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Evaluation of 64 Candidate Single Nucleotide Polymorphisms as Risk Factors for Neural Tube Defects in a Large Irish Study Population

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Abstract

Individual studies of the genetics of neural tube defects (NTDs) contain results on a small number of genes in each report. To identify genetic risk factors for NTDs, we evaluated potentially functional single nucleotide polymorphisms (SNPs) that are biologically plausible risk factors for NTDs but that have never been investigated for an association with NTDs, examined SNPs that previously showed no association with NTDs in published studies, and tried to confirm previously reported associations in folate-related and non-folate-related genes. We investigated 64 SNPs in 34 genes for association with spina bifida in up to 558 case-families (520 cases, 507 mothers, 457 fathers) and 994 controls in Ireland. Case-control and mother-control comparisons of genotype frequencies, tests of transmission disequilibrium, and log-linear regression models were used to calculate effect estimates. Spina bifida was associated with over-transmission of the *LEPR* (leptin receptor) rs1805134 minor C allele (genotype relative risk (GRR): 1.5; 95% confidence interval (CI): 1.0, 2.1; $P = 0.0264$) and the *COMT* (catechol-O-methyltransferase) rs737865 major T allele (GRR: 1.4; 95% CI: 1.1, 2.0; $P = 0.0206$). After correcting for multiple comparisons, these individual test *P*-values exceeded 0.05. Consistent with previous reports, spina bifida was associated with *MTHFR* 677C>T, T (Brachyury) rs3127334, *LEPR* K109R, and *PDGFRA* promoter haplotype combinations. The associations between *LEPR* SNPs and spina bifida suggest a possible mechanism for the finding that obesity is a NTD risk factor. The association between a variant in *COMT* and spina bifida implicates methylation and epigenetics in NTDs.

Keywords

congenital abnormalities; folic acid; neural tube defects; single nucleotide polymorphism; spina bifida

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INTRODUCTION

Neural tube defect (NTD) prevalence ranges between 0.5 and 5.0 per 1,000 pregnancies [Feuchtbaum et al., 1999; McDonnell et al., 1999]. The findings of increased prevalence (2–5%) in siblings of affected individuals [Sebold et al., 2005] and in same-sex twins (including monozygotic pairs) [Windham and Sever, 1982], compared with the general population, suggest that genetic factors contribute to NTD etiology. The observation that maternal periconceptional folic acid supplementation reduces the risk for NTDs by 50–70% has identified folate as an important environmental factor [MRC Vitamin Study Research Group, 1991; Czeizel and Dudas, 1992]. Homozygosity for a common variant in the folate gene 5,10-methylenetetrahydrofolate reductase, *MTHFR* 677C>T, has been associated with NTDs but only 11–26% of cases can be attributed to this variant [Kirke et al., 2004]. Animal, and some human, studies suggest that both folate-related and non-folate-related genes play a role in the etiology of NTDs.

We used several strategies to look for genetic risk factors for NTDs. First, we chose single nucleotide polymorphisms (SNPs) that have not been evaluated previously for NTD risk but that have potential functional consequences and are located in genes that could be biologically related to neural tube development. Second, we examined a group of biologically plausible candidate SNPs previously tested for association with NTDs but found to be unassociated. In these mostly small studies, this lack of association could have resulted from inadequate power. Third, we sought to confirm associations between NTDs and SNPs in folate-related and non-folate-related genes reported by others, often using smaller sample sizes than were available in this study. Fourth, now using a larger study population, we re-examined some SNPs that have been investigated by our research group.

MATERIALS AND METHODS

Cases and controls

Details of case and control recruitment have been described previously [Pangilinan et al., 2010; Kirke et al., 1993]. NTD-affected families (583 full and partial trios that included 544 cases, 531 mothers, and 478 fathers) were recruited in the Republic of Ireland from 1993 to 2004 with assistance from the Irish Association for Spina Bifida and Hydrocephalus and the Public Health Nurses. Controls (N = 999) were randomly selected from a bank of blood samples obtained between 1986 and 1990 from 56,049 pregnant women receiving prenatal care at the three main maternity hospitals in Dublin, excluding women whose past or current pregnancies were affected with an NTD. Informed consent was obtained for the collection of all blood and buccal samples. Buccal cells were collected onto cotton swabs and placed in stabilizing buffer before being processed. Ethical approval for the study was provided by the Research Ethics Committee of the Health Research Board of Ireland and the Institutional Review Board of the National Human Genome Research Institute.

SNP selection

We examined a total of 64 SNPs in 34 genes (for gene names and functions see supporting information Table I which may be found in the online version of this article). Six SNPs (*MTHFR* 677C>T and 1298A>C, *MTR* D919G, *T* (Brachyury) rs3127334, *UCP2* -866G>A and A55V) were previously evaluated by this research group, albeit with a smaller number of study subjects (<400 cases or case-families) [Brody et al., 1999; Shields et al., 1999; Shields et al., 2000; Parle-McDermott et al., 2003; Kirke et al., 2004; Mitchell et al., 2009]. Another group of 12 SNPs (*ALDH1A2* rs3784260, rs3784259, and rs16939660, *BHMT* rs3733890, *BRCA1* rs1799966, *CCL2* rs1024611, *ERCC2* rs13181, *LEPR* rs1137100, *NAT1* rs15561, *PDGFRA* rs6554162, rs1800812, and rs1135534) were previously reported

by other groups to be associated with NTDs [Joosten et al., 2001; Zhu et al., 2004; Deak et al., 2005; Jensen et al., 2005; Olshan et al., 2005; Jensen et al., 2006; King et al., 2007; Davidson et al., 2008; Shaw et al., 2009].

The remaining 46 SNPs (Table I) either were previously evaluated for NTD risk and no association was observed (5 SNPs: *BHMT2* rs626105, *CITED2* rs1131431, *LEPR* rs1137101 and rs8179183, *SHH* rs1233555), were examined as components of haplotypes associated with NTDs (4 SNPs: *NAT1* rs4986782, rs4986783, and rs56172717, *PEMT* rs7946), or had not been tested previously for an association with NTDs (37 SNPs). The 46 SNPs were selected from genes related to folate/homocysteine metabolism (*BHMT2*, *CBS*, *CTH*, *GGH*, *GNMT*, *PEMT*, *TK1*), craniofacial morphogenesis (*IRF6*, *MSX1*, *SHH*), obesity which is a risk factor for NTDs (*LEP*, *LEPR*), and genes that cause NTDs in animals (*BRCA1*, *CITED2*, *DNMT3B*, *LRP6*). We also included SNPs in genes that have been associated with other disease phenotypes (*ABCG2*, *CDKN2A*, *COMT*, *HSPA8*, *NAT1*, *NDOR1*, *NOS3*, *PADI4*, *RAC2*); these associations suggest that these SNPs could have functional consequences. We included SNPs from this last group of genes in our study because these genes regulate diverse cellular processes that could be involved in neural tube development and because one of our goals was to investigate novel candidate SNPs for NTDs.

Genotyping

Genomic DNA was extracted from blood or buccal samples using the QIAamp DNA Blood Mini Kit (Qiagen, Sussex, UK). Genotyping was performed by either of two methods: the Illumina GoldenGate assay (Illumina, San Diego, CA) or Matrix Assisted Laser Desorption/Ionization-Time-of-Flight (MALDI-TOF) mass spectrometry (Sequenom, San Diego, CA) of allele-specific extension products. Genotyping quality was assessed by repeat genotyping 2.5% of samples on the Illumina GoldenGate platform and 10% of samples using MALDI-TOF mass spectrometry; reproducibility exceeded 99.5% for each platform. For 10 SNPs, genotype concordance was assessed between the two genotyping methods. Concordance was observed among >98.5% of samples for each of the 10 SNPs. Of the 33 SNPs genotyped using the Illumina GoldenGate assay, the proportion of successful genotype calls was ≥95% for 25 SNPs, 90.0–94.9% for 7 SNPs (*COMT* rs737865, *IRF6* rs2235371, *MSX1* rs1042484, *PADI4* rs874881 and rs1748033, *PDGFRA* rs6554162, *UCP2* -866G>A), and 86% for 1 SNP (*MSX1* rs33946149). Of the 31 SNPs genotyped using MALDI-TOF mass spectrometry, ≥95% of genotypes were called successfully for 30 SNPs; 93% of genotypes were called successfully for one SNP (*LEP* rs2167270).

All SNPs were tested for Hardy-Weinberg equilibrium separately among controls, cases, case-mothers, and case-fathers. Using a cutoff of $P = 0.01$, one SNP was not in Hardy-Weinberg equilibrium among controls (*GGH* rs719235, $P = 0.00017$). There was no deviation from Hardy-Weinberg equilibrium among cases, case-mothers, and case-fathers for any of the SNPs tested ($P > 0.01$).

Exclusions

Individuals with discordant genotypes, or trios displaying non-Mendelian inheritance for one or two SNPs were excluded from analyses for those SNPs. Trios that showed non-Mendelian inheritance for >2 SNPs (20 cases and their parents) and individuals that had discordant genotypes for >2 SNPs (5 controls, 4 cases, 4 case-mothers, 1 case-father) were excluded from all analyses. The remaining 558 case families and 994 controls were included in the analysis.

Linkage disequilibrium

Linkage disequilibrium (LD) measures were estimated using Haploview (http://www.broadinstitute.org/haploview/haploview) [Barrett et al., 2005] based on the genotypes of control samples.

Statistical methods

Most of the case families had a child affected with spina bifida; therefore, the main analysis was restricted to this defect group. Analyses were repeated adding case families affected by other NTDs. Logistic regression was used to model dominant and recessive effects of genetic risk. These tests were applied to case-control and mother-control analyses to generate odds ratios (ORs) and 95% confidence intervals (CIs). Family-based analyses, which included the transmission disequilibrium test (TDT) and log-linear analysis, produced estimates of genotype relative risk (GRR) and 95% CIs. The log-linear models were fitted to test dominant and recessive effects of genetic risk; they assessed case effects as well as direct maternal effects. We adjusted for multiple comparisons using permutation (N = 9,999). Multivariate permuting of trios for the TDT and log-linear analysis was performed by treating the test as a one-sample test, and permuting the hypothetical risk allele. Permutations of trios were independent of those for cases and controls, and the results were combined by Bonferroni adjustment so that the resulting adjusted *P*-values accounted for all comparisons while controlling the probability of any false-positives at 5%.

The six SNPs previously examined by this group were tested using case-control, mother-control, and log-linear analyses. The 12 SNPs previously reported by other groups to be associated with NTDs were tested using analyses similar to those in the original reports [Joosten et al., 2001; Zhu et al., 2004; Deak et al., 2005; Jensen et al., 2005; Olshan et al., 2005; Jensen et al., 2006; King et al., 2007; Davidson et al., 2008; Shaw et al., 2009]. We performed case-control and mother-control analyses to test for the recessive effect of *PDGFRA* promoter haplotype combinations associated with low or high transcriptional activity. Low transcriptional activity was signified by either the joint presence of the A and T alleles for *PDGFRA* rs6554162 (-1467G>A) and rs1800812 (-794G>T), respectively, or the presence of the A allele for *PDGFRA* rs1135534 (-909C>A); other haplotype combinations indicated high transcriptional activity. Because we tested *a priori* hypotheses, the analyses for these 18 SNPs were not adjusted for multiple comparisons.

Of the other 46 SNPs (Table I), 37 were novel, unpublished candidates for NTD risk and nine were previously tested in mainly small study populations. We evaluated these SNPs by case-control, mother-control, and log-linear analyses, and the TDT. These analyses were adjusted for multiple comparisons by the permutation method described above.

RESULTS

The 558 case families included 449 full trios, 21 case-mother pairs, 5 case-father pairs, 45 cases only, and 38 families with parents only. A family history of NTDs was reported by 31.7% (177/558) of families. For the 31 SNPs genotyped by MALDI-TOF mass spectrometry, all samples were tested (558 case families and 994 controls). For the remaining 33 SNPs genotyped on the Illumina GoldenGate platform, 314 case families and 338 controls were tested (Table II). The genotype distribution for each SNP was obtained for controls, cases, case-mothers, and case-fathers (see supporting information Table II which may be found in the online version of this article).

For the six SNPs that we previously investigated, we increased the size of the case group by 100 or more subjects, compared with our earlier reports. In case-control analysis, spina bifida was associated with homozygosity for the *MTHFR* 677C>T (rs1801133, A222V)

minor T allele (OR: 1.3; 95% CI: 1.0, 1.6; $P = 0.0451$). The *T* (Brachyury) rs3127334 major A allele was also associated with spina bifida in case-control analysis (OR: 2.4; 95% CI: 1.1, 5.7; $P = 0.0288$; dominant model). There was no association with *MTHFR* 1298A>C (rs1801131, E429A), *MTR* D919G (rs1805087), *UCP2* -866G>A (rs659366), and *UCP2* A55V (rs660339), similar to our previous reports.

Our results for variants reported to be NTD risk factors by other groups (Table III) show that spina bifida was associated with transmission of the *LEPR* (leptin receptor) rs1137100 (K109R) major A allele (GRR: 1.4; 95% CI: 1.0, 1.8; $P = 0.0236$) and with *PDGFRA* (platelet-derived growth factor receptor, alpha polypeptide) haplotype combinations with high transcriptional activity (OR: 1.5; 95% CI: 1.0, 2.0); $P = 0.0252$; recessive effect). No association with spina bifida was observed for the other SNPs reported in previous studies.

We examined associations for the other 46 SNPs that either showed no association with NTDs in previous reports or were not tested previously for an association with NTDs (see supporting information Tables III and IV which may be found in the online version of this article); eight were associated with spina bifida (Table IV). Two of these SNPs were associated with spina bifida based on the results of two different statistical tests. Increased spina bifida risk was associated with the *LEPR* rs1805134 minor C allele when tested using the TDT (GRR: 1.5; 95% CI: 1.0, 2.1; $P = 0.0264$) and log-linear analysis (GRR: 1.7; 95% CI: 1.1, 2.6; $P = 0.0179$), and with the *COMT* (catechol-O-methyltransferase) rs737865 major T allele when tested using the TDT (GRR: 1.4; 95% CI: 1.1, 2.0; $P = 0.0206$) and log-linear analysis (GRR: 2.1; 95% CI: 1.1, 4.3; $P = 0.0364$). Correction for multiple comparisons performed for the 46 SNPs rendered these *P* values > 0.05. Adding case families with other NTDs produced few changes in the results obtained.

Multiple SNPs in *BRCA1* and *LEPR* that showed an association with spina bifida did not exhibit intragenic LD. *BRCA1* rs1799966 and rs799917 were in strong LD ($r^2 = 0.97$); neither was in strong LD with *BRCA1* rs3737559 ($r^2 < 0.22$). *LEPR* rs1137100, rs1805134, and rs8179183 were not in strong LD ($r^2 < 0.20$).

DISCUSSION

We used several strategies to select SNPs to assess as genetic risk factors for NTDs. We investigated potentially functional variants that have not previously been evaluated for NTD risk, examined candidates that previously showed no association but were deserving of further study, and attempted to confirm previously reported associations in folate-related and non-folate-related genes. We observed statistically significant associations between spina bifida and *MTHFR* 677C>T, *T* (Brachyury) rs3127334, *LEPR* K109R, and *PDGFRA* haplotype combinations with high transcriptional activity. Although not statistically significant after correction for multiple comparisons, spina bifida was associated with *COMT* rs737865 and *LEPR* S343S using both the TDT and log-linear analysis.

Among the candidate SNPs that have never been investigated as NTD risk factors or that previously showed no association with NTDs, most were not associated with spina bifida in our study population, but there were some notable exceptions. SNPs in *LEPR* and *COMT* showed associations with spina bifida using two different statistical tests. There are conflicting reports on the importance of *LEPR* as a risk factor for spina bifida [Shaw et al., 2000; Davidson et al., 2008]. We observed that the *LEPR* K109R major K allele was associated with increased spina bifida risk, confirming a previous report of over-transmission of the major K allele to spina bifida cases [Davidson et al., 2008]. Our finding of associations between spina bifida and three *LEPR* variants that are not in strong LD with each other, and the higher NTD risk in the offspring of obese women [Waller et al., 1994],

suggest that *LEPR* is a strong candidate for additional study. *COMT* encodes catechol-O-methyltransferase, an enzyme that transfers a methyl group from S-adenosylmethionine to catecholamines, and thus generates homocysteine. The V158M polymorphism (rs4680) occurs in up to 75% of Caucasians and gives rise to enzymes of substantially different activity such that MM homozygotes have between two and three times lower activity than VV homozygotes [Lavigne et al., 1997; Goodman et al., 2001]. The high activity enzyme (VV) has been linked to elevated plasma homocysteine, especially in the presence of the *MTHFR* TT genotype [Tunbridge et al., 2008]. SNPs in this gene have not been tested previously for NTD risk, but the finding that mothers carrying NTD-affected fetuses have higher homocysteine concentrations [Mills et al., 1995] makes *COMT* a good candidate gene for NTD risk assessment. We found no effect of the V158M polymorphism on spina bifida risk, but our observation of increased risk with the intronic SNP, rs737865, suggests that a role for this gene in NTD risk should be investigated further.

We also observed associations between spina bifida and other variants that have not been previously reported as NTD risk factors (*ABCG2* Q141K, *BRCA1* rs3737559 and P871L, *DNMT3B* rs6087990, and *SHH* rs1233555), although correction for multiple comparisons made these results not statistically significant. Nevertheless, it is biologically plausible that these variants could be related to NTDs. *ABCG2* is a protein involved in xenobiotic transport; and it can transport methotrexate, an antifolate, and mono- and poly-glutamyl folates across cell membranes [Chen et al., 2003]. *Brca1*-mutant mice have disorganized neuroepithelia and NTDs [Gowen et al., 1996]. *DNMT3B* encodes a DNA methyltransferase required for *de novo* DNA methylation during embryogenesis and *Dnmt3b*-deficient mice have NTDs [Okano et al., 1999]. *SHH* signaling is involved in patterning of the brain and spinal cord [Chiang et al., 1996] and *SHH* mutations are associated with craniofacial malformations in humans [Roessler et al., 2009]. While our results did not withstand correction, in combination with these reports, they suggest that variants in *ABCG2*, *BRCA1*, *DNMT3B*, and *SHH* could be involved in neural tube development.

Other studies have identified potential genetic risk factors for NTDs that require confirmation. We attempted to confirm some of these associations. In addition to our finding for *LEPR* K109R, we also observed an association with promoter haplotypes of *PDGFRA*, a gene associated with NTDs in animals [Soriano, 1997]. Haplotypes with low transcriptional activity have been associated with increased NTD risk in some human studies [Joosten et al., 2001; Zhu et al., 2004; Toepoel et al., 2009] but not all [Au et al., 2005]. In our study, being homozygous for *PDGFRA* haplotypes with high transcriptional activity was associated with spina bifida. We did not, however, confirm associations between spina bifida and a variant (R239Q) in *BHMT*, a folate/homocysteine enzyme, or other biologically plausible candidate SNPs (*ALDH1A2* rs3784260, rs3784259, and rs16939660, *BRCA1* rs1799966, *CCL2* rs1024611, *ERCC2* rs13181).

Using a larger study population, we evaluated variants in folate-related and non-folate-related genes that had been previously examined by our research group. Maternal folate deficiency has been identified as the most significant environmental factor influencing NTD risk; however, the mechanism through which this deficiency leads to NTDs is unknown. Our results bolster the evidence that *MTHFR* 677C>T, a variant in a folate enzyme gene, is associated with NTDs. There have been conflicting reports [Morrison et al., 1996; Richter et al., 2002; Speer et al., 2002] regarding an association with the rs3127334 variant of *T* (Brachyury) which encodes a transcription factor expressed in the notochord during neurulation [Herrmann, 1991]. In this study, we observed an association between this SNP and spina bifida, similar to our previous report [Shields et al., 2000].

A major strength of our study was the large number of affected families for which genotype data were available; this was valuable in our examination of previously reported associations, some of which were from studies with small sample sizes. Other strengths included the use of an ethnically homogenous study population and the ability to examine maternal effects as well as case effects. However, we lacked data on environmental exposures and the small number of families affected by anencephaly and encephalocele did not permit us to examine associations separately for these sub-groups. There were nine SNPs for which a genotype call rate of <95% was obtained, which could cause concern about the reliability of these data. Low call rates can be associated with poorly performing genotype assays. Such assays can introduce bias by over- or under-calling one allele. For these nine SNPs, additional data suggest that the genotypes are being accurately called. These SNP assays were highly reproducible (>99.5%) upon repeat genotyping, the SNPs were all in Hardy-Weinberg equilibrium, and the genotype and allele frequencies we obtained were consistent with those reported in reference populations (that is, HapMap). Although limited resources did not permit us to perform genotyping of all subjects on the Illumina GoldenGate platform, there was 80% power to detect an OR of at least 1.5 in case-control analyses for an allele frequency of 10%. The larger sample size genotyped by MALDI-TOF mass spectrometry further improved the power of the study: in case-control analyses, there was 80% power to detect an OR of at least 1.4 for an allele frequency of 10%, and an OR of at least 1.6 for an allele frequency of 5%.

In contrast to the NTD cases, which were recruited from the entirety of the Republic of Ireland, our controls were randomly selected from a large group of women attending their first prenatal care visit at the three main maternity hospitals in Dublin. Approximately one-third of all births in the Republic of Ireland, and 90% of all births in and around the Dublin area, occur in these hospitals. We investigated SNPs in genes located on autosomes, and previous reports indicate that, among this group of subjects, the allele frequencies of the genes on autosomes reflect those of the underlying Irish population [Parle-McDermott et al., 2003; Kirke et al., 2004]. Therefore, our results are unlikely to be biased by the use of this control group.

Our results add to our knowledge of NTD genetics. We confirmed associations in folate-related and other genes that were previously reported by others. In addition, we tested a number of variants that have not been previously examined for an association with NTDs. Among these novel candidate variants, SNPs in *LEPR* and *COMT* showed associations with spina bifida using two different statistical tests. Our results for the leptin receptor gene, *LEPR*, suggest that the increased risk of NTDs associated with obesity could involve leptin signaling. In a similar fashion, our findings for a variant in *COMT* implicate a specific methylation reaction that may interact with folate and should be investigated further in relation to NTDs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table I

Single nucleotide polymorphisms investigated for associations with neural tube defects.

Gene	SNP	Description	SNP association with protein function or disease phenotype	Reference[a]
ABCG2	rs2231142	Q141K	reduced *ABCG2* activity; associated with nonpapillary renal cell carcinoma	Mizuarai et al., 2004; Korenaga et al., 2005
BHMT2	rs626105	intron	NTD study examined rs626105 as a *BHMT2* marker	Zhu et al., 2005; Shaw et al., 2009
BRCA1	rs3737559	intron	breast cancer	Cox et al., 2005; Tommasi et al., 2005
BRCA1	rs8176166	intron		
BRCA1	rs16942	K1183R		
BRCA1	rs799917	P871L		
CBS	rs234706	Y233Y	reduced risk of coronary artery disease	Kruger et al., 2000
CDKN2A	rs3731249	A148T	malignant melanoma	Debniak et al., 2005
CITED2	rs1131431	3′UTR	present in some patients with congenital heart defects	Sperling et al., 2005
COMT	rs737865	intron	reduced *COMT* activity, plasma homocysteine concentration, schizophrenia	Lavigne et al., 1997; Goodman et al., 2001; Shifman et al., 2002; Chen et al., 2004; Tunbridge et al., 2008
COMT	rs4680	V158M		
CTH	rs1021737	S403I	plasma homocysteine concentration	Wang et al., 2004
DNMT3B	rs6087990	5′ near gene	evaluated for risk of primary lung cancer	Lee et al., 2005
GGH	rs11545078	T151I	evaluated because amino acid change might affect protein function	Cheng et al., 2004
GGH	rs719235	5′ near gene	SNPs in promoter region could affect *GGH* expression	Chave et al., 2003
GGH	rs3758149	5′ near gene		
GGH	rs719236	5′ near gene		
GNMT	rs10948059	5′ near gene	plasma homocysteine concentrations	Beagle et al., 2005
HSPA8	rs1136141	5′UTR	mild mental impairment	Butcher et al., 2005
IRF6	rs2235371	V274I	oral clefts	Zucchero et al., 2004
IRF6	rs2013162	S153S		
LEP	rs7799039	-2548G>A	overweight/obesity, glucose homeostasis	Mammes et al., 2000; Mammes et al., 2001; Yiannakouris et al., 2001; Lakka et al., 2004
LEP	rs2167270	-39G>A		
LEP	rs2060713	intron		
LEPR	rs3790435	5′UTR		
LEPR	rs1137101	Q223R		
LEPR	rs1805134	S343S		
LEPR	rs8179183	K656N		
LRP6	rs2302685	V1062I	bone fractures in elderly men	van Meurs et al., 2006
MSX1	rs33946149	intron	evaluated for association with oral clefts	Beaty et al., 2001; Fallin et al., 2003
MSX1	rs1042484	intron		
MSX1	rs34165410	G116G		
NAT1	rs4986782	R187Q	association with spina bifida when SNPs were tested as components of a haplotype	Jensen et al., 2006
NAT1	rs4986783	S214A		
NAT1	rs56172717	D251V		

Gene	SNP	Description	SNP association with protein function or disease phenotype	Reference[a]
NDOR1	rs62587579	V522I	reduced *NDOR1* activity	Finn et al., 2005
NOS3	rs2070744	intron	vascular invasion in breast cancer	Ghilardi et al., 2003
PADI4	rs11203366	S55G	rheumatoid arthritis	Suzuki et al., 2003; Harney et al., 2005
PADI4	rs11203367	A82V		
PADI4	rs874881	A112G		
PADI4	rs1748033	L117L		
PADI4	rs2240339	intron		
PEMT	rs7946	V175M	reduced *PEMT* activity; possible association with nonalcoholic fatty liver disease	Song et al., 2005
RAC2	rs13058338	intron	anthracycline-induced cardiotoxicity	Wojnowski et al., 2005
SHH	rs1233555	intron	associated with dideoxy DNA fingerprinting pattern of gene fragment containing exon 2 of *SHH*	Zhu et al., 2003
TK1	rs1132543	V106M	reduced enzyme stability	Berenstein et al., 2000

[a]References are listed in supplemental material (see supporting information Supplement I which may be found in the online version of this article)

Abbreviation: SNP – single nucleotide polymorphism

Table II

Irish case families that had a child affected with a neural tube defect.

Study subjects	Total	Subset genotyped on Illumina platform
Case families	558	314
Anencephaly	7	5
Spina bifida	528	298
Encephalocele	23	11
All neural tube defects		
Cases	520	295
Case mothers	507	284
Case fathers	457	270
Spina bifida		
Cases	498	284
Case mothers	477	268
Case fathers	436	259

Table III

Effect estimates for associations between spina bifida and specific SNPs or haplotypes previously reported to be associated with neural tube defects.

Gene	SNP/Haplotype	Description	Allele	Analysis	Effect estimate (95% CI)
ALDH1A2	rs3784259	intron	A (major allele)	TDT	GRR: 1.0 (0.8, 1.4)
ALDH1A2	rs3784260	intron	C (minor allele)	TDT	GRR: 0.9 (0.7, 1.2)
ALDH1A2	rs16939660	A151A	C (minor allele)	TDT	GRR: 1.3 (0.3, 6.0)
BHMT	rs3733890	R239Q	A (minor allele)	Case-control, recessive model	OR: 1.4 (0.9, 2.1)
BRCA1	rs1799966	S1613G	G (minor allele)	TDT	GRR: 1.2 (0.9, 1.6)
CCL2	rs1024611	5′ near gene	C (minor allele)	Mother-control, dominant model	GRR: 0.9 (0.7, 1.2)
ERCC2	rs13181	K751Q	G (minor allele)	Case-control, dominant model	OR: 1.1 (0.9, 1.4)
LEPR	rs1137100	K109R	A (major allele)	TDT	GRR: 1.4 (1.0, 1.8)
NAT1	rs15561	1095C>A	A (minor allele)	Log linear, case effect, dominant model	GRR: 1.0 (0.7, 1.3)
				Log linear, maternal effect, dominant model	GRR: 1.1 (0.8, 1.5)
PDGFRA	Promoter haplotype combinations that affect transcriptional activity	Low activity: (rs6554162 = A and rs1800812 = T) or rs1135534 = A; Other = high activity	-	Case-control analysis, recessive for low transcriptional activity	OR: 1.0 (0.5, 1.9)
				Mother-control analysis, recessive for low transcriptional activity	OR: 1.1 (0.6, 2.2)
				Case-control analysis, recessive for high transcriptional activity	OR: 1.5 (1.0, 2.0)
				Mother-control analysis, recessive for high transcriptional activity	OR: 0.7 (0.5, 1.0)

Abbreviations: SNP – single nucleotide polymorphism, TDT – transmission disequilibrium test, GRR – genotype relative risk, OR – odds ratio, CI – confidence interval

Table IV

Results of statistical analyses of associations between spina bifida and candidate single nucleotide polymorphisms.

Gene	SNP	Description	Allele	Analysis method	Effect estimate (95% CI)	Uncorrected *P*-value[a]
ABCG2	rs2231142	Q141K	C	Case-control, recessive model	OR: 1.5 (1.0, 2.1)	0.0449
ABCG2	rs2231142	Q141K	C	Mother-control, recessive model	OR: 1.5 (1.0, 2.3)	0.0312
BRCA1	rs3737559	intron	G	Case-control, recessive model	OR: 1.9 (1.2, 2.9)	0.0075
BRCA1	rs3737559	intron	G	Mother-control, recessive model	OR: 2.1 (1.3, 3.4)	0.0026
BRCA1	rs799917	P871L	C	Mother-control, dominant model	OR: 1.8 (1.1, 3.1)	0.0282
COMT	rs737865	intron	T	Mother-control, dominant model	OR: 0.4 (0.2, 0.8)	0.0105
COMT	rs737865	intron	T	Transmission disequilibrium test	GRR: 1.4 (1.1, 2.0)	0.0206
COMT	rs737865	intron	T	Log-linear, case effect, dominant model	GRR: 2.1 (1.1, 4.3)	0.0364
DNMT3B	rs6087990	5′ near gene	T	Log-linear, mother effect, recessive model	GRR: 1.4 (1.0, 1.9)	0.0356
LEPR	rs1805134	S343S	C	Transmission disequilibrium test	GRR: 1.5 (1.0, 2.1)	0.0264
LEPR	rs1805134	S343S	C	Log-linear, case effect, dominant model	GRR: 1.7 (1.1, 2.6)	0.0179
LEPR	rs8179183	K656N	C	Log-linear, case effect, recessive model	GRR: 2.4 (1.1, 5.5)	0.0439
SHH	rs1233555	intron	C	Log-linear, case effect, recessive model	GRR: 1.7 (1.2, 2.5)	0.0023

[a]After adjustment for multiple comparisons using permutation (N = 9,999), these *P*-values exceeded 0.05.

Abbreviations: SNP – single nucleotide polymorphism, OR – odds ratio, GRR – genotype relative risk, CI – confidence interval