Published in final edited form as:

Am J Med Genet A. 2008 October 15; 146A(20): 2617–2625. doi:10.1002/ajmg.a.32504.

Construction of a High Resolution Linkage Disequilibrium Map to Evaluate Common Genetic Variation in *TP53* and Neural Tube Defect Risk in an Irish Population

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Abstract

Genetic and environmental factors contribute to the etiology of neural tube defects (NTDs). While periconceptional folic acid supplementation is known to significantly reduce the risk of NTDs, folate metabolic pathway related factors do not account for all NTDs. Evidence from mouse models indicates that the tumor protein p53 (*TP53*) is involved in implantation and normal neural tube development. To determine whether genetic variation in the *TP53* might contribute to NTD risk in humans, we constructed a high resolution linkage disequilibrium (LD) map of the *TP53* genomic region based on genotyping 21 markers in an Irish population. We found that nine of these variants can be used to capture the majority of common variation in the *TP53* genomic region. In contrast, the 3-marker haplotype commonly reported in the *TP53* literature offers limited coverage of the variation in the gene. We used the expanded set of polymorphisms to measure the influence of *TP53* on NTDs using both case-control and family-based tests of association. We also assayed a functional variant in the p53 regulator *MDM2* (rs2279744). Alleles of three noncoding *TP53* markers were associated with NTD risk. A case effect was seen with the GG genotype of rs1625895 in intron 6 (OR = 1.37 [1.04-1.79], p=0.02). A maternal effect was seen with the 135/135 genotype of the intron 1 VNTR (OR = 1.86 [1.16-2.96], p=0.01) and the TT genotype of rs1614984 (RR = 0.58 [0.37-0.91], p=0.02). As multiple comparisons were made, these cannot be considered definitive positive findings and additional investigation is required.

Keywords

neural tube defects; spina bifida; p53; *TP53*; *MDM2*; linkage disequilibrium

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Introduction

Neural tube defects (NTDs) are one of the most common birth defects, with a prevalence of ~1 in 1000 live births in the US and Europe [1991a; Busby et al., 2005]. The neural tube is the precursor for the spinal cord and brain. Closure defects occurring during the fourth week post-fertilization can result in a range of NTD phenotypes. Spina bifida and anencephaly account for the majority of NTD cases, which can also include encephalocele, craniorachischisis and iniencephaly [Botto et al., 1999].

NTDs have complex origins that include environmental and genetic factors. A low level of maternal folate is a well-established risk factor [Daly et al., 1995], and periconceptional folic acid supplementation has been observed to reduce the risk of an NTD pregnancy by up to 70% [1991b; Czeizel and Dudas, 1992]. Variants of genes in the folate metabolic pathway have also been shown to act as risk factors for NTDs [Botto and Yang, 2000; Brody et al., 2002; De Marco et al., 2006; Parle-McDermott et al., 2006; Whitehead et al., 1995]. Other risk factors are known to exist, such as maternal diabetes [Becerra et al., 1990; Elwood et al., 1992], maternal use of the antiepileptic drug valproic acid [Lammer et al., 1987] and maternal obesity [Shaw et al., 1996; Waller et al., 1994; Watkins et al., 1996; Werler et al., 1996].

Mutated in approximately half of human cancers [Biggs et al., 1993; Greenblatt et al., 1994; Levine et al., 1994], the tumor protein p53 (*TP53*) gene is primarily known for its role in preventing tumorigenesis. As a cell cycle checkpoint protein, p53 is activated to achieve G1 growth arrest or apoptosis in response to DNA damage, aberrant growth signals, and other environmental insults [Vogelstein et al., 2000]. If compromise of p53 can lead to unchecked cellular differentiation, variant p53 function may adversely affect the cellular proliferation and apoptosis that occurs during development. Recently, Hu and colleagues [2007] reported that p53 regulates transcription of leukemia inhibitory factor (*LIF*). LIF is a maternally derived, autocrine/paracrine growth factor whose expression induces changes in the endometrial microenvironment to support blastocyst implantation [Cullinan et al., 1996]. LIF levels and implantation were reduced in pregnant p53^{-/-} mice, although treatment with LIF could restore implantation [Hu et al., 2007].

In addition to playing a role in producing a uterine environment that will support a successful pregnancy, p53 is important for embryonic development. Depending on strain background, 8-16% of female p53^{-/-} mice exhibited exencephaly [Armstrong et al., 1995; Patterson et al., 2006; Sah et al., 1995].

Mutation of *TP53* can modulate the NTD phenotypes of other mouse models. PAX3 is a family member of the paired box genes, which are developmental transcription factors. *Splootch* mice homozygous for a loss-of-function Pax3 allele develop exencephaly and/or spina bifida [Auerbach, 1954; Epstein et al., 1991]. Loss of p53 rescues this NTD phenotype [Pani et al., 2002], indicating a cooperative role for these genes in normal neural tube closure. Loss of p53 can also exacerbate a NTD phenotype. Gadd45a (growth arrest and DNA-damage-inducible protein alpha) is one of the downstream effectors induced by p53 to achieve a G2-M arrest. Similar to mice deleted for *TP53*, ~10% of mice deleted for Gadd45a are exencephalic [Hollander et al., 1999]. Gadd45a and *TP53* double knockouts exhibit increased levels of exencephaly [Patterson et al., 2006].

Evidence from these murine models suggests that p53 may play a role in the development of human NTDs. There is only a single report evaluating the connection between NTDs and p53. In this study, an unidentified intragenic multiallelic marker in *TP53* was not found to be associated with development of NTDs in a sample of 78 American Caucasian families [Melvin et al., 2000].

To more thoroughly evaluate whether genetic variation in p53 might contribute to NTD risk, we first created a high-resolution linkage disequilibrium (LD) map of 21 *TP53* markers. This map was used to identify a minimal set of polymorphisms to capture the majority of genetic variation in *TP53*. Our study design allowed employment of case-control and family-based association tests. Although several tests were suggestive of association, the candidate *TP53* polymorphisms tested in this study do not seem to be major contributors to NTD risk.

Materials and Methods

NTD Cases and Controls

NTD triads consisting of an NTD case and his/her parents (referred to as “NTD cases” [n=549], “NTD mothers” [n=532] and “NTD fathers” [n=481]) were recruited in the Republic of Ireland from 1993 to 2004 with the help of the Irish Association for Spina Bifida and Hydrocephalus and the Public Health Nurses. Ascertainment of controls has been previously described [Kirke et al., 1993]. Briefly, a bank of 56,049 blood samples was obtained from women recruited at their first prenatal visit at the three main maternity hospitals in Dublin from 1986 to 1990. Control samples (n=999) for this study were randomly drawn from the women in this collection, after exclusion of women who had given birth to a child with NTD or other known malformation, and exclusion of women whose past pregnancies were affected by NTDs. Informed consent and ethical approval were obtained for the use of these samples.

African American and Caucasian American Controls

Publicly available panels of 100 African American control DNAs (HD100AA) and 100 Caucasian control DNAs (HD100CAU) were obtained from the Coriell Cell Repositories (Camden, NJ).

DNA Isolation

Genomic DNA for all Irish samples was extracted from frozen blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, UK) according to the manufacturer's directions.

Genotyping

The intron 1 VNTR and the intron 3 16bp indel were genotyped by PCR amplification of a region encompassing the length polymorphism followed by fragment detection by an ABI 3100 Genetic Analyzer (Applied Biosystems). All other single nucleotide polymorphisms (SNPs) were genotyped by Matrix Assisted Laser Desorption/Ionization – Time-of-Flight (MALDI-TOF) mass spectrometry (Sequenom) of allele-specific extension products (primers sequences and assay conditions available upon request).

Genotyping consistency was determined by repeating a subset of samples. For the 112 control samples used to generate the high resolution LD map in the Irish population, ~5% of samples were repeated and 100% of the resulting genotypes were concordant for all 21 markers. The average genotyping call rate for these markers was 95.5% (87.5%-100%). For the 100 African American samples and 100 Caucasian American samples used to generate the LD maps of p53, ~10% of samples were repeated with 100% concordance for all 9 markers. The average genotyping call rate for these markers was 99.0% (95.0-100%) in the African American samples and 97.0% (92.0-100%) in the Caucasian American samples. For the 11 markers selected for NTD association analysis in the NTD family members and controls, at least 10% of samples were repeated with 94-99% concordance (average concordance of 96%). The average genotyping call rate for these markers was 96.7% (94.5%-99.1%).

The controls were in Hardy-Weinberg equilibrium for all tested variants ($p > 0.05$). Some NTD family member groups were observed to deviate from Hardy-Weinberg equilibrium: *DNAH2* rs3744258 cases ($\chi^2=4.57$, $p=0.03$), *TP53* rs8064946 fathers ($\chi^2=4.15$, $p=0.04$), *TP53* intron 1 VNTR mothers ($\chi^2=10.02$, $p=0.007$), *TP53* rs1642785 mothers ($\chi^2=7.23$, $p=0.01$), *TP53* rs1042522 R72P mothers ($\chi^2=8.97$, $p=0.003$), and *TP53* rs6503048 cases ($\chi^2=4.48$, $p=0.03$).

All discordant genotypes were excluded from analysis, as were the genotypes of NTD triads exhibiting non-Mendelian inheritance for any single marker. Samples with discrepancies for more than one marker (4.5%) were excluded from all analyses.

Statistical Methods

Single variant analysis—A log-linear model allowing for direct maternal effects along with case genetic effects was used to test for association for each polymorphism. The model allows for a separate relative multiplicative risk due to one or two copies of the index allele in the case, compared to no copies of the index allele. Similarly, the model allows for a separate relative multiplicative risk due to one or two maternal copies of the index allele, compared to no maternal copies of the index allele. The model for each polymorphism was fit by maximizing the likelihood of observed triads given the parental genes, using SAS PROC GENMOD [see [Weinberg et al., 1998] for how to implement such a model]. The hypothesis of no association for case genotype was tested using a two degree of freedom likelihood ratio test. A similar two degree of freedom test was used to test for no association for maternal genotype. Following a significant result of the two degree of freedom test, the hypotheses that one or two copies of the index allele is not associated with NTD were tested using a one degree of freedom likelihood ratio test. Bonferroni correction was used to adjust for multiple comparisons.

Using the same sample set, each polymorphism was also evaluated by comparing the genotypes of cases and controls or mothers and controls. Initially, we tested for a general association between case-control or mother-control status and genotype, using the Fisher's exact test for 2×3 contingency table. Following a significant result, sub-tables based on collapsing columns of the 2×3 table were tested using the chi-squared test for 2×2 tables in order to test dominant and recessive models of genetic risk.

Haplotype analysis—The structure of LD in the region was determined using Haploview (<http://www.broad.mit.edu/mpg/haploview/>, [Barrett et al., 2005]). Haplotype blocks were defined within Haploview based on D' estimates using the Solid Spine of LD option. Using PHASE 2.1 [Stephens and Donnelly, 2003; Stephens et al., 2001], haplotype frequency estimates based on these block definitions were then generated for NTD cases, NTD mothers and controls. Additionally, a permutation test within PHASE 2.1 was performed to ask whether haplotype frequencies differed between controls and NTD cases or NTD mothers.

Results

A High-Resolution LD Map of *TP53* Variants in the Irish Population

In order to characterize the genetic variation in *TP53* in the Irish population, we constructed a high-resolution LD map of the gene. Previously published markers and coding SNPs were selected based on the likelihood they may affect function. Additional markers were chosen to cover the gene and flanking regions. Previously reported *TP53* markers used in genetic association studies include an intron 1 VNTR [Hahn et al., 1993], as well as a three-marker haplotype featuring a 16 bp insertion/deletion in intron 3 (rs17878362), a coding

polymorphism in exon 4 (R72P, rs1042522) and a SNP in intron 6 (rs1625895) [Sjalander et al., 1995]. Another 32 *TP53* markers were selected from dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP/>), including 9 coding SNPs. Fourteen SNPs found to be monomorphic in >200 Irish individuals were dropped from the study (rs1800369 D21D, rs11575998 P34P, rs1800370 P36P, rs1800371 P47S, rs11540654 L110R, rs1800372 R213R, rs11575996 Q331Q, rs11540652 Q248R, rs8073436, rs1642791, rs1642783, rs858528, rs9903378, rs3021069). The remaining 21 biallelic polymorphisms were genotyped in 112 Irish controls. Haploview was used to estimate D' and r^2 measures of LD between these markers and construct an LD map (Figure 1a).

D' estimates of LD reveal two major haplotype blocks in the *TP53* gene. One very common haplotype (~90% of chromosomes) exists with a less common haplotype in the block spanning the 5' end of the gene through a large part of the first intron. Similarly, there is a single common haplotype (~70% of chromosomes) and three less common haplotypes in the second block spanning exon 4 through the penultimate intron.

Using pairwise r^2 values, we selected a reduced set of nine markers to capture the majority of the genetic variation in *p53* and include the much-studied three-marker haplotype [Sjalander et al., 1995]. The r^2 threshold was set at 0.8, so that all 21 markers show at least 80% genotype correlation with at least one of the nine markers chosen for NTD association analysis. An LD plot of these nine markers in the 112 Irish control samples reveals significant reduction in the number of markers in the haplotype blocks (Figure 1b). This set of nine markers was also typed in American Caucasian (n=100) and African American (n=100) samples (Figures 1c, 1d). Patterns of LD are very similar in the Irish population and American Caucasian populations. In comparison, the African American population exhibits less strong LD, especially in the 5' end of *p53*, consistent with an older population exhibiting relatively higher levels of genetic variability.

Evaluation of Individual Markers for Association with NTDs

In addition to the selected set of nine *TP53* markers, we also tested whether the *TP53* intron 1 VNTR influences NTD risk in the Irish population. Finally, we included a functional SNP (rs2279744) in *MDM2*, a direct negative regulator of *p53*. This SNP alters *MDM2* expression, which affects *p53* activity [Bond et al., 2004].

Allele and genotype frequencies are shown in Table I. Log-linear analysis was performed to detect a case or maternal genotype effect. Of the eleven tested markers, only the 135 allele of the intron 1 VNTR was associated with a case risk ($p=0.02$) but the subsequent one degree of freedom test was not significant for the 135/135 genotype (RR = 1.46 [0.79-2.70], $p=0.22$). Only rs1614984 was associated with a maternal NTD risk ($p = 0.04$) and the subsequent one degree of freedom test revealed a significant effect for the TT genotype (RR = 0.58 [0.37-0.91], $p=0.02$).

Case-control analysis was also performed for each marker. An initial χ^2 test of genotype distributions was used to compare NTD cases vs. controls or NTD mothers vs. controls. The only SNP exhibiting a case effect was rs1625895 in intron 6 ($p=0.03$), which also showed a significant effect for the GG genotype (OR = 1.37 [1.04-1.79], $p=0.02$). The only variant showing a maternal effect was the 135 allele of the intron 1 VNTR ($p=0.02$), which also showed a significant effect for the 135/135 genotype (OR = 1.86 [1.16-2.96], $p=0.01$).

Haplotype Analysis

Based on the solid spine of LD block definition in Haploview [Barrett et al., 2005], two haplotype blocks were identified in our set of nine *TP53* markers (Fig 1B). The first haplotype block consists of rs8064946 and rs2078486; the second haplotype block consists

of rs1642785, rs17878362, rs1042522 (R72P), rs1625895 and rs6503048. PHASE 2.1 was used to estimate haplotype frequencies and perform NTD case-control and NTD mother-control comparisons of haplotype frequencies within the blocks. No significant differences were detected for any of the candidate haplotype frequencies using a permutation test to compare NTD cases or NTD mothers with controls (Table II).

Discussion

Ours is the first study to evaluate whether common genetic variation in *TP53* contributes to NTD risk. Unlike most other NTD genetic association studies, ours evaluated a gene not directly involved in folate metabolism. Rather, *TP53* is of interest for its role in regulating cell cycle growth arrest and apoptosis. In particular, inhibition of p53 rescues the apoptosis and NTDs seen in Pax3-deficient mice [Pani et al., 2002], implying p53-dependent apoptosis can contribute to NTDs. Additionally, as a transcription factor and cell cycle checkpoint protein, expression of p53 influences blastocyst implantation and cell growth and proliferation during neural tube closure. This is supported by the role p53 plays in establishing a maternal microenvironment conducive to blastocyst implantation and by the observation that *TP53* $-/-$ mice exhibit exencephaly. Additionally, specific *TP53* genotypes modify the phenotype of NTDs found in mice in which Pax3 or Gadd45a have been deleted [Pani et al., 2002; Patterson et al., 2006].

This study includes one of the most comprehensive evaluations of genetic variation of the *TP53* gene to date. We created a high resolution LD map by genotyping a number of individuals for 21 variants in a region containing the *TP53* gene. We used these data to reduce the number of SNPs tested to a maximally informative candidate set of nine markers. These nine markers were chosen to “LD tag” the *TP53* locus and included the previously reported three-marker haplotype [Sjalander et al., 1995]. One of these SNPs results in an amino acid substitution. This SNP, R72P (rs1042522), was the only one of nine previously reported coding polymorphisms that we found to be polymorphic in the Irish population. This coding SNP has been evaluated for influence on the risk, onset and prognosis of many types of cancer [Pietsch et al., 2006]. As part of the three-marker haplotype, we also evaluated the intron 3 16bp indel (rs17878362), which has been associated with changes in steady-state RNA levels [Gemignani et al., 2004], DNA repair capacity and apoptosis [Wu et al., 2002] and many types of cancer risk, such as colon cancer [Gemignani et al., 2004], lung cancer [Wu et al., 2002], breast cancer [Wang-Gohrke et al., 1999; Weston and Godbold, 1997], and ovarian cancer [Runnebaum et al., 1995; Wang-Gohrke et al., 1999].

By mapping the linkage disequilibrium in this region we created a set of nine SNPs that capture the majority of common variation present in the *TP53* gene. Previously reported haplotype analyses of *TP53* involved a three-variant haplotype consisting of the intron 3 16bp indel (rs17878362), the exon 4 coding SNP R72P (rs1042522) and an intron 6 SNP (rs1625895). This haplotype has been evaluated for many phenotypes, including lung cancer [Birgander et al., 1995; Wu et al., 2002], colorectal cancer [Sjalander et al., 1995], nasopharyngeal cancer [Birgander et al., 1996], breast cancer [Sjalander et al., 1996; Weston et al., 1997], cervical cancer [Mittra et al., 2005], and oral squamous cell carcinoma [Hsieh et al., 2005].

According to our analysis of the Irish population, this three-marker haplotype contributes to variation within a single D' block. Additional polymorphisms flanking this haplotype represent additional common variation in this gene not covered by these markers. This includes regions of *TP53* that need to be considered when performing association studies. We advocate using a more complete set of markers as described in this study. The improved coverage obtained by using additional markers was demonstrated recently in two studies

from the NCI core genotyping group [Garcia-Closas et al., 2007; Mechanic et al., 2007]. Mechanic and colleagues described an association between a *TP53* haplotype and lung cancer that would not have been captured by the standard three-marker haplotype.

In this study, we evaluated ten *TP53* polymorphisms and one *MDM2* SNP in a large sample of Irish NTD cases, their parents and controls. We observed associations between three *TP53* noncoding variants and NTD risk. One SNP, rs1625895, was associated with the risk of being an NTD case. This SNP is located in intron 6, and is part of the historically tested three-marker haplotype. An increased risk of having a child with an NTD was observed in mothers carrying specific alleles of the intron 1 VNTR and the SNP rs1614984. There is no known functional consequence for these noncoding variants. Only rs1614984, located several hundred bases downstream from *TP53*, seems to be in a region that is well-conserved in mammals at the nucleotide level. None of these variants are predicted to alter splicing or transcription factor binding sites, or be targets of known regulatory miRNAs. Although these variants were associated with NTDs in this study, it also remains possible that these are not the causal variants but instead are linked to the functional variants responsible for NTD risk. In contrast, rs2279744 in the p53 inhibitor *MDM2* was a candidate variant of great interest since it has functional consequence; it alters an SP1 binding site, resulting in increased *MDM2* mRNA and protein levels which in turn further inhibits p53 activity [Bond et al., 2004]. Although it is the only variant evaluated in this study with molecular evidence of affecting p53 function, *MDM2* rs2279744 was not found to be associated with NTD risk.

Though statistically significant in univariate tests, the associations observed with the intron 1 VNTR, rs1625895, and rs1614984 do not remain significant after correcting the p-values for multiple hypothesis testing. While these variants can not be considered definitive NTD risk factors, the results of this study make them excellent candidates for future NTD studies which could avoid the penalty of performing multiple tests by focusing exclusively on these variants. For example, an independent study looking solely at rs1614984 in a Caucasian population with 700 NTD mothers and 700 controls would have 80% power to detect an effect similar to the one observed in this study.

In conclusion, it would be of great interest if these SNPs were to show significant association with NTD risk in an independent cohort. Additionally, the set of nine *TP53* variants described here can be used in other studies where variation in *TP53* may influence the phenotype in question.

Acknowledgments

These studies would not be possible without the participation of the affected families, and their recruitment by the Irish Association of Spina Bifida and Hydrocephalus and the Irish Public Health Nurses. The authors acknowledge research support from the intramural research programs of the National Human Genome Research Institute, the National Institute of Child Health and Human Development and the Health Research Board, Ireland. FP was supported by a Pharmacology Research Associate Fellowship from the National Institute of General Medical Sciences, National Institutes of Health.

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Figure 1.

LD maps of the *TP53* locus. Pairwise estimates of D' were generated and graphically displayed using Haploview. Figure 1a. A high resolution map of the *TP53* locus in an Irish population. Gene structure of *TP53* is shown (~17kb, transcription begins from the left). Relative distances of markers are shown with the exception of the double hatchmarks between the first and last marker pairs (~30kb and ~25kb, respectively). Gene names are included in the marker name if the variant falls in a gene. Gabriel block definitions are shown. Selected tagSNPs are boxed. Figures 1b-d. LD (D') maps of the nine marker tagSNP set in b) Irish, c) Caucasian and d) African American populations. Blocks were defined by a solid spine of LD.

Figure 1. LD maps of *TP53* in Irish, American Caucasian and African American populations.

Table I

Genotype Distributions and Allele Frequencies in Irish Controls and NTD Triads*

Marker	Controls	NTD Children	NTD Mothers	NTD Fathers
Genotypes	N (freq.)	N (freq.)	N (freq.)	N (freq.)
Alleles	Freq.	Freq.	Freq.	Freq.
DNAH2 rs3744258				
TT	381 (0.39)	193 (0.40)	180 (0.39)	161 (0.38)
TC	458 (0.47)	205 (0.43)	221 (0.47)	206 (0.49)
CC	128 (0.13)	82 (0.17)	65 (0.14)	57 (0.13)
T	0.63	0.62	0.62	0.62
C	0.37	0.38	0.38	0.38
TP53 rs8064946				
GG	796 (0.80)	398 (0.82)	374 (0.79)	349 (0.79)
GC	189 (0.19)	83 (0.17)	94 (0.20)	80 (0.18)
CC	5 (0.01)	6 (0.01)	8 (0.02)	10 (0.02)
G	0.90	0.90	0.88	0.89
C	0.10	0.10	0.12	0.11
TP53 rs2078486				
GG	839 (0.86)	425 (0.87)	405 (0.85)	364 (0.84)
GA	133 (0.14)	64 (0.13)	70 (0.15)	64 (0.15)
AA	3 (0.00)	1 (0.00)	4 (0.01)	3 (0.01)
G	0.93	0.93	0.92	0.92
A	0.07	0.07	0.08	0.08
TP53 intron 1 VNTR				
125/125	16 (0.02)	8 (0.02)	13 (0.03)	11 (0.03)
125/130	134 (0.14)	67 (0.14)	73 (0.16)	57 (0.13)
125/135	63 (0.07)	29 (0.06)	23 (0.05)	28 (0.07)
125/other	12 (0.01)	9 (0.02)	10 (0.02)	9 (0.02)
130/130	350 (0.36)	157 (0.32)	163 (0.35)	144 (0.34)
130/135	238 (0.25)	136 (0.28)	109 (0.23)	111 (0.26)
130/other	74 (0.08)	37 (0.08)	31 (0.07)	25 (0.06)
135/135 ^a	40 (0.04)	28 (0.06)	35 (0.07)	28 (0.07)
135/other	34 (0.04)	15 (0.03)	10 (0.02)	14 (0.03)
other/other	2 (0.00)	1 (0.00)	2 (0.00)	1 (0.00)
125	0.13	0.12	0.14	0.14
130	0.60	0.57	0.57	0.56
135	0.22	0.24	0.23	0.24
other	0.06	0.06	0.06	0.06
TP53 rs1642785				

Marker	Controls	NTD Children	NTD Mothers	NTD Fathers
GG	522 (0.55)	292 (0.60)	258 (0.54)	240 (0.56)
GC	374 (0.40)	169 (0.35)	198 (0.42)	161 (0.38)
CC	50 (0.05)	28 (0.06)	18 (0.04)	27 (0.06)
G	0.75	0.77	0.75	0.75
C	0.25	0.23	0.25	0.25
TP53 rs17878362 (indel)				
del del	731 (0.75)	378 (0.78)	369 (0.77)	325 (0.75)
del ins	224 (0.23)	100 (0.21)	106 (0.22)	98 (0.23)
ins ins	14 (0.01)	7 (0.01)	4 (0.01)	8 (0.02)
del	0.87	0.88	0.88	0.87
ins	0.13	0.12	0.12	0.13
TP53 rs1042522 R72P				
GG	539 (0.55)	294 (0.60)	261 (0.54)	235 (0.54)
GC	385 (0.39)	170 (0.34)	203 (0.42)	170 (0.39)
CC	58 (0.06)	29 (0.06)	17 (0.04)	27 (0.06)
G	0.74	0.77	0.75	0.74
C	0.26	0.23	0.25	0.26
TP53 rs1625895				
GG ^b	756 (0.77)	401 (0.82)	386 (0.81)	352 (0.80)
GA	222 (0.22)	83 (0.17)	90 (0.19)	81 (0.18)
AA	10 (0.01)	7 (0.01)	3 (0.01)	6 (0.01)
G	0.88	0.90	0.90	0.89
A	0.12	0.10	0.10	0.11
TP53 rs6503048				
CC	832 (0.85)	417 (0.86)	398 (0.86)	370 (0.86)
CT	138 (0.14)	61 (0.13)	64 (0.14)	54 (0.13)
TT	7 (0.01)	6 (0.01)	2 (0.00)	4 (0.01)
C	0.92	0.92	0.93	0.93
T	0.08	0.08	0.07	0.07
TP53 rs1614984				
CC	311 (0.32)	166 (0.34)	149 (0.32)	139 (0.33)
CT	478 (0.49)	234 (0.48)	248 (0.53)	200 (0.47)
TT ^c	192 (0.20)	87 (0.18)	73 (0.16)	84 (0.20)
C	0.56	0.58	0.58	0.57
T	0.44	0.42	0.42	0.43
MDM2 rs2279744				
TT	423 (0.43)	209 (0.43)	202 (0.43)	191 (0.44)
TG	439 (0.45)	235 (0.48)	221 (0.47)	198 (0.46)
GG	115 (0.12)	46 (0.09)	51 (0.11)	45 (0.10)

Marker	Controls	NTD Children	NTD Mothers	NTD Fathers
T	0.66	0.67	0.66	0.67
G	0.34	0.33	0.34	0.33

* Due to rounding, group frequencies may not add up to 1.

^a 135/135 mothers vs controls: OR = 1.86 [1.16-2.96, p=0.01]

^b GG cases vs. controls: OR = 1.37 [1.04-1.79, p=0.024]

^c TT NTD mothers: RR = 0.58 [0.37-0.91, p=0.019]

Table II

Estimated Haplotype Frequencies in Irish Controls, NTD Cases and NTD Mothers

	Haplotype	Control frequency*	NTD case frequency*	NTD mother frequency*
Block 1	G-G	0.897	0.899	0.882
	C-A	0.072	0.064	0.081
	C-G	0.029	0.033	0.035
			p**=0.73	p**=0.09
Block 2	G-A-G-G-C	0.725	0.723	0.723
	C-C-C-A-C	0.105	0.084	0.088
	C-A-C-G-C	0.069	0.069	0.075
	C-A-C-G-T	0.065	0.059	0.064
	G-C-G-G-C	0.015	0.021	0.023
	C-C-C-A-T	0.010	0.014	0.008
			p**=0.63	p**=0.50

Haplotype blocks are defined as shown in Figure 1b: Block 1 (rs8064946, rs2078486); Block 2 (rs1642785, rs17878362, rs1042522, rs1625895, rs6503048).

* Haplotype frequencies were estimated using Phase 2.1 as described in the Materials and Methods. Haplotypes with a frequency <0.01 are omitted so columns do not sum to 1.

** Result of permutation test for significant differences in haplotype frequencies in NTD cases vs. controls or NTD mothers vs. controls.