END (32.87 ± 4.7 years) and IVF (31.65 ± 3.2 years) groups. Hardy–Weinberg equilibrium was achieved in all study groups for PIN2, PIN3 and PEX4 (all $P > 0.05$, Supplemental Materials, Table S2. Genotypic and allelic frequencies of the *TP53* polymorphisms are shown in Table 1. In all four study subgroups, PIN3 and PEX4 allele frequencies did not differ significantly from those previously described in European populations (Supplementary Table S3).

Single marker analysis (Table 1) revealed a significant association between PIN2 (rs1642785) genotypes and IVF ($P = 0.016$), and a borderline association with the END group ($P = 0.052$) when compared with the Fertile group. There was an increased frequency of the PIN2 C allele in both the END and IVF groups. When analyzing *TP53* PIN3 (rs17878362) polymorphism, a clear difference between IVF and END groups was observed when compared with the Fertile group. Allele D (the duplicated allele) was enriched in patients in both groups as compared with Fertile ($P = 0.042$ and $P < 0.0004$ for the END and IVF groups, respectively). For *TP53* PEX4 (rs1042522), a statistically significant difference between both the END and IVF groups and the Fertile group was also demonstrated, with enrichment of the PEX4 C allele in both groups ($P = 0.007$ and $P = 0.009$, respectively).

When the Fertile and Unselected groups were compared, we observed that the allelic frequencies of PIN2 G and PEX4 G were significantly higher in the Fertile group, whereas PIN2 and PEX4 genotype distribution did not differ between groups.

Table 1 Genotypic and allelic frequencies of selected *TP53* polymorphisms between Fertility Unselected, Fertile, END and IVF groups

TP53		Unselected, *n* (%)	Fertile, *n* (%)	*P*-value[a]	END, *n* (%)	*P*-value[b]	*P*-value[c]	IVF, *n* (%)	*P*-value[d]	*P*-value[e]
PIN2	GG	166 (55.3)	88 (65.7)	0.114	53 (54.1)	0.304	0.052	63 (54.8)	0.049	0.016
rs1642785	GC	112 (37.3)	40 (29.9)		33 (33.7)			35 (30.4)		
	CC	22 (7.3)	6 (4.5)		12 (12.2)			17 (14.8)		
	G	444 (74.0)	216 (80.6)	0.007	139 (70.9)	0.397	0.015	161 (70.0)	0.245	0.005
	C	156 (26.0)	52 (19.4)		57 (29.1)			69 (30.0)		
PIN3	NN	222 (74.0)	94 (70.1)	0.658	49 (50.0)	<0.001	0.042	72 (62.6)	<0.001	0.004
rs17878362	ND	70 (23.3)	35 (26.1)		32 (32.7)			29 (25.2)		
	DD	8 (2.7)	5 (3.7)		17 (17.3)			14 (12.2)		
	N	514 (85.7)	223 (83.2)	0.350	130 (66.3)	<0.001	0.005	173 (75.2)	<0.001	0.027
	D	86 (14.3)	45 (16.8)		66 (33.7)			57 (24.8)		
PEX4	GG	158 (52.7)	89 (66.4)	0.013	50 (51.0)	0.535	0.007	63 (54.8)	0.159	0.009
rs1042522	GC	114 (38.0)	40 (29.9)		35 (35.7)			35 (30.4)		
	CC	28 (9.3)	5 (3.7)		13 (13.3)			17 (14.8)		
	G	430 (71.7)	218 (81.3)	0.002	135 (68.9)	0.455	0.001	161 (0.70)	0.635	0.003
	C	170 (28.3)	50 (18.7)		61 (31.1)			69 (0.30)		

Abbreviations: D, duplicated; END, endometriosis; IVF, *in vitro* fertilization; N, non-duplicated.
[a]χ^2-test, significant difference observed between women unselected for fertility and Fertile women.
[b]χ^2-test, significant difference observed between END patients and women unselected for fertility.
[c]χ^2-test, significant difference observed between END patients and Fertile group.
[d]χ^2-test, significant difference observed between IVF patients and women unselected for fertility.
[e]χ^2-test, significant difference observed between IVF patients and Fertile group.
IVF group: women with recurrent failure of IVF; END group: infertile women with minimal or mild endometriosis; Fertile: Fertile women; Unselected: women unselected with respect to fertility or infertility-related symptoms.

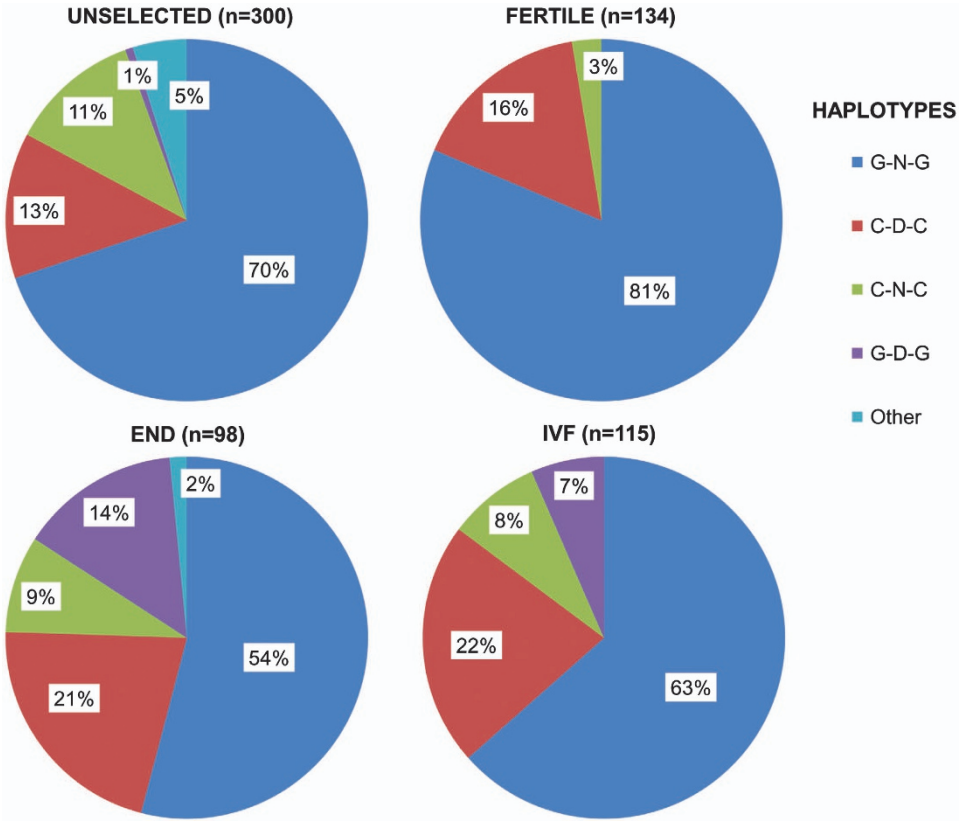

Figure 1 Distribution of the most frequent haplotypes among Unselected, Fertile, END and IVF groups. Haplotypes frequencies are shown as '%'. Haplotypes were constructed as PIN2 (G/C) – PIN3 (N/D) – PEX4 (C/G)

Similarly, PIN3 genotypic or allelic frequencies did not differ between groups (Table 1). For both the END and IVF groups, the allelic frequencies of PIN2, PIN3 and PEX4 differed significantly from those observed in the Fertile group. The allelic frequencies of PIN3 in infertile women (either END and IVF groups) differed significantly from both the Fertile and Unselected groups.

Haplotype analysis showed strong linkage disequilibrium between *TP53* PIN2 and PEX4 ($D' = 1$; $r^2 = 0.94$ in all studies groups, Supplementary Table S4) as previously described.[20] Therefore, we have only considered *TP53* PIN3 and *TP53* PEX4 in further analyses and in our discussion. We carried out a binary logistic regression analysis to evaluate the effect of *TP53* haplotypes with regard to END and IVF. Figure 1 shows the distribution of the most frequent haplotypes encountered (see Figure 1). Table 2 shows the odds ratios for the END and IVF groups of the most frequent haplotypes when compared with the reference N-G haplotype. Haplotypes D-C and N-C were related to higher risk for END ($P = 0.002$, $P = 0.001$, respectively) and failure of IVF ($P = 0.018$ and $P = 0.002$, respectively) when compared with the Fertile group. However, when the Unselected group (unselected for fertility) was used as the comparison group in the logistic regression model, the risk association with haplotypes D-C and N-C was not observed (data not shown).

Table 2 Binary logistic regression model for *TP53* haplotypes regarding PIN3 (N/D) and PEX4 (C/G) using the Fertile group as reference

	END		IVF	
Haplotype	***P*-value**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**
N-G				
D-C	0.002	2.1 (1.3–3.5)	0.018	1.7 (1.1–2.7)
N-C	0.001	4.9 (2.1–12.4)	0.002	4.1 (1.6–9.8)

Abbreviations: CI, confidence interval; D, duplicated; END, endometriosis; IVF, *in vitro* fertilization; N, non-duplicated; OR, odds ratio.
OR (95% CI) was calculated by binary logistic regression analysis. IVF group: women with recurrent failure of IVF; END group: infertile women with minimal or mild endometriosis.

Discussion

In this study, we have analyzed the distribution of three common polymorphisms in the *TP53* gene (PIN2, PIN3 and PEX4) in infertile women with failure of IVF treatment or with END-associated infertility. Our results demonstrate an association between these two forms of infertility and *TP53* alleles PIN3 D and PEX4 C, suggesting that variations in p53 activity specified by these polymorphisms may be involved in the pathogenesis of both conditions. These results support previously reported observations on associations between PEX4 and infertility, in particular IVF failure. Furthermore, these results provide clear evidence in favor of an association between *TP53* polymorphism and infertility-related END. Regarding *TP53* PIN3, several studies have evaluated the association between this polymorphism and lung[18] or breast cancer,[19] but to our knowledge, no previous study has analyzed its association with infertility or END.

Previous studies have shown associations between PEX4 C allele and END,[10,26,27] whereas others fail to demonstrate this association.[11,12] These controversies may be due to the environmental and genetic background of the studied populations but also because of differences in illness classifications (END is sometimes asymptomatic and often can only be diagnosed by laparoscopy). In our study, the END group was carefully diagnosed according to the American Society for Reproductive Medicine (ASRM) and women with END were excluded from both IVF and Fertile groups after laparoscopic examination. In addition, all four study groups described here were quite homogeneous in terms of self-reported skin color (a feature used as proxy for 'race' or ancestry background in Brazil) corroborating with previous population-based studies that demonstrate predominance of European genomes in this specific region.[28–32] This observation was further confirmed by comparative analysis of PIN3 and PEX4 allele frequencies encountered here and those previously described in European/European-derived and African/African-derived populations, showing that in all four study groups, allelic distribution was not statistically different from the observed in Europeans/Europeans-derived.

Kay *et al.*[33] were the first to associate *TP53* PEX4 C allele with women experiencing recurrent implantation failure. Other studies also associated the PEX4 C allele with the occurrence of idiopathic recurrent miscarriages[34] and implantation failure,[35] and Kang *et al.*[7] demonstrated that PEX4 C was significantly enriched among IVF patients, serving as risk factor for implantation failure. Our results are in agreement with these previous findings regarding the *TP53* PEX4 C allele and confirm this allele as a risk factor for both END-associated infertility and IVF failure in a different sample set.

These results and the findings of our study suggest that PIN3 and PEX4 polymorphisms present specific functional differences in p53 protein variants, having an impact on events that are critical for embryo implantation and/or early development. In the case of PEX4, there is experimental evidence from cell and animal studies that the p53 protein encoded by the PEX4 C allele (P72p53) is more efficient in initiating senescence that the product of the PEX4 G allele (R72p53), which in turn appears to have a stronger effect on p53-mediated apoptosis and suppression of cell transformation.[36] In the case of PIN3, presence of the *TP53* PIN3 D allele has been associated with reduced levels of *TP53* mRNA in lymphoblastoid cell lines.[17] Whether this effect also occurs *in vivo* remains to be determined. Marcel *et al.*[16] demonstrated that *TP53* PIN3 is located within a GC-rich region of intron 3 that form G-Quaduplex structures, which modulate splicing of intron 2. *In silico* models predict that PIN3 may alter the topology of these *G-quadruplex* structures, thus modifying the patterns of p53 mRNA isoform expression. The p53 isoform encoded by alternatively spliced p53 retaining intron 2 lacks the N-terminal domain containing the main transactivation activity of p53, thus resulting in an N-terminally truncated protein, which binds DNA but does not activate transcription through p53-response elements. It is important to emphasize that in our study *TP53* PIN3 presented an allelic distribution that was significantly different in infertile women (either END or IVF groups) when compared with women selected for fertility but also when compared with women from a community sample and unselected for fertility, suggesting that genetic variations in PIN3 may have a critical effect on infertility. Further experimental studies are needed to evaluate the possible impact of p53 isoforms in regulating these biological events, especially their impact on transactivation of key genes involved in the early stages of gestation, such as *LIF*. Haplotypes D-C and N-C were related to higher risk for END and IVF only when a group of women selected in favor of normal fertility (the Fertile group) was used as comparison group; this was not observed when the comparison group included women unselected for reproductive history. This observation suggests that specific haplotypes of *TP53* may be associated with high fertility features. Given the associations between specific SNPs and infertility, it is reasonable to assume that particular combinations of SNPs might provide a genetic marker for women with high fertility features.

Our results support that *TP53* polymorphisms have a role in both END-associated infertility and IVF failure; although current evidence points to a strong effect of the PEX4 polymorphism in embryo implantation and fertility, other SNPs in *TP53*, especially PIN3, may have a key role in the modulation of this process and in other biological processes related to early embryonic development. PIN3 was the only SNP that showed differential frequencies in infertile women (either END and IVF groups) when compared with either fertility-selected or unselected groups, whereas PIN2 and PEX4 only showed a differential distribution in END and IVF patients when compared with a group of patients at the other extreme of the phenotype (fertile group).

In conclusion, the data presented here add to the current evidence that variations in expression and activity of p53 may have an effect on the expression of key genes related to the control of cellular growth and invasion, which have been associated with END (*BAX, FAS, PIG11, PTEN*), as well as on genes associated with embryo implantation (*LIF*). Infertility associated to END could be related, at least in part, to embryo implantation failure in a mechanism similar to that seen in other infertile women without END. It may also involve other mechanisms affecting early embryonic development as well as cell–cell communications during the pre-implantation and implantation phases. In agreement with this hypothesis, previous studies have demonstrated lower implantation and pregnancy rates in endometriotic patients.[37] *TP53* polymorphisms, especially PIN3 and PEX4 may have an interest as biomarkers and could add to the development of a clinically relevant genetic profile that would be of great help for clinicians to identify patients at higher risk for IVF failure. The results of this study should be confirmed in larger cohorts with well defined phenotypes of END and infertility and long-term follow-up data. They also emphasize the importance of a clear definition of clinical phenotypes and of study design when analyzing the effects of specific polymorphisms on fertility.

Materials and Methods

Patients and subjects. All patients and subjects were informed about the procedures of the study when invited to participate and signed a consent form at inclusion. The research project was approved by the Institutional Ethics Committee (Hospital de Clinicas de Porto Alegre – GPPG 05-182; GPPG 09-430).

At inclusion, patients and subjects were also asked to provide a description of their perceived skin color. In Brazil, skin color is normally used to define an equivalent to 'race' or ancestry background.[32,38] We used the words 'White' and 'non-White'

to identify women who defined themselves with some term that suggests only European ancestry and with other terms that suggest some level of African ancestry (such as *mulato* or *pardo*), respectively. No term that reports some level of Amerindian ancestry was used by volunteers.

Patients and subjects were divided into four study groups. The IVF Group consisted of 115 women (<35 years) with at least one IVF failure, defined as a failure after IVF cycle treatment with transfer of two or more top quality embryos (8 cell embryos with $<20\%$ fragmentation). Briefly, inclusion criteria of this group were: age <35 years, exclusion of END by laparoscopy and the main factor was of mild masculine (oligospermia) or tubal origin. All patients in this group were submitted to conventional IVF. Patients with previous thyroid disease, positive anti-lupus or anti-cardiolipin antibodies and trombophilias were also excluded from our sample. Controlled ovarian hyperstimulation was performed with the use of recombinant human FSH and pituitary suppression with GnRh antagonist (fixed day-6 protocol). Ovulation was induced by 6500 IU recombinant hCG when at least three follicles had reached a diameter of >17 mm, and transvaginal follicle aspiration was performed 36 h later under ultrasound guidance. Embryos were classified according to the cumulative embryo classification, taking into account cleavage speed, blastomere symmetry, extent of fragmentation and the presence or absence of multinucleated blastomeres.

The END group comprised 98 infertile women with minimal or mild END as diagnosed by laparoscopy recruited at the Gynecology Service of Hospital de Clinicas de Porto Alegre (HCPA), in Southern Brazil. Infertility was defined as the inability of a couple to achieve pregnancy after 1 year of regular unprotected sexual intercourse.[39] Other causes of infertility were excluded by hysterosalpingography, sperm evaluation and hormonal measurements whenever necessary. END diagnosed during laparoscopy was categorized according to the classification proposed by the ASRM.[39]

The Fertile group consisted of 134 women with no history of infertility, who already had children without any difficulties or assisted reproduction and underwent laparoscopy for tubal ligation at HCPA. END was excluded in women from IVF and Fertile groups. In addition, we studied a group of 300 asymptomatic women, who volunteered for a community-based breast cancer screening program in Southern Brazil (from the same geographic recruitment area of the patients included in the IVF and Fertile groups). This group ('Unselected') was unselected with respect to fertility or infertility-related symptoms, as described elsewhere.[40]

Genotyping. Genomic DNA was extracted from peripheral blood using the Ilustra blood genomic Prep Mini spin Kit (GE Healthcare, Piscataway, NJ, USA) as described by the manufacturer. Genotypes and haplotypes defined by the three *TP53* gene polymorphisms (PIN2 rs1642785 G/C, PIN3 rs17878362 16 pb duplication and PEX4 rs1042522 C/G) were determined by Amplification Refractory Mutation System as previously described.[20]

Statistical analysis. The clinical characteristics of the women in all study groups were compared by one-way analysis of variance. Differences in genotype/allele distribution between IVF, END, Fertile and Unselected groups were evaluated using χ^2-analysis, also used to test for Hardy–Weinberg equilibrium. Linkage disequilibrium was assessed calculating D' value (the relative magnitude of D as compared with its theoretical maximum, calculated as D/D^{max}) as described by Lewontin.[41]

Binary logistic regression analysis was carried out to estimate the odds ratios with 95% confidence intervals in order to assess the influence of *TP53* haplotypes for END and IVF using the Fertile group as reference. Haplotype frequencies were calculated by direct count. Statistical analysis was performed using the SPSS 18.0 statistical package. All reported *P*-values are two-tailed and considered statistically significant when $0.05\geqslant$.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements. The work of DDP was supported by fellowships from CAPES and CNPQ (Brazil). The study was supported in part by grants from GlaxoSmithKline Oncology (Ethnic Research Initiative Grant Award 2009), UK; CNPq to PA-P (Grant 307779 2009-2), Brazil; FAPERGS-PPSUS (Grant no. 09/0103-0), FAPERGS PRONEX (Grant no. 10/0051-9) and Fundo de Incentivo a Pesquisa e Eventos, Hospital de Clínicas de Porto Alegre (GPPG no. 09430), Brazil.

1. Levine AJ, Hu W, Feng Z. The P53 pathway: what questions remain to be explored? *Cell Death Differ* 2006; **13**: 1027–1036.
2. Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi I, Kontgen F *et al.* Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature* 1992; **359**: 76–79.
3. Hu W, Feng Z, Teresky AK, Levine AJ. p53 regulates maternal reproduction through LIF. *Nature* 2007; **450**: 721–724.
4. Hu W, Feng Z, Atwal GS, Levine AJ. p53: a new player in reproduction. *Cell Cycle* 2008; **7**: 848–852.
5. Atwal GS, Bond GL, Metsuyanim S, Papa M, Friedman E, Distelman-Menachem T *et al.* Haplotype structure and selection of the MDM2 oncogene in humans. *Proc Natl Acad Sci USA* 2007; **104**: 4524–4529.
6. Atwal GS, Kirchhoff T, Bond EE, Montagna M, Menin C, Bertorelle R *et al.* Altered tumor formation and evolutionary selection of genetic variants in the human MDM4 oncogene. *Proc Natl Acad Sci USA* 2009; **106**: 10236–10241.
7. Kang HJ, Feng Z, Sun Y, Atwal G, Murphy ME, Rebbeck TR *et al.* Single-nucleotide polymorphisms in the p53 pathway regulate fertility in humans. *Proc Natl Acad Sci USA* 2009; **106**: 9761–9766.
8. Feng Z, Zhang C, Kang HJ, Sun Y, Wang H, Naqvi A *et al.* Regulation of female reproduction by p53 and its family members. *Faseb J* 2011; **25**: 2245–2255.
9. Ammendola M, Gloria-Bottini F, Sesti F, Piccione E, Bottini E. Association of p53 codon 72 polymorphism with endometriosis. *Fertil Steril* 2008; **90**: 406–408.
10. Chang CC, Hsieh YY, Tsai FJ, Tsai CH, Tsai HD, Lin CC. The proline form of p53 codon 72 polymorphism is associated with endometriosis. *Fertil Steril* 2002; **77**: 43–45.
11. Vietri MT, Molinari AM, Iannella I, Cioffi M, Bontempo P, Ardovino M *et al.* Arg72Pro p53 polymorphism in Italian women: no association with endometriosis. *Fertil Steril* 2007; **88**: 1468–1469.
12. Lattuada D, Vigano P, Somigliana E, Abbiati A, Candiani M, Di Blasio AM. Analysis of the codon 72 polymorphism of the TP53 gene in patients with endometriosis. *Mol Hum Reprod* 2004; **10**: 651–654.
13. Patounakis G, Treff N, Tao X, Lonczak A, Scott RT Jr, Frattarelli JL. The p53 codon 72 single nucleotide polymorphism lacks a significant effect on implantation rate in fresh in vitro fertilization cycles: an analysis of 1,056 patients. *Fertil Steril* 2009; **92**: 1290–1296.
14. Koshiol J, Hildesheim A, Gonzalez P, Bratti MC, Porras C, Schiffman M *et al.* Common genetic variation in TP53 and risk of human papillomavirus persistence and progression to CIN3/cancer revisited. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1631–1637.
15. Savage SA, Burdett L, Troisi R, Douglass C, Hoover RN, Chanock SJ. Germ-line genetic variation of TP53 in osteosarcoma. *Pediatr Blood Cancer* 2007; **49**: 28–33.
16. Marcel V, Tran PL, Sagne C, Martel-Planche G, Vaslin L, Teulade-Fichou MP *et al.* G-quadruplex structures in TP53 intron 3: role in alternative splicing and in production of p53 mRNA isoforms. *Carcinogenesis* 2011; **32**: 271–278.
17. Gemignani F, Moreno V, Landi S, Moullan N, Chabrier A, Gutierrez-Enriquez S *et al.* A TP53 polymorphism is associated with increased risk of colorectal cancer and with reduced levels of TP53 mRNA. *Oncogene* 2004; **23**: 1954–1956.
18. Wu X, Zhao H, Amos CI, Shete S, Makan N, Hong WK *et al.* p53 Genotypes and Haplotypes Associated With Lung Cancer Susceptibility and Ethnicity. *J Natl Cancer Inst* 2002; **94**: 681–690.
19. Wang-Gohrke S, Rebbeck TR, Besenfelder W, Kreienberg R, Runnebaum IB. p53 germline polymorphisms are associated with an increased risk for breast cancer in German women. *Anticancer Res* 1998; **18**: 2095–2099.
20. Marcel V, Palmero EI, Falagan-Lotsch P, Martel-Planche G, Ashton-Prolla P, Olivier M *et al.* TP53 PIN3 and MDM2 SNP309 polymorphisms as genetic modifiers in the Li-Fraumeni syndrome: impact on age at first diagnosis. *J Med Genet* 2009; **46**: 766–772.
21. Eskenazi B, Warner ML. Epidemiology of endometriosis. *Obstet Gynecol Clin North Am* 1997; **24**: 235–258.
22. Cunha-Filho JS, Gross JL, Lemos NA, Brandelli A, Castillos M, Passos EP. Hyperprolactinemia and luteal insufficiency in infertile patients with mild and minimal endometriosis. *Horm Metab Res* 2001; **33**: 216–220.
23. Podgaec S, Abrao MS, Dias JA Jr., Rizzo LV, de Oliveira RM, Baracat EC. Endometriosis: an inflammatory disease with a Th2 immune response component. *Hum Reprod* 2007; **22**: 1373–1379.
24. Bischoff F, Simpson JL. Genetic basis of endometriosis. *Ann N Y Acad Sci* 2004; **1034**: 284–299.
25. Giudice LC, Kao LC. Endometriosis. *Lancet* 2004; **364**: 1789–1799.
26. Hsieh YY, Lin CS. P53 codon 11, 72, and 248 gene polymorphisms in endometriosis. *Int J Biol Sci* 2006; **2**: 188–193.
27. Ribeiro CL Jr, Arruda JT, Silva CT, Moura KK. Analysis of p53 codon 72 gene polymorphism in Brazilian patients with endometriosis. *Genet Mol Res* 2009; **8**: 494–499.
28. Salzano FM, Bortolini MC. *The Evolution and Genetics of Latin American Populations.* Cambridge University Press: Cambridge, 2002, p 509.
29. Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD. Color and genomic ancestry in Brazilians. *Proc Natl Acad Sci USA* 2003; **100**: 177–182.
30. Marrero AR, Das Neves Leite FP, De Almeida Carvalho B, Peres LM, Kommers TC, Da Cruz IM *et al.* Heterogeneity of the genome ancestry of individuals classified as White in the state of Rio Grande do Sul, Brazil. *Am J Hum Biol* 2005; **17**: 496–506.

31. Zembrzuski VM, Callegari-Jacques SM, Hutz MH. Application of an African Ancestry Index as a genomic control approach in a Brazilian population. *Ann Hum Genet* 2006; **70**(Pt 6): 822–828.
32. Pena SD, Di Pietro G, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy Fde S *et al.* The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. *PLoS One* 2011; **6**: e17063.
33. Kay C, Jeyendran RS, Coulam CB. p53 tumour suppressor gene polymorphism is associated with recurrent implantation failure. *Reprod Biomed Online* 2006; **13**: 492–496.
34. Pietrowski D, Bettendorf H, Riener EK, Keck C, Hefler LA, Huber JC *et al.* Recurrent pregnancy failure is associated with a polymorphism in the p53 tumour suppressor gene. *Hum Reprod* 2005; **20**: 848–851.
35. Goodman C, Jeyendran RS, Coulam CB. P53 tumor suppressor factor, plasminogen activator inhibitor, and vascular endothelial growth factor gene polymorphisms and recurrent implantation failure. *Fertil Steril* 2009; **92**: 494–498.
36. Vousden KH, Prives C. Blinded by the Light: The Growing Complexity of p53. *Cell* 2009; **137**: 413–431.
37. Pellicer A, Oliveira N, Ruiz A, Remohi J, Simon C. Exploring the mechanism(s) of endometriosis-related infertility: an analysis of embryo development and implantation in assisted reproduction. *Hum Reprod* 1995; **10**(Suppl 2): 91–97.
38. Guerreiro-Junior V, Bisso-Machado R, Marrero A, Hunemeier T, Salzano FM, Bortolini MC. Genetic signatures of parental contribution in black and white populations in Brazil. *Genet Mol Biol* 2009; **32**: 1–11.
39. ASRM. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. *Fertil Steril* 1997; **67**: 817–821.
40. Palmero EI, Schuler-Faccini L, Caleffi M, Achatz MI, Olivier M, Martel-Planche G *et al.* Detection of R337H, a germline TP53 mutation predisposing to multiple cancers, in asymptomatic women participating in a breast cancer screening program in Southern Brazil. *Cancer Lett* 2008; **261**: 21–25.
41. Lewontin RC. On measures of gametic disequilibrium. *Genetics* 1988; **120**: 849–852.

Supplementary Information accompanies the paper on Cell Death and Disease website (http://www.nature.com/cddis)