Maternal SNPs in the p53 pathway: Risk factors for trisomy 21?

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Abstract. The p53 family and its regulatory pathway play an important role as regulators of developmental processes, limiting the propagation of aneuploid cells. Its dysfunction or imbalance can lead to pathological abnormalities in humans. The aim of this study was to evaluate the effect of maternal polymorphisms \textit{TP53} c.215G>C (P72R), \textit{TP73} c.-30G>A and 14 c.-20C>T, \textit{MDM2} c.14+309T>G (SNP309), \textit{MDM4} c.753+572C>T and \textit{USP7} c.2719-234G>A as risk factors for Down Syndrome (DS) birth. A case-control study was conducted with 263 mothers of DS children and 196 control mothers. The distribution of these genotypic variants was similar between case and control mothers. However, the combined alleles \textit{TP53} \textit{Ca} and \textit{MDM2} \textit{G}, and \textit{TP53} \textit{Ca} and \textit{USP7} \textit{A} increased the risk of having offspring with DS (OR = 1.84 and 1.77; 95% CI; \textit{P}< 0.007 and 0.018, respectively). These results suggest that, although the individual polymorphisms were not associated with DS birth, the effect of the combined genotypes among \textit{TP53}, \textit{MDM2} and \textit{USP7} genes indicates a possible role of \textit{TP53} and its regulatory pathway as a risk factor for aneuploidy.

Keywords: Down syndrome, \textit{TP53}, \textit{TP73}, \textit{MDM2}, \textit{MDM4}, \textit{USP7}

1. Introduction

Down Syndrome (DS), characterized by trisomy of chromosome 21, is the most common cause of mental retardation in humans [23], occurring in 1 in 700–800 births [30]. The meiotic nondisjunction is the main cause of free 21 trisomy, event responsible for the aneuploidy 21 in 95% of affected individuals [3]. In 95% of cases, the nondisjunction occurs during maternal meiosis [1], mainly in the first meiotic division [2,59]. It is well established that advanced maternal age is a risk factor for aneuploidy and is associated specifically with errors that occur during oogenesis [59].

Given the important role played by \textit{p53} family proteins as regulators of crucial developmental processes, their dysfunction or imbalance can lead to pathological abnormalities in humans. Genomic instability, aneuploidy and copy number polymorphisms that originate in the female germline and contribute to a number of developmental defects can be explored through investigations of the \textit{TP53} gene family [26]. Encoded by \textit{TP53} gene, the \textit{p53} protein has known importance in the prevention of tumors and genomic stability in somatic cells, acting as a transcription factor that regulates a large number of genes in response to cell damage, including activation of oncogenes and DNA damage [12,35,45]. When activated, \textit{p53} initiates cellular responses, such as cell cycle arrest, DNA repair, senescence and apoptosis [22,27]. The loss of \textit{p53} allows the accumulation of aneuploid cells as a result of chromosomal instability. Thus, \textit{p53} and its regulatory pathway play a critical role in limiting the propagation of aneuploidy and preserving the nature of diploid human cells [55].

The central control of the \textit{p53} regulatory pathway consists of three major genes and their products:
MDM2 (Mouse double minute p53 binding protein homolog 2), MDM4 (Mouse double minute p53 binding protein homolog 4) and USP7 (ubiquitin specific peptidase 7 (herpes virus-associated)), also known as HAUSP [10,29]. The main negative regulator of the TP53 is the protein MDM2, which acts on the p53 as an E3 ubiquitin ligase, leading to degradation of p53 [9,31]. MDM2 is upregulated by TP53, where the increase in p53 levels leads to increased transcription of MDM2. Thus, the product degrades p53 by inhibiting their levels, resulting in a negative feedback loop. This process maintains the p53 protein at low level in the presence of stress signals, allowing normal cell proliferation [42,47]. Participating in the same metabolic pathway, the TP73 gene plays a crucial role in maintaining the rate of ovulation and acting on the spindle checkpoint, reducing aneuploidy in the offspring [57]. p73 plays an important role in maintaining genomic integrity as well, which is particularly important when p53 function is compromised [5].

Single nucleotide polymorphisms (SNPs) in genes of the p53 regulatory pathway have been targeted for study in research relating to human reproduction [16,17,29]. A common polymorphism in TP53 c.215G>C (P72R, rs1042522) [11], a substitution at codon 72 that makes the induction of apoptosis less efficient [15,54]. The MDM2 gene has an important functional polymorphism c.14+309T>G (SNP309, rs2279744), the result of a thymine to guanine change in its promoter region [7], increasing MDM2 expression and attenuating the p53 function [7,16,29]. A substitution in intron 9 of MDM4 gene c.753+572C>T (rs1563828) is correlated with human reproduction, as well as c.2179-234G>A change in intron 25 of USP7 gene (rs1529916) [29]. In TP73, two closely linked polymorphisms in position 4 c.-30G>A and 14 c.-20C>T (rs2273953, rs1801173) are located before the initiating codon in exon 2. This region can form a clamp-shaped structure with the potential to interfere in gene expression [28].

Thus, we hypothesized that polymorphisms related to TP53 and TP73 genes and their regulatory pathway – MDM2, MDM4 and USP7 – may be closely associated with human reproduction, where its fine regulation is extremely important in maintaining genomic stability of germline cells avoiding aberrations in its genome, as aneuploidies. This study investigated the influence of the TP53 gene family and their regulators as risk factors for aneuploidy of chromosome 21. We analyzed the role of TP53 c.215G>C polymorphism (rs1042522), TP73 c.-30G>A (rs2273953) and c.-20C>T (rs1801173), MDM2 c.14+309T>G (rs2279744), MDM4 c.753+572C>T (rs1563828) and USP7 c.2719-234G>A (rs1529916) as maternal risk factors for DS birth in a case-control study.

2. Materials and methods

2.1. Subjects

All cases were identified through Medical Genetic Service of Hospital de Clínicas de Porto Alegre (HCPA) and local support groups of DS (APAES). The control group consisted of women with healthy children who were randomly selected to participate in this study during the blood collection for routine laboratorial analyzes in the HCPA. The case-control study was conducted with 263 case mothers and 196 control mothers. Further details on the selection and sample characteristics can be found in our previously published work [8].

This study was approved by the Ethics Committee of HCPA. All mothers who participated in the study signed an informed consent form. We collected 5 mL of peripheral blood in EDTA tubes for genetic analysis.

2.2. Analysis of polymorphisms

DNA was extracted from blood samples as described by Lahiri and Nurnberger [32]. The SNPs of five genes of the p53 signaling pathway were genotyped, including TP53, TP73, MDM2, MDM4 and USP7. Genotypes were determined by using the following allelic discrimination Taqman probes (Applied Biosystems): C2403545_10 (TP53 gene), C9493064_10 (MDM4 gene) and C9688119_1 (USP7 gene). Since these two loci are closely linked, the genotype determination of polymorphisms c.-30G>A (rs2273953) and c.-20C>T (rs1801173) of TP73 gene was performed with the probe C16180357_10 determining polymorphism c.-30G>A (rs2273953), a method previously used by Hamajima and colleagues (2002) [21] and Scacchi and colleagues (2009) [51]. To determine the genotype of MDM2 gene c.14+309T>G, probes were used which were labeled with FAM-TCCCGCCGCCGCAG and VIC-CTCCGGCGCGCGAAG fluorescence and primers forward 5’-CGGGAGTTCAGGGTAAAGGT-3’ and reverse 5’-ACAGGACCTGCGATCATC-3’. The real-time PCR reactions were performed in 96 well plates in each reaction containing: 10 ng of genomic DNA, 2x MasterMix Genotyping TaqMan (Applied Biosystems), probes specific for each polymorphism (40x) and enough water to reach 8 μL. The re-
actions were conducted in the StepOnePlus™ PCR Real-Time System, with an initial cycle of 10 minutes at 95°C, followed by 45 cycles at 95°C for 15 s and 63°C for 1 minute. The reactions for c.14+309T>G MDM2 gene were also conducted in 96 well plates in each reaction containing: 10ng of genomic DNA, 2x MasterMix Genotyping TaqMan (Applied Biosystems), 1 μM of each primer and probe, and sufficient water to reach 25 μL. This reaction was also conducted in StepOnePlus™ PCR Real-Time Systems, with the initial cycle of 2 min at 50°C for 10 minutes and heating at 95°C, followed by 45 cycles at 95°C for 15 s and 60°C for 1 minute. The reaction products were analyzed on StepOne V2.2.2 Software.

2.3. Statistical analysis

Statistical analysis were performed using SPSS software, version 14.0. The chi-square was used to test the Hardy-Weinberg equilibrium, to compare allelic and genotypic frequencies, to compare the ethnicity, and the frequency of spontaneous abortions between groups. The gene-gene additive effect was also analyzed by chi-square test and logistic regression models were used to control the effect of maternal age at the time of conception. For maternal age, a dichotomous variable was used (< 35 or ≥ 35 years) due to high prevalence of women under 35 years of age at the time of conception. The ORs were used to quantify the association between the age of 35 years at the time of conception showed to be higher in case mothers (34.75 years ± 5.99, P = 0.000002) as well as the prevalence of mothers over 35 years of age in the case group (57% vs. 20%, OR = 5.31, 95% CI = 3.45–8.17, P < 0.000001) (6 missing values in control group). The case group contained 237 (90.1%) mothers classified as Euro-descendants, 17 (6.5%) as African-descent and 9 (3.4%) classified as other ethnicity. In the control group, 175 (89.7%) mothers were classified as Euro-descendants, 10 (5.15%) as African-descent and 10 (5.15%) as other ethnicity (1 missing value). The ethnic groups did not differ significantly between groups (P = 0.569). A higher frequency of spontaneous abortion was observed in the case group (21.7% vs. 9.7%, P < 0.001).

Table 1 shows the distribution of genotypes and allele frequencies of the studied polymorphisms between case and control groups, as well as in other European and euro-descendant populations. The allelic and genotypic frequencies of polymorphisms were in Hardy-Weinberg equilibrium and did not differ between case and control groups when analyzed separately, even when controlled for maternal age, ethnicity and spontaneous abortion. Our observed allele frequencies are in accordance to those described for euro-descendants.

The gene-gene additive effect was analyzed by a combination of TP53 risk allele with the risk alleles of other genes of its pathway (TP53 C and MDM2 G; TP53 C and MDM4 T; TP53 C and USP7 A; TP53 C and TP73 A/T). As shown in Table 2, the risk of having a child with DS in women with risk alleles for TP53 + MDM2 and TP53 + USP7 is 1.84 and 1.77 times higher, respectively (95% CI and P < 0.007 and 0.018), when adjusted for maternal age. We additionally tested the interaction of TP53, MDM2 and USP7 risk alleles together. The risk of having a DS child with this genotype is 2.04 higher (95% CI and P < 0.020) when controlled for maternal age. Interestingly, women under the age of 35 years at the time of conception showed a higher frequency of TP53 C allele associated with USP7 A allele (OR = 1.99; 95% CI = 1.08–3.66; P < 0.026). When applying the Bonferroni’s correction for multiple comparisons, the TP53/MDM2 additive effect maintained its statistical significance both in the unadjusted and in adjusted for maternal age model. However, the gene-gene additive effect including USP7 kept its significance only in the unadjusted analysis.

4. Discussion

Recent evidence has shown the important role played by p53 family as regulators of crucial processes related to human reproduction [14,26]. Our working hypothesis was based on two main pieces of evidence: 1) The loss of p53 function or genes that regulate this metabolic pathway may be related to the accumulation of aneuploid cells, increasing the risk of DS birth in women with these polymorphisms [55]. This same loss
of function could also decrease the action of apoptotic mechanisms that would eliminate aneuploid embryos in women with wild alleles [56]. Even if the inactivation of p53 is not the primary cause of aneuploidy, its dysfunction strongly facilitates a tolerance to this chromosomal instability [5] and, 2) Loss of p73 function due to polymorphisms in its encoding gene may interfere in the control of the meiotic spindle during oogenesis, increasing the risk of aneuploidy in the offspring [56,57]. The expression of TP73 naturally decreases with age, therefore the loss of TP73 function may contribute to the increase of aneuploidy produced by old oocytes [26,56,57]. In our sample about 43% of women were younger than 35 years at the time of the trisomic fetus conception. Thus, it becomes evident that mutations affecting the function of TP73 may be related to increased frequency of aneuploid pregnancies in young women. Supporting this hypothesis, a recent study showed that mice deficient in p73 presented spindle abnormalities, aneuploidy and little competence in fetal development [17].

The present study showed that polymorphisms in TP53, TP73, MDM2, MDM4 and USP7 genes do not represent a risk factor in the process of aneuploidy when analyzed separately, even when controlled for advanced maternal age. The distributions of allelic and genotypic frequencies found in this study are consistent with that expected for populations with European ancestry. Despite the ethnic admixture present in the Brazilian population, Southern Brazil has strong European ancestry, and the majority of our cases and controls were classified as Euro-descendants. This was confirmed by the similarity of allele frequencies observed in our sample compared to 1000 Genomes [52] and HapMap [53] databases for europeans and euro-descendants.

Taking into account that the p53 pathway studied depends on multiple protein interactions, we found that the combination of TP53 and MDM2 polymorphisms,

### Table 1
Allelic and genotypic frequencies of SNPs in the TP53 pathway

| Gene          | Genotype/Allele | Case n (%) | Control n (%) | Expected allele frequency |
|---------------|-----------------|------------|---------------|--------------------------|
| **TP53**      |                 |            |               |                          |
| (rs1042522)   | GG              | 116 (44.1) | 99 (50.5)     | 0.322                    |
|               | GC              | 123 (46.8) | 78 (39.8)     |                          |
|               | CC              | 24 (9.1)   | 19 (9.7)      |                          |
|               | G               | 355 (67.5) | 276 (70.4)    | 0.086                    |
|               | TT              | 104 (39.5) | 82 (41.9)     | 0.442                    |
|               | TG              | 123 (46.8) | 81 (41.3)     |                          |
|               | GG              | 36 (13.7)  | 33 (16.8)     |                          |
|               | T               | 331 (62.9) | 245 (62.5)    | 0.949                    |
| **MDM2**      |                 |            |               |                          |
| (rs2279744)   | CC              | 88 (33.5)  | 70 (35.7)     | 0.832                    |
|               | TT              | 104 (39.5) | 82 (41.9)     | 0.086                    |
|               | CG              | 123 (47.1) | 87 (44.4)     |                          |
| **MDM4**      |                 |            |               |                          |
| (rs1563828)   | CC              | 88 (33.5)  | 70 (35.7)     | 0.832                    |
|               | TT              | 104 (39.5) | 82 (41.9)     | 0.086                    |
|               | CT              | 124 (47.1) | 87 (44.4)     |                          |
| **USP7**      |                 |            |               |                          |
| (rs1529916)   | CC              | 88 (33.5)  | 70 (35.7)     | 0.832                    |
|               | TT              | 104 (39.5) | 82 (41.9)     | 0.086                    |
|               | CT              | 124 (47.1) | 87 (44.4)     |                          |
| **TP73**      |                 |            |               |                          |
| (rs2273953 and rs1801173) | CC/CT | 161 (61.2) | 109 (55.6)    | 0.381                    |
|               | GA/CT           | 87 (33.1)  | 71 (36.2)     |                          |
|               | AA/TT           | 15 (5.7)   | 16 (8.2)      |                          |

*Chi-square; **Data from european and euro-descendants populations; NA = not available.

### Table 2
Risk allele combinations in the TP53 pathway

| Alleles† | Case n (%) | Control n (%) | P** | OR (IC 95%) | P** | OR (IC 95%) |
|----------|------------|---------------|-----|-------------|-----|-------------|
| TP53 + MDM2G | 94 (35.7) | 46 (33.2) | 0.006 | 1.81 (1.17–2.81) | 0.007 | 1.84 (1.18–2.89) |
| TP53 + MDM4T | 96 (36.5) | 65 (35.3) | 0.521 | 1.16 (0.77–1.74) | 0.536 | 1.14 (0.75–1.74) |
| TP53 + USP7A | 80 (30.4) | 37 (18.9) | 0.007 | 1.88 (1.18–3.00) | 0.018 | 1.77 (1.10–2.85) |
| TP53 + TP73A/T | 58 (22.0) | 43 (21.9) | 0.933 | 1.01 (0.63–1.64) | 0.760 | 1.08 (0.66–1.75) |
| TP53 C + MDM2G + USP7A | 51 (19.4) | 19 (9.7) | 0.006 | 2.24 (1.24–4.10) | 0.020 | 2.04 (1.12–3.71) |

†Includes the presence of the allele in homozygosis or heterozygosis.
*Chi-square (Yates correction); **P and OR adjusted for maternal age by logistic regression.
and possibly TP53 and USP7 contributes to this increased risk. The effect of TP53 C allele associated with USP7 A allele is probably maternal age independent as women under the age of 35 years at the time of conception showed a higher frequency of TP53 C allele associated with USP7 A allele. These results indicate a synergistic effect between genes that act in the same pathway in a multifactorial way. The allele P72 reduces the efficiency of p53 to induce apoptosis. Acting in the same signaling pathway, the G allele increases the expression of MDM2, degrading more p53 and negatively influencing the induction of apoptosis in these cells [16]. Some studies showed that a large amount of p53 protein is produced by the human placenta in abnormal pregnancies. It is suggested that p53 is an important factor in the pathogenesis of diseases through the induction of trophoblast apoptosis [19,24, 37,48]. Thus, the interaction of these polymorphisms could decrease the levels of the pro-apoptotic p53 protein, making it less functional in response to cell damage. As a consequence, the reaction would be attenuated by trophoblastic apoptosis and promote greater tolerance of aneuploidy.

Although these polymorphisms have never been investigated as possible risk factors for aneuploidy in humans, some studies showed an association between SNPs of p53 pathway and fertility, suggesting a specific role of p53 in the regulation of human reproduction. Pietrowski and colleagues (2005) [46] reported an association of the P72 allele and recurrent miscarriages. However, Fang and colleagues (2011) [16] did not find any difference in genotype and allele frequency of TP53 P72 and R72 forms through a case-control study involving women with miscarriages. They also reported that women with TP53 P72/P72 genotype and MDM2 G/G have a significantly higher expression of the MDM2 protein, which may attenuate the response of apoptosis after DNA damage. The genes MDM2, MDM4 and USP7, which produce proteins that regulate p53 level, had their minor alleles enriched in women seeking clinics for in vitro fertilization for the same SNPs studied [29,36].

The R72 and P72 forms of TP53 have different biological effects: R72 is more efficient in inducing apoptosis while P72 can promote a G1 arrest. This polymorphism seems to increase the chance of miscarriage in healthy women [46]. Thus, it is possible that genes that undergo selection in the TP53 pathway may affect human reproduction [29]. The G allele of MDM2 c.14+309T>G SNP is associated with a high risk of spontaneous abortion [16], which supports the combined effect between this MDM2 polymorphism and R72P of p53 [7,58]. The MDM4 gene, that is structurally homologous with the MDM2 gene, is also involved in the regulation of p53. The MDM4 protein indirectly affects p53, modulating its levels as well as MDM2 activity [42], not only stimulating the ubiquitination of p53 mediated by MDM2, but also the self-ubiquitination of MDM2 [39]. The transcriptional activation of TP53 by MDM4 can be inhibited regardless of MDM2 [18], by binding to the TP53 transactivation domain, that contributes to the total inhibition of p53 [41]. The T allele of this polymorphism is associated with fertility in women, suggesting that MDM4 can regulate reproduction in a dependent and independent TP53 pathways, and may also interact with TP63 and TP73 [29]. Another important regulator of the p53 signaling pathway, the USP7 gene, acts in the stabilization of MDM2, MDM4 and p53 by deubiquitinating these proteins [10,25,38]. Other studies also reported an association of TP73 polymorphisms with increased risk for Alzheimer’s disease, leukoplakia and several types of cancer [40,43,51].

There are no functional studies regarding the polymorphism c.2719-234G>A of the USP7 gene. Kang and colleagues [29] found an association of the mutated A allele with infertility, showing the impact of this SNP on human fertility through the attenuation of the p53 pathway. As in this work, we also found a higher frequency of this allele in women younger than 35 years. The MDM2, MDM4 and USP7 proteins maintain the levels and activity of p53 that are critical to an appropriate transcriptional response signal after cell stress [29].

In humans, the p53 gene family appears to play other important roles in reproduction: TP53 is involved in the regulation of the blastocyst and TP73 regulates the integrity of germ line cells [17]. Some evidence supports the involvement of p53 as functional in germline cells. Studies in C. elegans showed similar functions of that analogous to the p53 protein: cep-1 (C. elegans p53-like-1). During the development of germ cells cep-1 ensures correct meiotic segregation [13]. In addition, the expression of CEP-1 at the end of pachytene, associated with the establishment of apoptotic competence, ensures that the germline cells with DNA damage or defects in meiotic recombination are eliminated before oogenesis. This process ensures that only healthy germ cells advance to the next generation [50]. Additionally, cks2 proteins, that are essential components of cyclins complexes, and are involved in cell cycle control, have their expression negatively controlled by p53 at
transcriptional and proteic level. This regulation contributes to control the transition from metaphase I to anaphase I in mammals’ meiosis [49].

Despite the well established association between DS and advanced maternal age, the biochemical and molecular basis of nondisjunction are still not well understood. Altered patterns of recombination are known risks for nondisjunction [33,34]. More recently, Oliver and colleagues [44] have added support to the multifactorial etiology of nondisjunction in human meiosis. Their data suggested that pericentromeric chromatid exchanges during meiosis in females interact with maternal age-related risk factors, altering the susceptibility to nondisjunction. One year later, Gosh and colleagues [20] tested this hypothesis in an independent and ethnically different population in India and their results were consistent with those of Oliver and colleagues [44]. In our study we searched for different susceptibility factors that could predispose to aneuploidy, and we looked also for a possible interaction with advanced maternal age. However, a logistic regression analysis considering maternal age failed to show an interaction between p53 pathway, maternal age and aneuploidy.

In this article we presented recent evidence linking the role of the TP53 family and its regulators in the maintenance spindle stability during meiosis and embryonic development. All evidence suggests that the lack of studies investigating the relationship of these polymorphisms with the genesis of aneuploidy in humans, this work is the first to establish a relationship between polymorphisms in the TP53 gene family and its regulatory pathway as a risk factor for aneuploidy of 21. Future studies in other populations should be conducted to confirm our findings, especially to clarify the factors independent of maternal age which may be involved in the development of aneuploid cells.

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### Supplementary material

#### Table S1
Allelic and genotypic frequencies of SNPs in the TP53 pathway in euro-descendants

| Gene | Genotype/Allele | Case n (%) | Control n (%) | \(P^\ast\) | Expected allele frequency** |
|------|----------------|------------|---------------|---------|-----------------------------|
|      |                |            |               |         | 1000genomes [52] | HapMap [53] |
| TP53 (rs1042522) | GG | 106 (44.7) | 92 (52.6) | 0.164 |              |
|      | GC | 113 (47.7) | 67 (38.3) |              |
|      | CC | 18 (7.6) | 16 (9.1) |              |
|      | G  | 325 (68.6) | 251 (71.7) | 0.369 | 78.9 | 76.7 |
| MDM2 (rs2279744) | TT | 96 (40.5) | 75 (42.9) | 0.666 |              |
|      | TG | 109 (46.0) | 73 (41.7) |              |
|      | GG | 32 (13.5) | 27 (15.4) |              |
|      | T  | 301 (63.5) | 223 (63.7) | 0.991 | 64.0 | NA |
| MDM4 (rs1563828) | CC | 83 (35.0) | 63 (36.0) | 0.978 |              |
|      | CT | 111 (46.8) | 81 (42.3) |              |
|      | TT | 43 (18.2) | 31 (17.7) |              |
|      | C  | 277 (58.4) | 207 (59.1) | 0.895 | 67.9 | 65.0 |
| USP7 (rs1529916) | GG | 111 (46.8) | 87 (49.7) | 0.482 |              |
|      | GA | 111 (46.8) | 73 (41.7) |              |
|      | AA | 15 (6.4) | 15 (8.6) |              |
|      | G  | 333 (70.3) | 247 (70.6) | 0.982 | 67.3 | 68.9 |
| TP73 (rs2273953 and rs1801173) | CC | 149 (62.9) | 98 (56.0) | 0.269 |              |
|      | CT | 76 (32.0) | 63 (36.0) |              |
|      | AA/TT | 12 (5.1) | 14 (8.0) |              |
|      | G/C | 374 (78.9) | 259 (74.0) | 0.117 | 79.9 | NA |

*Chi-square; **Data from european and euro-descendants populations. NA = not available.

#### Table S2
Risk allele combinations in the TP53 pathway showing data for euro-descendants only

| Alleles† | Case n (%) | Control n (%) | \(P^\ast\) | OR (IC 95%)** | P** | OR (IC 95%)** |
|----------|------------|---------------|---------|-------------|------|-------------|
| TP53C + MDM2G | 82 (34.6) | 38 (21.7) | 0.006 | 1.91 (1.19–3.06) | 0.009 | 1.90 (1.17–3.06) |
| TP53C + MDM4T | 82 (34.6) | 55 (31.4) | 0.569 | 1.15 (0.75–1.79) | 0.551 | 1.14 (0.73–1.79) |
| TP53C + USP7A | 72 (30.4) | 32 (18.3) | 0.007 | 1.95 (1.19–3.22) | 0.016 | 1.85 (1.12–3.06) |
| TP53C + TP73A/T | 50 (21.1) | 34 (19.4) | 0.770 | 1.11 (0.66–1.86) | 0.502 | 1.19 (0.71–2.02) |
| TP53 C + MDM2G + USP7A | 45 (19.0) | 15 (8.6) | 0.005 | 2.50 (1.29–4.88) | 0.016 | 2.23 (1.16–4.60) |

†Includes the presence of the allele in homozygosis or heterozygosis. *Chi-square (Yates correction). **P and OR adjusted for maternal age by logistic regression.