

Genetics of human neural tube defects

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Neural tube defects (NTDs) are common, severe congenital malformations whose causation involves multiple genes and environmental factors. Although more than 200 genes are known to cause NTDs in mice, there has been rather limited progress in delineating the molecular basis underlying most human NTDs. Numerous genetic studies have been carried out to investigate candidate genes in cohorts of patients, with particular reference to those that participate in folate one-carbon metabolism. Although the homocysteine remethylation gene *MTHFR* has emerged as a risk factor in some human populations, few other consistent findings have resulted from this approach. Similarly, attention focused on the human homologues of mouse NTD genes has contributed only limited positive findings to date, although an emerging association between genes of the non-canonical Wnt (planar cell polarity) pathway and NTDs provides candidates for future studies. Priorities for the next phase of this research include: (i) larger studies that are sufficiently powered to detect significant associations with relatively minor risk factors; (ii) analysis of multiple candidate genes in groups of well-genotyped individuals to detect possible gene–gene interactions; (iii) use of high throughput genomic technology to evaluate the role of copy number variants and to detect ‘private’ and regulatory mutations, neither of which have been studied to date; (iv) detailed analysis of patient samples stratified by phenotype to enable, for example, hypothesis-driven testing of candidate genes in groups of NTDs with specific defects of folate metabolism, or in groups of fetuses with well-defined phenotypes such as craniorachischisis.

INTRODUCTION

Congenital malformations are the leading cause of infant mortality in developed countries and a major cause of health problems in surviving children. Neural tube defects (NTDs) are a common group of central nervous system anomalies affecting 0.5–2 per 1000 pregnancies worldwide. NTDs arise when the neural tube, the embryonic precursor of the brain and spinal cord, fails to close during neurulation. The cranial region (anencephaly) or the low spine (open spina bifida; myelomeningocele) are most commonly affected although, in the severe NTD craniorachischisis, almost the entire neural tube remains open, from midbrain to low spine.

Most individuals who survive with NTDs (particularly myelomeningocele) have a multiple system handicap and a limited life expectancy. However, despite the high prevalence and traumatic consequences for affected individuals and their families, the causes of NTD are poorly understood. Identification of causative factors is confounded by the fact that the

majority of these malformations appear to result from a combination of genetic and environmental factors. A strong genetic component is indicated by the high recurrence risk for siblings of affected individuals (1,2). Syndromic cases of NTD also exist, often associated with chromosomal anomalies, but these represent <10% of all defects (1,3–5). The majority of NTDs are sporadic, with recurrence fitting a multifactorial polygenic or oligogenic pattern, rather than models on the basis of single gene dominant or recessives, with reduced penetrance (2).

GENETIC ANALYSIS OF HUMAN NTDS

Positional cloning strategies have been hampered by the paucity of large families with multiple affected members. Nevertheless, genome-wide studies using collections of smaller multiplex families have implicated chromosomes 2, 7 and 10 as harbouring candidate risk loci for spina bifida

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(6–8). Although the causative genes are yet to be identified, these studies may result in identification of candidate sequences that can be evaluated in larger populations. An alternative approach exploits the association of NTDs with chromosomal anomalies such as trisomies 13 and 18 (9), suggesting that gene-dosage can affect neural tube closure. Rearrangements involving deletions, duplications or balanced translocations are likely to be most informative, with fine mapping of chromosomal breakpoints enabling identification of specific loci (10).

In some studies, direct mutation screening of candidate genes has been carried out in cohorts of patients (11), but the vast majority involve statistical association analysis of sequence variants in or near candidate genes. Most work has involved case–control analysis, comparing the frequency of ‘risk’ alleles in affected individuals and/or mothers with a matched unaffected cohort. More sophisticated studies have used the transmission disequilibrium test (TDT) in family trios (mother, father and affected child), which is less dependent on population structure. In the remainder of this article, we review the main candidate gene studies which have arisen primarily from analysis of folate metabolic pathways and mouse models of NTDs. Boyles *et al.* (11) published a comprehensive review of this field up to 2004, and an updated candidate gene list is presented in Table 1.

CANDIDATE GENES FROM FOLATE METABOLISM

Epidemiological studies provide an opportunity to identify risk factors for NTDs, such as dietary or teratogenic agents, to which susceptibility may be modified by genetic predisposition (12–14). Among environmental factors, folate status plays a key role in determining NTD risk (15,16). Maternal supplementation with folic acid during pregnancy reduces NTD frequency (17,18) whereas reduced serum folate and/or elevated homocysteine (an inverse indicator of folate status) are observed in some mothers of NTD fetuses, and are considered risk factors for NTDs (19–21). However, NTDs are not simply a condition of folate deficiency: maternal folate levels in most human NTD-affected pregnancies are in the ‘normal’ range (22), suggesting that low folate status may increase susceptibility but is not directly causative. Similarly, in mice dietary folate deficiency can cause significant embryonic growth retardation but does not cause NTDs (16,23,24). Hence, sub-optimal folate status may pre-dispose to NTDs in combination with additional factors, either environmental or genetic.

The intricate interplay and cross-regulation between elements of one-carbon (folate) metabolism (Fig. 1) complicates the teasing out of events that impinge on neural tube closure. In mice, key cellular functions in the developing embryo include methylation reactions and biosynthesis of nucleotides that support rapid cellular proliferation (2,25). Cranial NTDs arise when the methylation cycle is inhibited (26,27), and in null embryos for DNA methyltransferase 3B (28). In contrast, exogenous homocysteine does not cause NTDs (29–31), even in genetically predisposed *plotch* embryos (24) and may be an indicator of impaired folate or methylation cycle activity.

If folate status interacts with genetic factors in the causation of NTDs, this could involve either folate-related or folate-independent genes. To date, most emphasis has been placed on the evaluation of folate-related genes as NTD candidates (32,33) (Table 1). Further support comes from analysis of primary cell lines obtained from NTD fetuses, which indicates that a genetically-determined abnormality of folate metabolism is present, in at least a proportion of cases (34). However, identifying specific NTD-predisposing genetic lesions has proven far from straight forward. Although a number of variants have been widely studied, inconsistent results between different cohorts and populations (Table 1) indicate that very few, if any, have a major causative effect. Below, we sub-divide the candidate folate-related genes into three functional categories.

Methylation related genes

Among folate-related genes, 5,10-methylene tetrahydrofolate reductase (MTHFR) has been the principal focus of attention, following reports that the 677C>T (A222V; rs1801133) polymorphism is associated with increased risk of NTDs in Dutch and Irish populations (35–37). Other populations show no association (38,39) or even a protective effect (40,41) (Table 1). A meta-analysis, including genotype data from 27 studies up to 2004, suggests that the 677TT genotype confers an overall 1.9 times increase in NTD risk (Odds ratio: 1.9; 95% confidence interval: 1.6–2.2) (15). A more recent meta analysis (42) found a positive association only in non-latin groups, principally the Irish population.

The action of MTHFR generates 5-methylTHF for remethylation of homocysteine, at the expense of other folates required for purine and pyrimidine biosynthesis (Fig. 1). The A222V variant protein has reduced function and is associated with elevated plasma homocysteine (36). Nullizygosity for MTHFR in mice also results in elevated homocysteine and diminished DNA methylation (43), although NTDs are not observed under either normal or folate-deficient conditions. Moreover, MTHFR nullizygosity does not exacerbate the folate-responsive *plotch* mutation (43–45). These data suggest that in populations where MTHFR is a risk factor, additional interacting factors are likely to be present.

The link between reduced methylation/elevated homocysteine and NTDs has prompted analysis of variants in other genes that could influence the methylation cycle through remethylation (*MTR*, *MTRR*, *BHMT* and *BHMT2*) or trans-sulfuration (*CBS*) of homocysteine (11) (Table 1). In general, mildly elevated risks have been identified in some studies but rarely replicated. *MTRR* (methionine synthase reductase) functions to maintain activity of *MTR* (methionine synthase), and a variant form (I22M, encoded by 66A>G) was reported as an NTD case and maternal risk factor in some studies, but not others (Table 1). Mouse studies do not support a role for these genes in NTDs: targeted deletion of *Mtr* is embryonic lethal prior to neurulation stages and heterozygotes do not show NTDs (46). Similarly, reduced activity of *Mtrr* and loss of *cbs* function do not cause NTDs, although elevated plasma homocysteine is observed (47,48).

Table 1. Candidate gene analysis in human NTDs

Human gene	Type of candidate	Reference	Population studied	Sample size	Type of study	Summary of results/conclusion
<i>AHCY</i> (S-adenosylhomocysteine hydrolase)	One carbon metabolism	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>ALDH1L1</i> (Aldehyde dehydrogenase 1, member L1)	One carbon metabolism	(56)	Dutch	180 SB patients, 190 controls	Case-control study	Nominally significant association with Asp793Glu variant ^a
<i>ALDH1A2</i> (Retinaldehyde dehydrogenase Type 2, RALDH2)	Retinol metabolism	(104)	USA	318 SB families	Family based association study	One polymorphism associated with increased SB risk (tentative association for two others)
<i>AMD1</i> (Adenosyl methionine decarboxylase 1)	One carbon metabolism	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>APE1</i> (apurinic endonuclease1)	DNA repair	(112)	Mixed USA	380 SB cases, 350 controls	Case-control study	Suggestion of reduced risk for Asp148Glu variant
<i>BHMT</i> (betaine-homocysteine methyltransferase)	One carbon metabolism	(113)	Mixed USA	252 SB cases, 337 controls	Case-control study	No association for Arg239Gln polymorphism
		(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
		(103)	Mixed USA	259 SB cases, 359 controls	Case-control study	Modest increase in SB risk associated with 1 SNP (of eight tested)
<i>BHMT2</i> (betaine-homocysteine methyltransferase 2)	One carbon metabolism	(56)	Dutch	180 SB patients, 190 controls	Association study	No association detected
<i>BRCA1</i> (breast cancer 1)	NTDs in mouse mutant	(103)	Mixed USA	259 SB cases, 359 controls	Case-control study	No association for 7 SNPs tested
		(114)	USA	268 SB patients and parents	Family based association study (TDT)	Association with SB for two microsatellite markers and A4956G SNP. Proposed polymorphisms affect level of lesion, not causative
<i>CAT</i> (catalase)	Oxidative stress	(115)	Mixed USA	507 SB cases, 185 controls	Case-control study	No association
<i>CBS</i> (cystathionine beta-synthase)	Folate metabolism	(116)	UK	207 NTD cases (200 mothers, 93 fathers), 601 controls, 542 control mothers	Case-control study	No association for 844ins68
		(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
		(103)	Mixed USA	259 SB cases, 359 controls	Case-control (9 SNPs)	Modest increase in SB risk associated with 2 SNPs
<i>CFL1</i> (<i>n</i> -cofilin)	NTDs in mouse mutant	(117)	Mixed USA	246 SB cases, 336 controls	Case-control SNPs	Mildly elevated risk of NTDs in non-Hispanic whites
<i>CHKA</i> (choline kinase A)	One carbon metabolism	(118)	Mixed USA	103 SB cases, 338 controls	Case-control study	Possible association with reduced SB risk for 1 of 2 SNPs studied
<i>CITED2</i>	NTDs in mouse mutant	(119)	Mixed USA	64 SB cases, 72 controls	Mutation screen and case-control	No mutations. No association of three 5'-UTR SNPs with risk
<i>COQ3</i> (Coenzyme Q3 homolog, methyltransferase)	Methylation	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>CRABPI</i> (cellular retinoic acid binding protein I)	Retinol metabolism	(104)	USA	230 SB cases, 318 SB families	Mutation screen and family based association study	No mutations. No association (3 SNPs tested)
<i>CRABPII</i> (cellular retinoic acid binding protein II)	Retinol metabolism	(104)	USA	230 SB cases	Mutation screen	No mutations
<i>CTH</i> (Cystathionase)	One carbon metabolism	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>CUBN</i> (cubulin)	Endocytosis (folate transport)	(56)	Dutch	179 SB patients, 190 controls	Case-control study	GG genotype for rs1907362 significantly associated with reduced SB risk
<i>CYP26A1</i> (cytochrome P450)	Retinol metabolism	(104)	USA	230 SB cases	Mutation screen	No mutations
<i>CYP26B1</i> (cytochrome P450)	Retinol metabolism	(104)	USA	230 SB cases, 318 SB families	Mutation screen and Family based association study	No mutations. No association (5 SNPs tested)

Continued

Table 1. Continued

Human gene	Type of candidate	Reference	Population studied	Sample size	Type of study	Summary of results/conclusion
<i>DHFR</i> (Dihydrofolate reductase)	Folate metabolism	(120)	Mixed USA	61 SB cases and parents (multi-affected families) and 219 controls	Case-control study of 19-bp intron-1 deletion	The del/del genotype was more frequent in mothers of SB cases, compared with controls. No association in fathers or patients
		(121)	Irish	283 cases (and 280 mothers, 279 fathers) and 256 controls. SB (95%) or encephalocele (5%)	Case-control study. 19-bp deletion and two 3'-UTR variants.	19-bp Intron deletion shows protective effect. May increase mRNA levels
		(122)	Dutch	109 patients, 121 mothers (SB). 234 paediatric controls, 292 control women	Case-control study. 19-bp deletion and 9-bp repeat in 5'-UTR	19-bp Intron deletion not associated with NTDs. No effect on expression
		(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
		(103)	Mixed USA	259 SB cases, 359 controls	Case-control study	No association for 9 SNPs tested. Intron deletion not tested
		(41)	Mixed UK	126 SB (open); 103 SB (closed); 49 anencephalic; 192 controls	Case-control study	No association
<i>FOLR1</i> (Folate receptor 1)	Folate transport	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>FOLR2</i> (Folate receptor 2)	Folate transport	(103)	Mixed USA	259 SB cases, 359 controls	Case-control study	No association for 3 SNPs tested
		(56)	Dutch	180 SB patients, 190 controls	Association study	No association
<i>FOLR3</i> (Folate receptor 3)	Folate transport	(103)	Mixed USA	259 SB cases, 359 controls	Case-control study	No association for 3 SNPs tested
<i>FOLR3</i> (Folate receptor 3)	Folate transport	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>FPGS</i> (Folypolyglutamate synthase)	Cellular folate retention	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>FTCD</i> (Forminotransferase cyclodeaminase)	One carbon metabolism	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>GAMT</i> (Guanidinoacetate N-methyl transferase)	One carbon metabolism	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>GAPD</i> (glyceraldehyde 3 phosphate dehydrogenase)	Glucose metabolism	(115)	Mixed USA	507 SB cases, 185 controls	Case-control study	No association
<i>GART</i> (Phosphoribosylglycinamide formyltransferase, phosphoribosyl glycinamide synthetase, phosphoribosyl aminoimidazole synthetase)	Purine biosynthesis (one carbon metabolism)	(56)	Dutch	180 SB patients, 190 controls	Association study	No association
<i>GCPH</i> (glutamate carboxypeptidase), <i>FOLH1</i> (Folate hydrolase)	Folate metabolism	(116)	UK	208 NTD cases (200 mothers, 92 fathers). 600 child, 531 mother controls	Case-control study	No association for 1561C>T
		(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>GGH</i> (Gamma-glutamyl hydrolase)	Folate metabolism	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>GLUT1</i> (glucose transporter 1)	Glucose metabolism	(115)	Mixed USA	507 SB cases, 185 controls	Case-control study	Pro196 silent SNP associated with risk in TDT test
		(115)	Mixed USA	507 SB cases, 185 controls	Case-control study	No association
<i>GLUT4</i> (glucose transporter 4)	Glucose metabolism	(115)	Mixed USA	507 SB cases, 185 controls	Case-control study	No association
<i>HK1</i> (hexokinase 1)	Glucose metabolism	(115)	Mixed USA	507 SB cases, 185 controls	Case-control study	Lys481 SNP variant associated with risk in TDT test
		(115)	Mixed USA	507 SB cases, 185 controls	Case-control study	No association
<i>HK2</i> (hexokinase 2)	Glucose metabolism	(115)	Mixed USA	507 SB cases, 185 controls	Case-control study	No association
<i>ICMT</i> (Isoprenylcysteine carboxyl methyltransferase)	Protein methylation	(56)	Dutch	180 SB patients, 190 controls	Association study	No association
<i>INS</i> (insulin)	Glucose metabolism	(115)	Mixed USA	507 SB cases, 185 controls	Case-control study	No association
<i>INSR</i> (insulin receptor)	Glucose metabolism	(115)	Mixed USA	507 SB cases, 185 controls	Case-control study	No association
<i>LEP</i> (leptin)	Glucose metabolism	(115)	Mixed USA	507 SB cases, 185 controls	Case-control study	Arg109Lys variant associated with risk in TDT test
		(115)	Mixed USA	507 SB cases, 185 controls	Case-control study	Arg109Lys variant associated with risk in TDT test
<i>LEPR</i> (leptin receptor)	Glucose metabolism	(115)	Mixed USA	507 SB cases, 185 controls	Case-control study	Arg109Lys variant associated with risk in TDT test
<i>MAT1A</i> (Methionine adenosyltransferase I, alpha)	One carbon metabolism	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association

<i>MAT2A</i> (Methionine adenosyltransferase II, alpha)	One carbon metabolism	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>MGMT</i> (O-6-Methylguanine DNA methyltransferase)	One carbon metabolism DNA methylation	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>MTHFD1</i> (methylene tetrahydrofolate dehydrogenase/ methylene tetrahydrofolate-cyclohydrolase/ formyl tetrahydrofolate synthetase)	Folate metabolism	(50)	Irish	176 NTD cases (Mostly SB, few encephalocele). 245 mothers, 127 fathers (also includes parents of anencephalic cases). 770 controls	Case-control to evaluate Arg653Gln (1958G>A; dbSNP rs 1801133) polymorphism	Maternal AA genotype confers increased risk to offspring
		(51)	Italian	142 NTD cases (open and closed SB) (125 mothers, 108 fathers). 523 controls.	Case-control study and family based association study (TDT) for 1958G>A	Mildly increased risk for AA and GA genotypes in cases (and mothers). TDT shows excess transmission of A allele to cases
		(52)	Dutch	103 SB cases, 113 mothers, 98 fathers. 460 controls.	Case-control and TDT for 1958G>A	No association
		(54)	Irish	509 cases (with 485 mothers and 439 fathers)—mostly open SB. 966 controls.	Case-control study for SNP rs1076991C>T	Promoter variant not independent risk factor. Case and maternal risk factor when combined with Arg653Gln variant
		(56) (103)	Dutch Mixed USA	180 SB patients, 190 controls 259 SB cases, 359 controls	Case-control study Case-control (10 SNPs)	No association Increased SB risk for one SNP, decreased risk for three others
		(41)	Mixed UK	126 SB (open); 103 SB (closed); 49 anencephalic; 192 controls	Case-control study	No association
		<i>MTHFD2</i>	Folate metabolism	(56) (103)	Dutch Mixed USA	180 SB patients, 190 controls 259 SB cases, 359 controls
<i>MTHFR</i> (5,10-methylene tetrahydrofolate reductase)	Folate metabolism	(116)	UK	200 NTD cases (186 mothers, 92 fathers). 578 child, 512 mother controls	Case-control study	Mildly elevated risk for 677C>T. No association for 1298A>C
		(123)	Polish	20 NTD cases, 262 controls.	Case-control for C677T and A1298C	No association
		(124)	Irish	471 cases (451 SB, 20 encephalocele). Triads comprise >1300 samples. 922 controls.	Mutation screen. Case-control and TDT for 116C>T (P39P) and 1793G>A (R594Q)	Possible association with SB for variants tested. Unlikely to be independent risk factors (association likely due to linkage disequilibrium with 677C>T)
		(125)	Mixed USA	350 cases, 328 mothers, 245 fathers, 167 siblings	Family-based association study	No association for 677C>T
		(126)	Italian	15 cases (open SB), 18 fathers, 60 mothers. 43 control children and 100 control adults	Case-control screen for C677T and A1298C	T allele more frequent in cases than controls. A1298C not different between groups
		(127)	Mexican	118 NTD case mothers (all anencephalic), 112 control mothers	Case-control study for C677T and A1298C	Maternal TT confers higher risk than CC for anencephaly. A1298C not associated with NTDs
(128)	Indian	83 mothers of NTD cases. 60 controls	Case-control for C677T and A1298C	677T more frequent in mothers of cases than in controls (only for lower defects). No difference in A1298C frequency		

Continued

Table 1. Continued

Human gene	Type of candidate	Reference	Population studied	Sample size	Type of study	Summary of results/conclusion
		(129)	Mexican (Yucatan)	108 cases (97 SB, 4 anencephalic, 7 encephalocele), with 147 parents. 120 controls	Case-control, screen for A1298C	No association
		(130)	French	77 NTD mothers. 61 controls	Case-control study	No association for 677C>T. Reduced risk for 1298A>C allele
		(131)	Chinese	38 mothers of NTD cases. 80 controls	Case-control to evaluate 677C>T	TT genotype less frequent in case mothers (but small numbers)
		(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association for 677C>T or 1298A>C
		(103)	Mixed USA	259 SB cases, 359 controls	Case-control (13 SNPs)	Increased risk, OR2.0, of SB associated with 677C>T
		(41)	Mixed UK	126 SB (open); 103 SB (closed); 49 anencephalic; 192 controls	Case-control for C677T and A1298C	Slight protective effect for open SB of 677TT genotype
<i>MTHFS</i> (5,10-methylenetetrahydrofolate synthetase)	Folate metabolism	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>MTR</i> (methionine synthase)	One carbon metabolism (homocysteine remethylation)	(105)	Hispanic USA	43 NTD cases, 122 mothers. 124 control infants and 127 mothers	Case-control study for 2756A>G	Not independent risk factor. May be associated with NTDs in combination with MTRR 66A>G
		(130)	French	77 NTD mothers. 61 controls	Case-control study	No association for 66A>G
		(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
		(103)	Mixed USA	259 SB cases, 359 controls	Case-control study	No association with SB for 21 SNPs tested (including 2756A>G)
		(41)	Mixed UK	126 SB (open); 103 SB (closed); 49 anencephalic; 192 controls	Case-control study	No association
<i>MTRR</i> (methionine synthase reductase)	One carbon metabolism (homocysteine remethylation)	(105)	Hispanic USA	43 NTD cases, 122 mothers. 124 control infants and 127 mothers	Case-control study for 66A>G (I22M)	G allele associated with increased risk. Additional risk in combination with MTR 2756G allele
		(116)	UK	201 NTD cases (203 mothers, 88 fathers). 601 child, 532 mother controls	Case-control study	Mildly reduced risk associated with 66A>G
		(132)	Irish	575 NTD families 95% SB). 487 controls.	Case-control and family-based analysis for three variants	No association for 66A>G (I22M) with SB risk (except possible paternal effect). No association for S175L or K350R
		(133)	Dutch	109 cases (open SB) and parents. 234 control children, 292 control women.	Case-control screen for 66A>G	In this study and meta-analysis (including previous studies) maternal GG is associated with increased risk in offspring, but GG in child not associated with NTDs
		(130)	French	77 NTD mothers. 61 controls	Case-control study	Marginally increased risk associated with 66G allele
		(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
		(103)	Mixed USA	259 SB cases, 359 controls	Case-control study	Modest increase in SB risk for three linked SNPs, but not 66A>G

		(41)	Mixed UK	126 SB (open); 103 SB (closed); 49 anencephalic; 192 controls	Case-control study	Marginal increased risk for combined NTDs
<i>MUT</i> (methylmalonyl-CoA mutase)	One carbon metabolism	(134)	Irish	279 NTD triads (mostly SB), 256 controls	Case-control and family-based study	No association with NTDs for three variants tested
<i>NATI</i> (N-acetyltransferase 1)	Folate metabolism and acetylation reactions	(135)	Mixed USA	354 NTD families	Family based association study	1095C>A not associated with SB risk (except in combination with maternal smoking)
		(136)	Mixed USA	374 NTD families	Family based association study	Composite genotype (6 SNPs) related to reduced function associated with lower SB risk for cases and mothers
<i>NAT2</i> (N-acetyltransferase 2)		(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>NNMT</i> (Nicotinamide N-methyltransferase)	Methylation reactions	(137)	Mixed USA	252 SB cases, 335 controls	Case-control study	No association between NNMT variants and SB risk
<i>NCAM1</i> (neural cell adhesion molecule1)	Embryonic cell adhesion	(56) (138)	Dutch USA	180 SB patients, 190 controls 204 SB families	Association study Family based association study	No association Risk of SB associated with intronic SNP (of 11 tested) in first 132 families, not in further 72 families
<i>NOS1</i> (nitric oxide synthase 1)	Possible effect on one carbon metabolism	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association for repeat polymorphisms
<i>NOS2</i> (nitric oxide synthase 2A)		(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association for SNPs and repeat polymorphism
<i>NOS3</i> (nitric oxide synthase 3 endothelial)		(139)	Mixed US	301 families with SB	Family based association study	No association by TDT analysis. Maternal 894G>T associated with SB risk by log-linear modelling
		(140)	Dutch	109 SB cases, 121 mothers, 103 fathers. 500 controls	Case-control and TDT	894G>T not independent risk factor. Possible risk interaction with <i>MTHFR</i> 677TT
		(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association detected for SNPs and repeat polymorphism
<i>hOGG1</i> (8-hydroxyguanine DNA-glycosylase1)	DNA repair	(103)	Mixed USA	259 SB cases, 359 controls	Case-control study	No association
<i>PAX3</i>	NTDs in mouse mutant	(112)	Mixed USA	380 SB cases, 350 controls	Case-control study	No association
		(70)	Mixed USA	74 SB cases, 87 controls	Mutation screen and case-control study	rs16863657 and related haplotype associated with increased SB risk
<i>PCMT1</i> (l-isoaspartate O-methyltransferase)	Methylation reactions	(141)	Mixed USA	152 SB cases, 423 controls	Case-control study of Ile120Val	Val/Val genotype associated with possible reduction in SB risk
<i>PDGFRA</i> (Platelet derived growth factor receptor alpha)	NTDs in <i>Patch</i> mouse mutant	(56) (142)	Dutch US Hispanic	180 SB patients, 190 controls 43 NTD cases, 122 NTD mothers. 124 control infants, 127 control mothers	Case-control study Case-control study for promoter haplotypes	No association for Ile120Val P1 promoter haplotypes with lower activity may be associated with maternal risk. Case numbers too small for conclusion
		(143)	Mixed USA	407 parent-child triads (SB). 164 controls	Case-control and family based association (TDT)	No association for P1 promoter haplotypes
		(108)	Dutch	88 SB cases, 56 SB mothers. 74 controls, 72 control mothers	Evaluated H1 and H2 promoter haplotypes	H1 promoter may be more frequent in cases than controls. Suggestion that BMI, glucose and inositol differentially interact with H1/H2
<i>PEMT</i> (Phosphatidylethanolamine N-methyltransferase)	One carbon and choline metabolism	(144)	Mixed USA	360 SB cases, 595 controls	Case-control study	No association with SB for two non-synonymous SNPs

Continued

Table 1. Continued

Human gene	Type of candidate	Reference	Population studied	Sample size	Type of study	Summary of results/conclusion
<i>PRKACA</i> , <i>PRKACB</i> (cAMP-dependent protein kinase A catalytic subunits)	NTDs in mouse mutants	(145)	Mixed USA	207 SB cases, 209 controls	Mutation screen and case-control study	No mutation. No association
<i>PRMT1</i> (Protein arginine methyltransferase 1)	Methylation reactions	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>PRMT2</i> (Protein arginine methyltransferase 2)	Methylation reactions	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>PYCT1A</i> (CTP:phosphocholine cytidyltransferase)	One carbon metabolism	(118)	Mixed USA	103 SB cases, 338 controls	Case-control study	Weak association with SB risk for 1 of 2 SNPs studied
<i>RNMT</i> (RNA (guanine-7-) methyltransferase)	Methylation reactions	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association
<i>SARDH</i> (Sarcosine dehydrogenase)	One carbon metabolism	(56)	Dutch	180 SB patients, 190 controls	Case-control study	Nominally significant association with two synonymous SNPs ^a
<i>SHMT</i> (Serine hydroxyl methyltransferase I)	One carbon metabolism	(146)	UK	97 NTD mothers, 190 controls	Case-control study	1420C>T associated with protective maternal effect
<i>SLCA19A1</i> , <i>RFC-1</i> (reduced folate carrier),	Folate transport	(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association for Leu474Phe
		(116)	UK	206 NTD cases (186 mothers, 92 fathers), 578 child, 512 mother controls	Case-control study	No association for 80G>A (H27R)
		(125)	Mixed USA	350 cases, 328 mothers, 245 fathers, 167 siblings.	Family-based association study	No association for 80G>A
		(147)	Irish	437 NTD families, 852 controls	Case-control and family based association	No association for 80G>A. 61-bp polymorphism under-represented in cases
		(131)	Chinese	38 mothers of cases. 80 controls	Case-control for 80G>A	Association of GG genotype with risk of NTD offspring
		(148)	Chinese	104 NTD families, 100 control families	Case-control for 80G>A	Elevated risk for GG genotype of cases or mother (if folic acid not used)
		(56)	Dutch	180 SB patients, 190 controls	Case-control study	Nominally significant association for 80AA genotype and reduced risk ^a
		(103)	Mixed USA	259 SB cases, 359 controls	Case-control study	No association with risk of SB for 6 SNPs tested. 80G>A not tested
		(41)	Mixed UK	126 SB (open); 103 SB (closed); 49 anencephalic; 192 controls	Case-control study	No association
		<i>SOD2</i> (superoxide dismutase 2)	Oxidative stress	(115)	Mixed USA	507 SB cases, 185 controls
<i>T</i> (Brachyury)	Axial development in mouse	(149)	Mixed USA	316 SB families	Family-based association study	<i>TIVS7</i> T/C allele more frequent in cases than expected
<i>TCNII</i> (transcobalamin II)	One carbon metabolism	(150)	Irish	~350 NTD families, ~700 controls.	Case-control and family based association study	No association with SB risk for 6 SNPs
		(130)	French	77 NTD mothers. 61 controls	Case-control study	No association for 776C>G
		(56)	Dutch	180 SB patients, 190 controls	Case-control study	No association for Arg259Pro
<i>TXN2</i> (thioredoxin2)	NTDs (SB) in mouse knockout	(151)	Mixed USA	48 SB cases, 48 controls	Case-control study	A2 promoter allele associated with risk of NTDs but small sample size
<i>TP53</i> (p53)	NTDs in mouse mutant	(152)	Irish	549 NTD cases, 532 mothers, 481 fathers. 999 controls	Case-control and family based association studies	Two non-coding variants associated with case NTD risk and two variants with maternal risk (but no multiple testing correction)
<i>TRDMT1</i> (tRNA aspartic acid methyltransferase 1)	One carbon metabolism	(115)	Mixed USA	507 SB cases, 185 controls	Case-control study	No association
		(56)	Dutch	180 SB patients, 190 controls	Case-control study	Nominally significant association for one SNP ^a

<i>TYMS</i> (thymidylate synthase)	Folate metabolism and pyrimidine biosynthesis	(110)	Mixed USA	264 SB cases, 259 controls.	Mutation screen and case-control for 28-bp repeat in 5'-UTR and 6-bp deletion in 3'-UTR	3'-UTR polymorphism associated with increased SB risk in non-Hispanic white population. Further increased risk with 5'-UTR polymorphism
		(153)	UK	197 NTD cases, 194 mothers, 93 fathers, 179 control infants, 177 control mothers	Case-control study of 28-bp repeat	No association for TYMS
		(56) (103)	Dutch Mixed USA	180 SB patients, 190 controls 259 SB cases, 359 controls	Case-control study Case-control study	No association Modest increase in SB risk for three linked SNPs (of 5 tested)
<i>UCP2</i> (uncoupling protein 2)	Energy metabolism Previous study indicates association with NTDs	(154)	Irish	169 cases, 163 mothers, 167 fathers	Evaluated 866G/A, A55V, 3'-UTR 45-bp ins/del	UCP2 not associated with NTD risk. Different frequency of risk allele in control population compared with previous study
<i>VANGL1</i> (van gogh-like 1)	PCP gene homologue; Parologue of <i>VANGL2</i>	(95)	Mixed UK and USA	66 (21 craniorachischisis, 24 SB, 21 anencephalic) and 200 controls	Mutation screen	No causative mutations. One missense variant, present in controls
		(96)	Italian and French	144 (80 SB, 7 craniorachischisis, 22 closed spinal dysraphism, 35 caudal regression). 172 Italian and CEPH controls	Mutation screen	Three missense mutations (two in myelomeningocele, R274Q and M328T; 1 in caudal regression, V239I). V239I has functional effect on interaction with Dvl proteins
		(97)	Italian and USA	673 cases (15 open cranial dysraphisms, 456 SB, 202 closed spinal dysraphisms)	Mutation screen	Ten missense variants in 13 individuals (absent in 1187–1462 controls). Five in highly conserved residues (two in myelomeningocele, three in closed spinal dysraphism/caudal regression syndrome)
<i>VANGL2</i> (van gogh-like 2)	Mouse model: Craniorachischisis in <i>loop-tail</i> mice	(95)	Mixed UK and USA	66 (21 craniorachischisis, 24 SB, 21 anencephalic) and 200 controls	Mutation screen	No causative mutations identified. 7 bp duplication in intron six in one craniorachischisis
		(96)	Italian and French	144 (80 SB, 7 craniorachischisis, 22 closed spinal dysraphism, 35 caudal regression) and 172 Italian and CEPH controls	Mutation screen	No coding mutations
<i>XPB</i> (DNA excision repair protein ERCC-2)	DNA repair	(112)	Mixed USA	380 SB cases, 350 controls	Case-control study	Mildly elevated risk associated with 751Gln
<i>XRCC1</i> (X-ray repair cross-complementing)	DNA repair	(112)	Mixed USA	380 SB cases, 350 controls	Case-control study	No association
<i>XRCC3</i> (X-ray repair cross-complementing)	DNA repair	(112)	Mixed USA	380 SB cases, 350 controls	Case-control study	No association
<i>ZIC1</i>	Brain defects in mouse mutant	(155)	Dutch	117 NTDs (SB, Anencephaly, Encephalocele), 364 controls	Mutation screen	No mutations
<i>ZIC2</i>	NTDs in mouse mutant	(155)	Dutch	117 NTDs (SB, Anencephaly, Encephalocele), 364 controls	Mutation screen; case-control study	Alanine deletion in one patient. Frequent polymorphism (1059C>T) has no association
<i>ZIC3</i>	NTDs in mouse mutant	(155)	Dutch	117 NTDs (SB, Anencephaly, Encephalocele), 364 controls	Mutation screen	One silent variant (858G>A) in one patient

^aNominally significant association which does not stand after correction for multiple testing. SB, defined as spina bifida (myelomeningocele) in study criteria. For studies labelled NTDs, populations were mixed (multiple types of NTDs) or undefined.

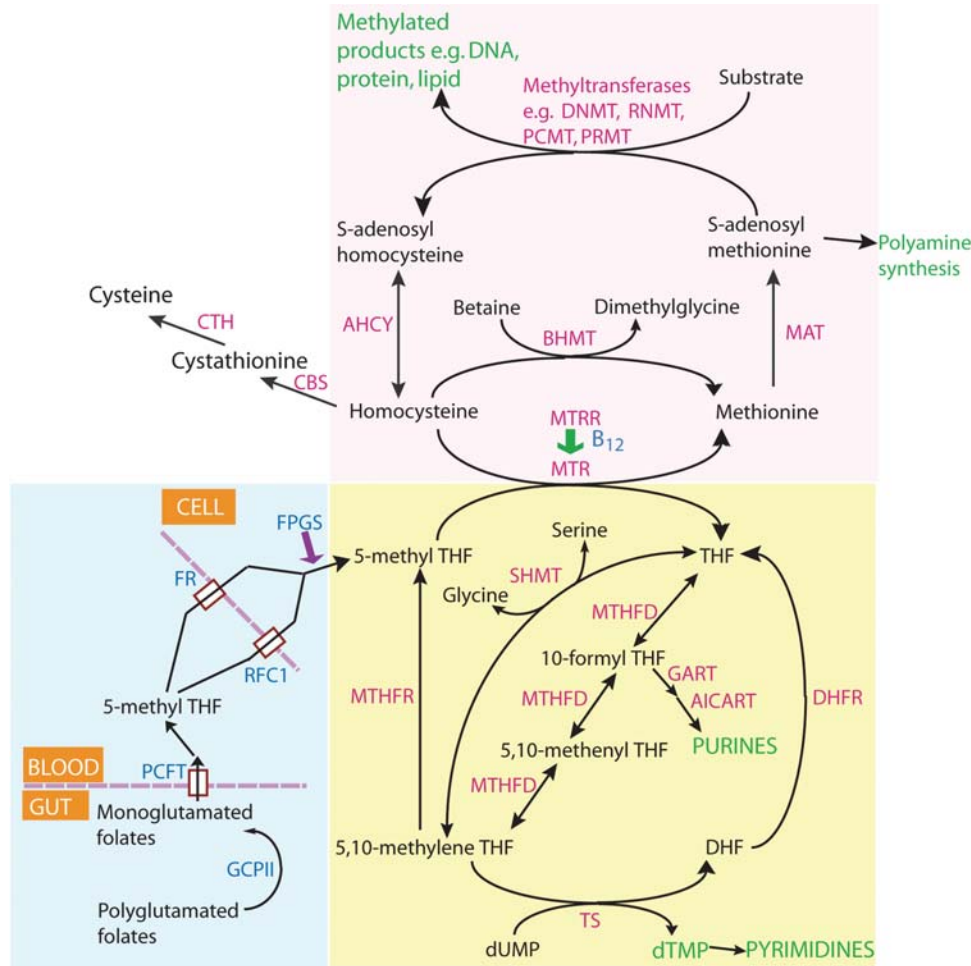


Figure 1. Summary of folate one-carbon metabolism showing the main pathways and reactions that have been subject to analysis in the context of NTDs. Blue shading: proteins involved in processing of folates in the digestive tract, transport and cellular retention (by addition of glutamates). Yellow shading: the major part of the cycle involving transfer of 1C groups between folate molecules, as required for purine and pyrimidine biosynthesis. Pink shading: reactions of the methylation cycle. For clarity, mitochondrial reactions that include generation of formate and cleavage of glycine have been omitted. For explanation of abbreviations, see Table 1.

Folate cycle enzymes required for nucleotide biosynthesis

MTHFD1 encodes the cytoplasmic trifunctional C1THF synthase enzyme. A polymorphism (1958G>A; rs2236225) which results in an R653Q substitution in the 10-formylTHF synthetase domain was found to be both a maternal and NTD case risk factor, in the Irish and Italian populations (49–51), although not in the Dutch (51,52) or British (41). The R653Q polymorphism causes reduced C1THF synthase activity in cell lines, resulting in diminished purine biosynthesis (53). A promoter polymorphism (rs1076991C>T) in *MTHFD1*, that reduces transcriptional activity *in vitro*, was also associated with NTD case and maternal risk, in combination with R653Q (54).

Folate transport

Another attractive group of candidate genes are those encoding proteins required for transport, uptake and cellular retention of folates. This includes folate receptors *FR α* (*Folr1* in

mice), *FR β* and *FR γ* , *RFC1* (reduced folate carrier), *GCPII* (folyl- γ -glutamate carboxypeptidase) and *FPGS* (folylpolyglutamate synthetase) (32,33). Increased risks associated with variants in *RFC1* and *GCPII* are not reproduced in all studies (Table 1), although the recently identified proton-coupled folate transporter *PCFT* (*SLC46a1*) is not required for embryonic survival or neural tube closure in mice (55), but has not yet been investigated in humans. A recent case–control study revealed a possible association with reduced risk of spina bifida for a polymorphism in *CUBN* (Cubulin), which encodes a membrane-associated multi-ligand endocytic receptor expressed in the neural folds and yolk sac (56). Together, cubulin and its partner protein megalin are involved in binding and endocytic uptake of a large number of different proteins, several of which could be important for neural tube closure, including the intrinsic factor-cobalamin complex (IF-B₁₂) and folate binding protein (folate receptor) (57,58). Intriguingly, *Cubn* was one of the most up-regulated genes in a microarray analysis of *Rfc1* null mouse embryos (59), which may

reflect a compensatory mechanism to enhance endocytic folate uptake via Folr1. Hence, *CUBN* merits further attention as a potential risk factor, especially in conjunction with *RFC1*.

In view of the apparent resilience of mouse neurulation to specific genetic disturbance of the methylation cycle, analysis of compound mutants with other folate-related or NTD susceptibility alleles would be of considerable interest. In our analysis of NTD cell lines, impaired folate cycle activity did not correlate with known variants in *MTHFR*, *MTHFD1*, *DHFR*, *GCPII*, *MTR*, *MTRR* or *RFC1* (34), encouraging the view that currently unknown genetic influences on folate metabolism remain to be identified in many NTD cases.

CANDIDATE GENES FROM THE MOUSE

The potential complexity of NTD genetics is illustrated by the fact that 200 or more different mouse genes result in NTD phenotypes either through naturally occurring, induced or targeted mutations (2,25). Many of the NTD-causing mouse mutations implicate specific signalling pathways such as non-canonical Wnt signalling (see below), maintenance of the cell cycle, regulation of the actin cytoskeleton, chromatin organization or epigenetic modifications including methylation and acetylation. Recently, NTDs were observed in mice null for *Mib2* (60), *Smurf1/2* (61) and *Hectd1* (62), which all encode E3 ubiquitin ligases, suggesting a possible role in neurulation for protein ubiquitination and targeted degradation. The human homologues of some of these mouse NTD genes have been examined in case-control association studies or directly sequenced in mutation screens, although with very few significant findings to date (Table 1).

It is important to ask how appropriate are the mouse models as paradigms for human NTDs? At the embryonic level, the events of neurulation appear extremely similar between mice and humans. For example, the initial fusion event, Closure 1, occurs at a closely similar stage and body axial level in both species, as does initiation of closure in the forebrain (Closure 3) and completion of spinal closure at the posterior neuropore. One point of variation concerns *de novo* initiation of closure at the forebrain/midbrain boundary (Closure 2 in mice) which may be absent from human neurulation (63). Hence, brain closure could be a rather simpler process in humans than mice.

Another potential difference between mouse models and human NTDs is that many gene-specific homozygous null mouse embryos exhibit phenotypes additional to NTDs, such as prenatally lethal heart defects. Such syndromic examples do not appear particularly close models for human NTDs which are primarily non-syndromic (64). On the other hand, detailed analysis of a few of the mouse mutants suggests that isolated NTDs can also result from the effect of hypomorphic alleles, combinations of heterozygous mutations, genetic background effects and/or gene-environmental interactions. This partial loss of function or multifactorial aetiologies may more closely resemble human NTDs. For example, NTDs in *spotch* mice result from homozygosity for mutations in *Pax3* (23,65) but can also occur, or be exacerbated, as a result of interaction with mutations in other genes including

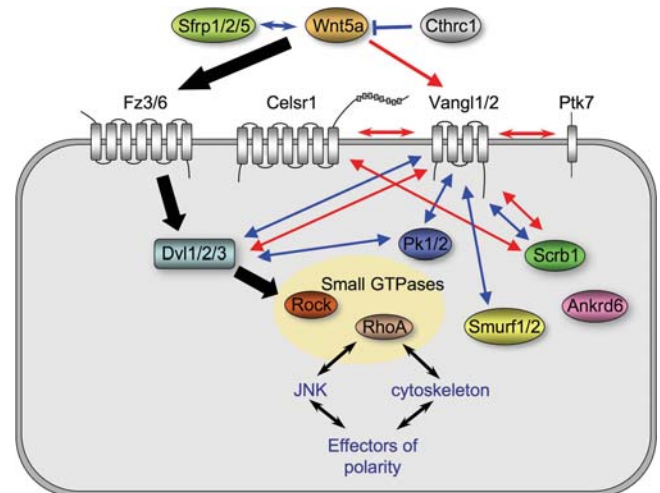


Figure 2. Diagrammatic representation of non-canonical Wnt signalling in a mammalian cell. Black arrows indicate the signalling pathway necessary for establishment of planar cell polarity (PCP). Known biochemical interactions are indicated by blue arrows and genetic interactions are shown by red arrows. Ankr6 is the mammalian homologue of Diego which in *Drosophila* interacts with Fz, Vang and Pk, but has not been studied in vertebrates.

neurofibromin1 (66) and *grainyhead-like-3* (67). Environmental factors including folate deficiency and arsenic can exacerbate NTDs in homozygous *spotch* mutants, or induce NTDs in the usually unaffected heterozygotes (24,68). Although association studies in humans have provided little evidence to implicate *PAX3* mutations in human NTDs (69,70), the possible contribution of gene-gene and gene-environment interactions indicates that larger scale studies may be needed before a role for *PAX3* in human NTDs can be completely ruled out.

The *curly tail* mouse also exhibits features typical of the multifactorial aetiology of human NTDs (71). Spinal NTDs are partially penetrant in homozygous *ct/ct* mutant embryos, with the frequency of defects strongly affected by genetic modifiers (72). The major *ct* gene is a hypomorphic allele of *Grhl3*, whose knockouts display completely penetrant spina bifida (73–75). The *ct* mutation appears to affect a regulatory region, emphasising the need for consideration of possible non-coding mutations in human NTDs. Moreover, there is a strong effect of environmental factors, including a protective effect of supplemental inositol (76). A key role for inositol in neural tube closure is supported by the finding that inositol deficiency *in vitro* causes NTDs (77), inositol may prevent diabetes-associated NTDs (78) and the recent finding of NTDs in embryos carrying a hypomorphic allele of inositol 1,3,4-trisphosphate 5/6-kinase (*Itpk1*), a key enzyme in inositol phosphate metabolism (79).

Planar cell polarity signalling and NTDs

A major advance in understanding the genetic basis of neurulation has been the finding that initiation of closure at the hindbrain-cervical boundary (Closure 1) requires non-canonical Wnt signalling: the so-called planar cell polarity (PCP) pathway (Fig. 2). PCP signalling was defined originally

Table 2. Genes of the planar cell polarity pathway—involvement in mouse NTDs

Gene	Mutant/genotype	Protein	NTD phenotype	References
Single gene effects				
<i>Vangl2</i>	<i>Loop-tail (Lp, Lp^{m1Jus}); Lp/Lp</i>	Transmembrane protein	Craniorachischisis. Occasional spina bifida in <i>Lp/+</i>	(91,92,156,157)
<i>Scrb1</i>	<i>Circle-tail; Crc/Crc</i>	Cytoplasmic polarity protein	Craniorachischisis	(93)
<i>Celsr1</i>	<i>Crash; Crsh/Crsh</i>	Seven-pass transmembrane protein	Craniorachischisis	(158)
	<i>Spin-cycle; Scy/Scy</i>		Craniorachischisis	
<i>Ptk7 (CKK-4)</i>	<i>Ptk7^{mu/mu} (truncating gene-trap allele)</i>	Transmembrane receptor tyrosine kinase-like protein	Craniorachischisis	(94)
Paralogous gene interactions				
<i>Dvl1/2</i>	<i>Dvl1^{-/-}; Dvl2^{-/-}</i>	Cytoplasmic signalling proteins	Craniorachischisis	(159)
<i>Dvl2/3</i>	<i>Dvl2^{-/-}; Dvl3^{+/-} (Dvl2^{-/-}; 3^{-/-} die before E8.5)</i>		Craniorachischisis	(101)
<i>Fz3/6</i>	<i>Fz3^{-/-}; Fz6^{-/-}</i>	Transmembrane receptor protein	Craniorachischisis	(160)
<i>Smurf1/2</i>	<i>Smurf1^{-/-}; Smurf2^{-/-}</i> <i>Smurf1^{-/-}; 2^{+/-} or 1^{+/-}; 2^{-/-}</i>	Ubiquitin ligase	No closure at E8.5 Spina bifida and/or exencephaly	(61)
Interactions between different genes				
<i>Vangl2/Scrb1</i>	<i>Lp/+; Crc/+</i>	See above	Craniorachischisis	(102)
<i>Vangl2/Dvl3</i>	<i>Lp/+; Dvl3^{-/-}</i> <i>Lp/+; Dvl3^{+/-}</i>	See above	Craniorachischisis Craniorachischisis or exencephaly	(101)
<i>Vangl2/Vangl1</i>	<i>Lp/+; Vangl1^{gt/+}</i>	Transmembrane protein	Craniorachischisis	(98)
<i>Vangl2/Ptk7</i>	<i>Lp/+; Ptk7^{+mu}</i>	See above	Spina bifida	(94)
<i>Vangl2/Wnt5a</i>	<i>Lp/+; Wnt5a^{-/-}</i>	Secreted signalling protein	Craniorachischisis	(161)
<i>Vangl2/Sfrp1,2,5</i>	<i>Lp/+; Sfrp1^{-/-}; Sfrp2^{-/-}; Sfrp5^{+/-}</i> <i>Lp/+; Sfrp1^{-/-}; Sfrp2^{+/-}; Sfrp5^{-/-}</i>	Secreted Wnt antagonist	Craniorachischisis Spina bifida	(162)
<i>Vangl2/Cthrc1</i>	<i>Lp/+; Cthrc1^{LacZ/LacZ}</i>	Secreted glycoprotein, Wnt co-factor	Exencephaly	(100)
<i>Vangl2/Grhl3</i>	<i>Lp/+; Grhl3^{ct/ct}</i>	Transcription factor	Severe spina bifida	(163)
<i>Vangl2/cobl</i>	<i>Lp/+; cobl^{C101/C101}</i>	Actin nucleator	Exencephaly	(99)

in *Drosophila*, as a genetic cascade involving the transmembrane receptor frizzled and the cytoplasmic protein dishevelled, but without a requirement for β -catenin (80–83). This pathway is required to specify planar polarity in epithelia including the wing and compound eye. In vertebrates, non-canonical Wnt signalling is highly conserved, underpinning tissue and cellular polarity during morphogenesis in systems as diverse as gastrulation and the coordinated orientation of stereociliary bundles in inner ear hair cells (84–90).

A potential role for PCP in NTDs first came to light following positional cloning of *Vangl2* (the homologue of *Drosophila strabismus/Van gogh*) in the *loop-tail* mouse mutant which exhibits the severe NTD, craniorachischisis (91,92). Subsequently, the same NTD phenotype was found in other mouse mutants and targeted gene knockouts (Table 2) almost all of which have been implicated biochemically in the PCP pathway (e.g. *Celsr1*, *Dvl*) or which interact genetically with recognized PCP genes (e.g. *Scrb*, *Ptk7*) (93,94). Interestingly, the double knockout for *Smurf1* and *Smurf2* was recently found to display craniorachischisis and other characteristic PCP defects. These genes encode ubiquitin ligases whose targets include Prickle1 (Fig. 2), supporting the crucial nature of PCP signalling for initiation of neural tube closure (61).

In view of these findings in mice, PCP genes emerge as excellent candidates for causation of craniorachischisis in humans. Nevertheless, sequence analysis has so far failed to identify mutations in human *VANGL2* or its paralogue *VANGL1* in a group of patients with craniorachischisis (95,96). Reports of other PCP gene analysis in similar patients are awaited. Although craniorachischisis is the obvious NTD phenotype for study, the *VANGL* genes have also been

analysed in patients with anencephaly and open and closed spina bifida. No mutations were reported in *VANGL2* (95,96) but several highly conserved and unique, heterozygous missense variants were identified in *VANGL1* in patients with either myelomeningocele or closed spina bifida, as well as caudal regression syndrome (96,97). To date a functional effect has been demonstrated for one of these putative mutations, where V239I (identified in caudal regression syndrome) results in loss of interaction between *VANGL1* and *DVL* proteins (96).

Interestingly, loss of *Vangl1* function is insufficient to cause NTDs in mice, although compound heterozygotes with *Vangl2* (*loop-tail*) develop craniorachischisis (98). Nevertheless, there is increasing evidence that PCP genes can contribute to NTDs other than craniorachischisis (Table 2). For example, double heterozygotes carrying both *Vangl2* and *Ptk7* develop spina bifida (94) although *Vangl2* double mutants with *cordobleu^{C101}* or *Cthrc1* develop exencephaly (99,100). In contrast, *Vangl2:Scrb* and *Vangl2:Dvl3* double heterozygotes develop craniorachischisis (101,102). It remains to be determined why *Vangl2* displays this variable phenotypic behaviour when combined with different PCP and other mutants. Hence, although non-canonical Wnt signalling has been firmly linked with Closure 1 in mice, it is possible that genes in this pathway play more diverse roles in human neural tube closure.

CONCLUSIONS

The identification of genetic risk factors for human NTDs is complicated by the multiplicity of genes participating

in neurulation, and the importance of gene–environment interactions. Sequence analysis of candidate genes implicated from their role in mouse models has revealed putative mutations in a few genes, but each in only a small number of patients. Association studies of common polymorphic variants, particularly related to folate one-carbon metabolism, indicate risk factors such as *MTHFR*. However, no specific folate-related gene has yet been implicated as a major determinant of risk for NTDs. Large-scale studies will be required to provide sufficient statistical power to convincingly test whether such variants are truly NTD susceptibility factors (56,103). It will also be essential, to evaluate multiple genes (folate-related and others) in the same individuals in order to detect possible compounding effects of combinations of risk alleles that, individually, might not be statistically significant (11,39). To date, very few studies have been sufficiently large to overcome issues of multiple testing bias in screening for gene–gene interactions (39,56,104). Examination of specific hypotheses may be fruitful where fewer NTD cases are available, particularly if combined with stratified sample sets in which cases are sub-divided on the basis of phenotype. For example, NTDs with abnormal folate metabolism have enabled a combined analysis of *MTR* and *MTRR* (105), and fetuses with craniorachischisis provide a focus for determining the role of *PCP* genes. Gene–environment interactions appear likely to contribute to NTD predisposition, with examples including interactions of *MTHFR* with multivitamin use (106), *MTRR* with vitamin B₁₂ (107) and *PDGFRA* with inositol and zinc (108).

One limitation of the association studies of multiple folate-related candidate genes in NTDs is the predominant focus on known polymorphisms. In future, it will be necessary also to consider the possible existence of ‘private’ disease-causing mutations. Moreover, the potential for deleterious gene expression changes resulting from promoter mutations or copy number variation has been addressed in relatively few studies (10,108–110). Emerging technologies for high throughput sequencing and analysis of genomic deletions and copy number variations (111) offer the prospect, in the coming years, of progress in identification of candidate genes and screening for novel mutations in human NTDs.

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Conflict of Interest statement. None declared.

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REFERENCES

1. Dtrait, E.R., George, T.M., Etchevers, H.C., Gilbert, J.R., Vekemans, M. and Speer, M.C. (2005) Human neural tube defects: developmental biology, epidemiology, and genetics. *Neurotoxicol. Teratol.*, **27**, 515–524.
2. Harris, M.J. and Juriloff, D.M. (2007) Mouse mutants with neural tube closure defects and their role in understanding human neural tube defects. *Birth Defects Res. A Clin. Mol. Teratol.*, **79**, 187–210.
3. Kennedy, D., Chitayat, D., Winsor, E.J.T., Silver, M. and Toi, A. (1998) Prenatally diagnosed neural tube defects: ultrasound, chromosome, and autopsy or postnatal findings in 212 cases. *Am. J. Med. Genet.*, **77**, 317–321.
4. Chen, C.P. (2007) Chromosomal abnormalities associated with neural tube defects (I): full aneuploidy. *Taiwan J. Obstet. Gynecol.*, **46**, 325–335.
5. Chen, C.P. (2007) Chromosomal abnormalities associated with neural tube defects (II): partial aneuploidy. *Taiwan J. Obstet. Gynecol.*, **46**, 336–351.
6. Rampersaud, E., Bassuk, A.G., Enterline, D.S., George, T.M., Siegel, D.G., Melvin, E.C., Aben, J., Allen, J., Aylsworth, A., Brei, T. *et al.* (2005) Whole genomewide linkage screen for neural tube defects reveals regions of interest on chromosomes 7 and 10. *J. Med. Genet.*, **42**, 940–946.
7. Stamm, D.S., Rampersaud, E., Slifer, S.H., Mehlretter, L., Siegel, D.G., Xie, J., Hu-Lince, D., Craig, D.W., Stephan, D.A., George, T.M. *et al.* (2006) High-density single nucleotide polymorphism screen in a large multiplex neural tube defect family refines linkage to loci at 7p21.1-pter and 2q33.1–q35. *Birth Defects Res. A Clin. Mol. Teratol.*, **76**, 499–505.
8. Stamm, D.S., Siegel, D.G., Mehlretter, L., Connelly, J.J., Trott, A., Ellis, N., Zismann, V., Stephan, D.A., George, T.M., Vekemans, M. *et al.* (2008) Refinement of 2q and 7p loci in a large multiplex NTD family. *Birth Defects Res. A Clin. Mol. Teratol.*, **82**, 441–452.
9. Goetzinger, K.R., Stamilio, D.M., Dicke, J.M., Macones, G.A. and Odibo, A.O. (2008) Evaluating the incidence and likelihood ratios for chromosomal abnormalities in fetuses with common central nervous system malformations. *Am. J. Obstet. Gynecol.*, **199**, 285–286.
10. Gustavsson, P., Schoumans, J., Staaf, J., Borg, A., Nordenskjold, M. and Anneren, G. (2007) Duplication 16q12.1–q22.1 characterized by array CGH in a girl with spina bifida. *Eur. J. Med. Genet.*, **50**, 237–241.
11. Boyles, A.L., Hammock, P. and Speer, M.C. (2005) Candidate gene analysis in human neural tube defects. *Am. J. Med. Genet. C Semin. Med. Genet.*, **135**, 9–23.
12. Frey, L. and Hauser, W.A. (2003) Epidemiology of neural tube defects. *Epilepsia*, **44**, 4–13.
13. Mitchell, L.E. (2005) Epidemiology of neural tube defects. *Am. J. Med. Genet.*, **135C**, 88–94.
14. Carmichael, S.L., Witte, J.S. and Shaw, G.M. (2009) Nutrient pathways and neural tube defects: a semi-Bayesian hierarchical analysis. *Epidemiology*, **20**, 67–73.
15. Blom, H.J., Shaw, G.M., Den Heijer, M. and Finnell, R.H. (2006) Neural tube defects and folate: case far from closed. *Nat. Rev. Neurosci.*, **7**, 724–731.
16. Beaudin, A.E. and Stover, P.J. (2007) Folate-mediated one-carbon metabolism and neural tube defects: balancing genome synthesis and gene expression. *Birth Defects Res. C Embryo. Today*, **81**, 183–203.
17. Smithells, R.W., Sheppard, S., Schorah, C.J., Seller, M.J., Nevin, N.C., Harris, R., Read, A.P. and Fielding, D.W. (1980) Possible prevention of neural-tube defects by periconceptional vitamin supplementation. *Lancet*, **1**, 339–340.
18. Wald, N., Sneddon, J., Densen, J., Frost, C. and Stone, R.M.C. Vitamin Study Res Group (1991) Prevention of neural tube defects: results of the medical research council vitamin study. *Lancet*, **338**, 131–137.
19. Kirke, P.N., Mollooy, A.M., Daly, L.E., Burke, H., Weir, D.G. and Scott, J.M. (1993) Maternal plasma folate and vitamin B₁₂ are independent risk factors for neural tube defects. *Q. J. Med.*, **86**, 703–708.
20. Steegers-Theunissen, R.P.M., Boers, G.H.J., Trijbels, F.J.M., Finkelstein, J.D., Blom, H.J., Thomas, C.M.G., Borm, G.F., Wouters, M.G.A.J. and Eskes, T.K.A.B. (1994) Maternal hyperhomocysteinemia: a risk factor for neural tube defects. *Metabolism*, **43**, 1475–1480.
21. Mills, J.L., McPartlin, J.M., Kirke, P.N., Lee, Y.J., Conley, M.R., Weir, D.G. and Scott, J.M. (1995) Homocysteine metabolism in pregnancies complicated by neural-tube defects. *Lancet*, **345**, 149–151.

22. Scott, J.M. (1999) Folate and vitamin B12. *Proc. Nutr. Soc.*, **58**, 441–448.
23. Greene, N.D., Massa, V. and Copp, A.J. (2009) Understanding the causes and prevention of neural tube defects: insights from the splotch mouse model. *Birth Defects Res. A Clin. Mol. Teratol.*, **85**, 322–330.
24. Burren, K.A., Savery, D., Massa, V., Kok, R.M., Scott, J.M., Blom, H.J., Copp, A.J. and Greene, N.D.E. (2008) Gene–environment interactions in the causation of neural tube defects: folate deficiency increases susceptibility conferred by loss of *Pax3* function. *Hum. Mol. Genet.*, **17**, 3675–3685.
25. Copp, A.J., Greene, N.D.E. and Murdoch, J.N. (2003) The genetic basis of mammalian neurulation. *Nat. Rev. Genet.*, **4**, 784–793.
26. Dunlevy, L.P.E., Burren, K.A., Chitty, L.S., Copp, A.J. and Greene, N.D.E. (2006) Excess methionine suppresses the methylation cycle and inhibits neural tube closure in mouse embryos. *FEBS Lett.*, **580**, 2803–2807.
27. Dunlevy, L.P.E., Burren, K.A., Mills, K., Chitty, L.S., Copp, A.J. and Greene, N.D.E. (2006) Integrity of the methylation cycle is essential for mammalian neural tube closure. *Birth Defects Res. (Part A)*, **76**, 544–552.
28. Okano, M., Bell, D.W., Haber, D.A. and Li, E. (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*, **99**, 247–257.
29. VanAerts, L.A.G.J.M., Blom, H.J., Deabreu, R.A., Trijbels, F.J.M., Eskes, T.K.A.B., Peereboom-Stegeman, J.H.J.C. and Noordhoek, J. (1994) Prevention of neural tube defects by and toxicity of L-homocysteine in cultured postimplantation rat embryos. *Teratology*, **50**, 348–360.
30. Greene, N.D.E., Dunlevy, L.E. and Copp, A.J. (2003) Homocysteine is embryotoxic but does not cause neural tube defects in mouse embryos. *Anat. Embryol.*, **206**, 185–191.
31. Bennett, G.D., Vanwaes, J., Moser, K., Chaudoin, T., Starr, L. and Rosenquist, T.H. (2006) Failure of homocysteine to induce neural tube defects in a mouse model. *Birth Defects Res. B Dev. Reprod. Toxicol.*, **77**, 89–94.
32. Molloy, A.M., Brody, L.C., Mills, J.L., Scott, J.M. and Kirke, P.N. (2009) The search for genetic polymorphisms in the homocysteine/folate pathway that contribute to the etiology of human neural tube defects. *Birth Defects Res. A Clin. Mol. Teratol.*, **85**, 285–294.
33. Beaudin, A.E. and Stover, P.J. (2009) Insights into metabolic mechanisms underlying folate-responsive neural tube defects: a minireview. *Birth Defects Res. A Clin. Mol. Teratol.*, **85**, 274–284.
34. Dunlevy, L.P.E., Chitty, L.S., Doudney, K., Burren, K.A., Stojilkovic-Mikic, T., Stanier, P., Scott, R., Copp, A.J. and Greene, N.D.E. (2007) Abnormal folate metabolism in foetuses affected by neural tube defects. *Brain*, **130**, 1043–1049.
35. Frosst, P., Blom, H.J., Milos, R., Goyette, P., Sheppard, C.A., Matthews, R.G., Boers, G.J.H., Den Heijer, M., Kluijtmans, L.A.J., Van den Heuvel, L.P. *et al.* (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat. Genet.*, **10**, 111–113.
36. Van der Put, N.M.J., Steegers-Theunissen, R.P.M., Frosst, P., Trijbels, F.J.M., Eskes, T.K.A.B., Van den Heuvel, L.P., Mariman, E.C.M., Den Heijer, M., Rozen, R. and Blom, H.J. (1995) Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. *Lancet*, **346**, 1070–1071.
37. Shields, D.C., Kirke, P.N., Mills, J.L., Ramsbottom, D., Molloy, A.M., Burke, H., Weir, D.G., Scott, J.M. and Whitehead, A.S. (1999) The ‘thermolabile’ variant of methylenetetrahydrofolate reductase and neural tube defects: an evaluation of genetic risk and the relative importance of the genotypes of the embryo and the mother. *Am. J. Hum. Genet.*, **64**, 1045–1055.
38. Stegmann, K., Ziegler, A., Ngo, E.T.K.M., Kohlschmidt, N., Schröter, B., Ermert, A. and Koch, M.C. (1999) Linkage disequilibrium of MTHFR genotypes 677C/T-1298A/C in the German population and association studies in probands with neural tube defects (NTD). *Am. J. Med. Genet.*, **87**, 23–29.
39. Boyles, A.L., Billups, A.V., Deak, K.L., Siegel, D.G., Mehlretter, L., Slifer, S.H., Bassuk, A.G., Kessler, J.A., Reed, M.C., Nijhout, H.F. *et al.* (2006) Neural tube defects and folate pathway genes: family-based association tests of gene–gene and gene–environment interactions. *Environ. Health Perspect.*, **114**, 1547–1552.
40. De Marco, P., Calevo, M.G., Moroni, A., Arata, L., Merello, E., Finnell, R.H., Zhu, H., Andreussi, L., Cama, A. and Capra, V. (2002) Study of MTHFR and MS polymorphisms as risk factors for NTD in the Italian population. *J. Hum. Genet.*, **47**, 319–324.
41. Doudney, K., Grinham, J., Whittaker, J., Lynch, S.A., Thompson, D., Moore, G.E., Copp, A.J., Greene, N.D.E. and Stanier, P. (2009) Evaluation of folate metabolism gene polymorphisms as risk factors for open and closed neural tube defects. *Am. J. Med. Genet.*, **149A**, 1585–1589.
42. Amorim, M.R., Lima, M.A., Castilla, E.E. and Orioli, I.M. (2007) Non-Latin European descent could be a requirement for association of NTDs and MTHFR variant 677C>T: a meta-analysis. *Am. J. Med. Genet. A*, **143A**, 1726–1732.
43. Chen, Z.T., Karaplis, A.C., Ackerman, S.L., Pogribny, I.P., Melnyk, S., Lussier-Cacan, S., Chen, M.F., Pai, A., John, S.W.M., Smith, R.S. *et al.* (2001) Mice deficient in methylenetetrahydrofolate reductase exhibit hyperhomocysteinemia and decreased methylation capacity, with neuropathology and aortic lipid deposition. *Hum. Mol. Genet.*, **10**, 433–443.
44. Li, D., Pickell, L., Liu, Y., Wu, Q., Cohn, J.S. and Rozen, R. (2005) Maternal methylenetetrahydrofolate reductase deficiency and low dietary folate lead to adverse reproductive outcomes and congenital heart defects in mice. *Am. J. Clin. Nutr.*, **82**, 188–195.
45. Li, D., Pickell, L., Liu, Y. and Rozen, R. (2006) Impact of methylenetetrahydrofolate reductase deficiency and low dietary folate on the development of neural tube defects in splotch mice. *Birth Defects Res. A Clin. Mol. Teratol.*, **76**, 55–59.
46. Swanson, D.A., Liu, M.L., Baker, P.J., Garrett, L., Stitzel, M., Wu, J.M., Harris, M., Banerjee, R., Shane, B. and Brody, L.C. (2001) Targeted disruption of the methionine synthase gene in mice. *Mol. Cell. Biol.*, **21**, 1058–1065.
47. Elmore, C.L., Wu, X., Leclerc, D., Watson, E.D., Bottiglieri, T., Krupenko, N.I., Krupenko, S.A., Cross, J.C., Rozen, R., Gravel, R.A. *et al.* (2007) Metabolic derangement of methionine and folate metabolism in mice deficient in methionine synthase reductase. *Mol. Genet. Metab.*, **91**, 85–97.
48. Watanabe, M., Osada, J., Aratani, Y., Kluckman, K., Reddick, R., Malinow, M.R. and Maeda, N. (1995) Mice deficient in cystathionine β -synthase: Animal models for mild and severe homocyst(e)inemia. *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 1585–1589.
49. Brody, L.C., Conley, M., Cox, C., Kirke, P.N., McKeever, M.P., Mills, J.L., Molloy, A.M., O’Leary, V.B., Parle-McDermott, A., Scott, J.M. *et al.* (2002) A polymorphism, R653Q, in the trifunctional enzyme methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthetase is a maternal genetic risk factor for neural tube defects: report of the Birth Defects Research Group. *Am. J. Hum. Genet.*, **71**, 1207–1215.
50. Parle-McDermott, A., Kirke, P.N., Mills, J.L., Molloy, A.M., Cox, C., O’Leary, V.B., Pangilinan, F., Conley, M., Cleary, L., Brody, L.C. *et al.* (2006) Confirmation of the R653Q polymorphism of the trifunctional C1-synthase enzyme as a maternal risk for neural tube defects in the Irish population. *Eur. J. Hum. Genet.*, **14**, 768–772.
51. De Marco, P., Merello, E., Calevo, M.G., Mascelli, S., Raso, A., Cama, A. and Capra, V. (2006) Evaluation of a methylenetetrahydrofolate-dehydrogenase 1958G>A polymorphism for neural tube defect risk. *J. Hum. Genet.*, **51**, 98–103.
52. Van der Linden, I., Heil, S.G., Kouwenberg, I.C., den, H.M. and Blom, H.J. (2007) The methylenetetrahydrofolate dehydrogenase (MTHFD1) 1958G>A variant is not associated with spina bifida risk in the Dutch population. *Clin. Genet.*, **72**, 599–600.
53. Christensen, K.E., Rohlicek, C.V., Andelfinger, G.U., Michaud, J., Bigras, J.L., Richter, A., Mackenzie, R.E. and Rozen, R. (2009) The MTHFD1 p.Arg653Gln variant alters enzyme function and increases risk for congenital heart defects. *Hum. Mutat.*, **30**, 212–220.
54. Carroll, N., Pangilinan, F., Molloy, A.M., Troendle, J., Mills, J.L., Kirke, P.N., Brody, L.C., Scott, J.M. and Parle-McDermott, A. (2009) Analysis of the MTHFD1 promoter and risk of neural tube defects. *Hum. Genet.*, **125**, 247–256.
55. Jakubowski, H., Perla-Kajan, J., Finnell, R.H., Cabrera, R.M., Wang, H., Gupta, S., Kruger, W.D., Kraus, J.P. and Shih, D.M. (2009) Genetic or nutritional disorders in homocysteine or folate metabolism increase protein N-homocysteinylolation in mice. *FASEB J.*, **23**, 1721–1727.

56. Franke, B., Vermeulen, S.H., Steegers-Theunissen, R.P., Coenen, M.J., Schijvenaars, M.M., Scheffer, H., den, H.M. and Blom, H.J. (2009) An association study of 45 folate-related genes in spina bifida: Involvement of cubilin (CUBN) and tRNA aspartic acid methyltransferase 1 (TRDMT1). *Birth Defects Res. A Clin. Mol. Teratol.*, **85**, 216–226.
57. Birn, H., Zhai, X., Holm, J., Hansen, S.I., Jacobsen, C., Christensen, E.I. and Moestrup, S.K. (2005) Megalin binds and mediates cellular internalization of folate binding protein. *FEBS J.*, **272**, 4423–4430.
58. Christensen, E.I. and Birn, H. (2002) Megalin and cubilin: multifunctional endocytic receptors. *Nat. Rev. Mol. Cell Biol.*, **3**, 256–266.
59. Gelineau-van Waes, J., Maddox, J.R., Smith, L.M., van, W.M., Wilberding, J., Eudy, J.D., Bauer, L.K. and Finnell, R.H. (2008) Microarray analysis of E9.5 reduced folate carrier (RFC1; Slc19a1) knockout embryos reveals altered expression of genes in the cubilin–megalin multiligand endocytic receptor complex. *BMC Genomics*, **9**, 156.
60. Wu, J.I., Rajendra, R., Barsi, J.C., Durfee, L., Benito, E., Gao, G., Kuruvilla, M., Hrdlickova, R., Liss, A.S. and Artzt, K. (2007) Targeted disruption of Mib2 causes exencephaly with a variable penetrance. *Genesis*, **45**, 722–727.
61. Narimatsu, M., Bose, R., Pye, M., Zhang, L., Miller, B., Ching, P., Sakuma, R., Luga, V., Roncari, L., Attisano, L. et al. (2009) Regulation of planar cell polarity by Smurf ubiquitin ligases. *Cell*, **137**, 295–307.
62. Zohn, I.E., Anderson, K.V. and Niswander, L. (2007) The Hectd1 ubiquitin ligase is required for development of the head mesenchyme and neural tube closure. *Dev. Biol.*, **306**, 208–221.
63. Greene, N.D. and Copp, A.J. (2009) Development of the vertebrate central nervous system: formation of the neural tube. *Prenat. Diagn.*, **29**, 303–311.
64. Harris, M.J. (2001) Why are the genes that cause risk of human neural tube defects so hard to find? *Teratology*, **63**, 165–166.
65. Epstein, D.J., Vekemans, M. and Gros, P. (1991) Splotch (Sp^{2H}), a mutation affecting development of the mouse neural tube, shows a deletion within the paired homeodomain of Pax-3. *Cell*, **67**, 767–774.
66. Lakkis, M.M., Golden, J.A., O'Shea, K.S. and Epstein, J.A. (1999) Neurofibromin deficiency in mice causes exencephaly and is a modifier for Splotch neural tube defects. *Dev. Biol.*, **212**, 80–92.
67. Estibeiro, J.P., Brook, F.A. and Copp, A.J. (1993) Interaction between splotch (*Sp*) and curly tail (*ct*) mouse mutants in the embryonic development of neural tube defects. *Development*, **119**, 113–121.
68. Martin, L.J., Machado, A.F., Loza, M.A., Mao, G.E., Lee, G.S., Hovland, D.N. Jr, Cantor, R.M. and Collins, M.D. (2003) Effect of arsenite, maternal age, and embryonic sex on spina bifida, exencephaly, and resorption rates in the splotch mouse. *Birth Defects Res. (Part A)*, **67**, 231–239.
69. Trembath, D., Sherbondy, A.L., Vandyke, D.C., Shaw, G.M., Todoroff, K., Lammer, E.J., Finnell, R.H., Marker, S., Lerner, G. and Murray, J.C. (1999) Analysis of select folate pathway genes, PAX3, and human T in a Midwestern neural tube defect population. *Teratology*, **59**, 331–341.
70. Lu, W., Zhu, H., Wen, S., Laurent, C., Shaw, G.M., Lammer, E.J. and Finnell, R.H. (2007) Screening for novel PAX3 polymorphisms and risks of spina bifida. *Birth Defects Res. A Clin. Mol. Teratol.*, **79**, 45–49.
71. Van Straaten, H.W.M. and Copp, A.J. (2001) Curly tail: a 50-year history of the mouse spina bifida model. *Anat. Embryol.*, **203**, 225–237.
72. Neumann, P.E., Frankel, W.N., Letts, V.A., Coffin, J.M., Copp, A.J. and Bernfield, M. (1994) Multifactorial inheritance of neural tube defects: localization of the major gene and recognition of modifiers in *ct* mutant mice. *Nat. Genet.*, **6**, 357–362.
73. Ting, S.B., Wilanowski, T., Auden, A., Hall, M., Voss, A.K., Thomas, T., Parekh, V., Cunningham, J.M. and Jane, S.M. (2003) Inositol- and folate-resistant neural tube defects in mice lacking the epithelial-specific factor Grhl-3. *Nat. Med.*, **9**, 1513–1519.
74. Gustavsson, P., Greene, N.D., Lad, D., Pauws, E., de Castro, S.C., Stanier, P. and Copp, A.J. (2007) Increased expression of Grainyhead-like-3 rescues spina bifida in a folate-resistant mouse model. *Hum. Mol. Genet.*, **16**, 2640–2646.
75. Gustavsson, P., Copp, A.J. and Greene, N.D. (2008) Grainyhead genes and mammalian neural tube closure. *Birth Defects Res. A Clin. Mol. Teratol.*, **82**, 728–735.
76. Greene, N.D.E. and Copp, A.J. (1997) Inositol prevents folate-resistant neural tube defects in the mouse. *Nat. Med.*, **3**, 60–66.
77. Cockcroft, D.L., Brook, F.A. and Copp, A.J. (1992) Inositol deficiency increases the susceptibility to neural tube defects of genetically predisposed (curly tail) mouse embryos in vitro. *Teratology*, **45**, 223–232.
78. Khandelwal, M., Reece, E.A., Wu, Y.K. and Borenstein, M. (1998) Dietary myo-inositol therapy in hyperglycemia-induced embryopathy. *Teratology*, **57**, 79–84.
79. Wilson, M.P., Hugge, C., Bielinska, M., Nicholas, P., Majerus, P.W. and Wilson, D.B. (2009) Neural tube defects in mice with reduced levels of inositol 1,3,4-trisphosphate 5/6-kinase. *Proc. Natl. Acad. Sci. U. S. A.*, **106**, 9831–9835.
80. Krasnow, R.E., Wong, L.L. and Adler, P.N. (1995) Dishevelled is a component of the frizzled signaling pathway in *Drosophila*. *Development*, **121**, 4095–4102.
81. Strutt, D.I., Weber, U. and Mlodzik, M. (1997) The role of RhoA in tissue polarity and Frizzled signalling. *Nature*, **387**, 292–295.
82. McEwen, D.G. and Peifer, M. (2000) Wnt signaling: Moving in a new direction. *Curr. Biol.*, **10**, R562–R564.
83. Strutt, D. (2008) The planar polarity pathway. *Curr. Biol.*, **18**, R898–R902.
84. Wallingford, J.B., Rowning, B.A., Vogeli, K.M., Rothbacher, U., Fraser, S.E. and Harland, R.M. (2000) Dishevelled controls cell polarity during *Xenopus* gastrulation. *Nature*, **405**, 81–85.
85. Park, M. and Moon, R.T. (2002) The planar cell-polarity gene stbm regulates cell behaviour and cell fate in vertebrate embryos. *Nat. Cell Biol.*, **4**, 20–25.
86. Jessen, J.R., Topczewski, J., Bingham, S., Sepich, D.S., Marlow, F., Chandrasekhar, A. and Solnica-Krezel, L. (2002) Zebrafish trilobite identifies new roles for Strabismus in gastrulation and neuronal movements. *Nat. Cell Biol.*, **4**, 610–615.
87. Montcouquiol, M., Rachel, R.A., Lanford, P.J., Copeland, N.G., Jenkins, N.A. and Kelley, M.W. (2003) Identification of *Vangl2* and *Scrb1* as planar polarity genes in mammals. *Nature*, **423**, 173–177.
88. Henderson, D.J., Conway, S.J., Greene, N.D.E., Gerrelli, D., Murdoch, J.N., Anderson, R.H. and Copp, A.J. (2001) Cardiovascular defects associated with abnormalities in midline development in the *loop-tail* mouse mutant. *Circ. Res.*, **89**, 6–12.
89. Phillips, H.M., Rhee, H.J., Murdoch, J.N., Hildreth, V., Peat, J.D., Anderson, R.H., Copp, A.J., Chaudhry, B. and Henderson, D.J. (2007) Disruption of planar cell polarity signaling results in congenital heart defects and cardiomyopathy attributable to early cardiomyocyte disorganization. *Circ. Res.*, **101**, 137–145.
90. Vandenberg, A.L. and Sassoon, D.A. (2009) Non-canonical Wnt signaling regulates cell polarity in female reproductive tract development via van gogh-like 2. *Development*, **136**, 1559–1570.
91. Kibar, Z., Vogan, K.J., Groulx, N., Justice, M.J., Underhill, D.A. and Gros, P. (2001) *Ltap*, a mammalian homolog of *Drosophila Strabismus/Van Gogh*, is altered in the mouse neural tube mutant Loop-tail. *Nat. Genet.*, **28**, 251–255.
92. Murdoch, J.N., Doudney, K., Paternotte, C., Copp, A.J. and Stanier, P. (2001) Severe neural tube defects in the *loop-tail* mouse result from mutation of *Lpp1*, a novel gene involved in floor plate specification. *Hum. Mol. Genet.*, **10**, 2593–2601.
93. Murdoch, J.N., Henderson, D.J., Doudney, K., Gaston-Massuet, C., Phillips, H.M., Paternotte, C., Arkell, R., Stanier, P. and Copp, A.J. (2003) Disruption of *scribble (Scrb1)* causes severe neural tube defects in the *circletail* mouse. *Hum. Mol. Genet.*, **12**, 87–98.
94. Lu, X., Borchers, A.G., Jolicoeur, C., Rayburn, H., Baker, J.C. and Tessier-Lavigne, M. (2004) PTK7/CCK-4 is a novel regulator of planar cell polarity in vertebrates. *Nature*, **430**, 93–98.
95. Doudney, K., Ybot-Gonzalez, P., Paternotte, C., Stevenson, R.E., Greene, N.D., Moore, G.E., Copp, A.J. and Stanier, P. (2005) Analysis of the planar cell polarity gene *Vangl2* and its co-expressed paralogue *Vangl1* in neural tube defect patients. *Am. J. Med. Genet. A*, **136A**, 90–92.
96. Kibar, Z., Torban, E., McDearmid, J.R., Reynolds, A., Berghout, J., Mathieu, M., Kirillova, I., De Marco, P., Merello, E., Hayes, J.M. et al. (2007) Mutations in *VANGL1* associated with neural-tube defects. *N. Engl. J. Med.*, **356**, 1432–1437.
97. Kibar, Z., Bosoi, C.M., Kooistra, M., Salem, S., Finnell, R.H., De, M.P., Merello, E., Bassuk, A.G., Capra, V. and Gros, P. (2009) Novel mutations in *VANGL1* in neural tube defects. *Hum. Mutat.*, **30**, E706–E715.

98. Torban, E., Patenaude, A.M., Leclerc, S., Rakowiecki, S., Gauthier, S., Andelfinger, G., Epstein, D.J. and Gros, P. (2008) Genetic interaction between members of the Vangl family causes neural tube defects in mice. *Proc. Natl. Acad. Sci. U. S. A.*, **105**, 3449–3454.
99. Carroll, E., Gerrelli, D., Gasca, S., Berg, E., Beier, D., Copp, A. and Klingensmith, J. (2003) Cordon-bleu is a conserved gene involved in neural tube formation. *Dev. Biol.*, **262**, 16–31.
100. Yamamoto, S., Nishimura, O., Misaki, K., Nishita, M., Minami, Y., Yonemura, S., Tarui, H. and Sasaki, H. (2008) Cthrc1 selectively activates the planar cell polarity pathway of Wnt signaling by stabilizing the Wnt-receptor complex. *Dev. Cell*, **15**, 23–36.
101. Etheridge, S.L., Ray, S., Li, S., Hamblet, N.S., Lijam, N., Tsang, M., Greer, J., Kardos, N., Wang, J., Sussman, D.J. *et al.* (2008) Murine dishevelled 3 functions in redundant pathways with dishevelled 1 and 2 in normal cardiac outflow tract, cochlea, and neural tube development. *PLoS Genet.*, **4**, e1000259.
102. Murdoch, J.N., Rachel, R.A., Shah, S., Beermann, F., Stanier, P., Mason, C.A. and Copp, A.J. (2001) *Circletail*, a new mouse mutant with severe neural tube defects: Chromosomal localisation and interaction with the *loop-tail* mutation. *Genomics*, **78**, 55–63.
103. Shaw, G.M., Lu, W., Zhu, H., Yang, W., Briggs, F.B., Carmichael, S.L., Barcellos, L.F., Lammer, E.J. and Finnell, R.H. (2009) 118 SNPs of folate-related genes and risks of spina bifida and conotruncal heart defects. *BMC Med. Genet.*, **10**, 49.
104. Deak, K.L., Dickerson, M.E., Linney, E., Enterline, D.S., George, T.M., Melvin, E.C., Graham, F.L., Siegel, D.G., Hammock, P., Mehlretter, L. *et al.* (2005) Analysis of *ALDH1A2*, *CYP26A1*, *CYP26B1*, *CRABP1*, and *CRABP2* in human neural tube defects suggests a possible association with alleles in *ALDH1A2*. *Birth Defects Res. A Clin. Mol. Teratol.*, **73**, 868–875.
105. Zhu, H., Wicker, N.J., Shaw, G.M., Lammer, E.J., Hendricks, K., Suarez, L., Canfield, M. and Finnell, R.H. (2003) Homocysteine remethylation enzyme polymorphisms and increased risks for neural tube defects. *Mol. Genet. Metab.*, **78**, 216–221.
106. Volcik, K.A., Shaw, G.M., Lammer, E.J., Zhu, H.P. and Finnell, R.H. (2003) Evaluation of infant methylenetetrahydrofolate reductase genotype, maternal vitamin use, and risk of high versus low level spina bifida defects. *Birth Defects Res. (Part A)*, **67**, 154–157.
107. Wilson, A., Platt, R., Wu, Q., Leclerc, D., Christensen, B., Yang, H., Gravel, R.A. and Rozen, R. (1999) A common variant in methionine synthase reductase combined with low cobalamin (vitamin B12) increases risk for spina bifida. *Mol. Genet. Metab.*, **67**, 317–323.
108. Toepoel, M., Steegers-Theunissen, R.P., Ouborg, N.J., Franke, B., Ladd, A.M., Joosten, P.H. and van Zoelen, E.J. (2009) Interaction of PDGFRA promoter haplotypes and maternal environmental exposures in the risk of spina bifida. *Birth Defects Res. A Clin. Mol. Teratol.*, **85**, 629–636.
109. Joosten, P.H., Toepoel, M., Mariman, E.C. and Van Zoelen, E.J. (2001) Promoter haplotype combinations of the platelet-derived growth factor alpha-receptor gene predispose to human neural tube defects. *Nat. Genet.*, **27**, 215–217.
110. Volcik, K.A., Shaw, G.M., Zhu, H.P., Lammer, E.J., Laurent, C. and Finnell, R.H. (2003) Associations between polymorphisms within the thymidylate synthase gene and spina bifida. *Birth Defects Res. Part A Clin. Mol. Teratol.*, **67**, 924–928.
111. Henrichsen, C.N., Chaignat, E. and Reymond, A. (2009) Copy number variants, diseases and gene expression. *Hum. Mol. Genet.*, **18**, R1–R8.
112. Olshan, A.F., Shaw, G.M., Millikan, R.C., Laurent, C. and Finnell, R.H. (2005) Polymorphisms in DNA repair genes as risk factors for spina bifida and orofacial clefts. *Am. J. Med. Genet.*, **135A**, 268–273.
113. Zhu, H., Curry, S., Wen, S., Wicker, N.J., Shaw, G.M., Lammer, E.J., Yang, W., Jafarov, T. and Finnell, R.H. (2005) Are the betaine-homocysteine methyltransferase (BHMT and BHMT2) genes risk factors for spina bifida and orofacial clefts? *Am. J. Med. Genet. A*, **135**, 274–277.
114. King, T.M., Au, K.S., Kirkpatrick, T.J., Davidson, C., Fletcher, J.M., Townsend, I., Tyerman, G.H., Shimmin, L.C. and Northrup, H. (2007) The impact of BRCA1 on spina bifida meningomyelocele lesions. *Ann. Hum. Genet.*, **71**, 719–728.
115. Davidson, C.M., Northrup, H., King, T.M., Fletcher, J.M., Townsend, I., Tyerman, G.H. and Au, K.S. (2008) Genes in glucose metabolism and association with spina bifida. *Reprod. Sci.*, **15**, 51–58.
116. Relton, C.L., Wilding, C.S., Pearce, M.S., Laffling, A.J., Jonas, P.A., Lynch, S.A., Tawn, E.J. and Burn, J. (2004) Gene-gene interaction in folate-related genes and risk of neural tube defects in a UK population. *J. Med. Genet.*, **41**, 256–260.
117. Zhu, H., Enaw, J.O., Ma, C., Shaw, G.M., Lammer, E.J. and Finnell, R.H. (2007) Association between CFL1 gene polymorphisms and spina bifida risk in a California population. *BMC Med. Genet.*, **8**, 12.
118. Enaw, J.O., Zhu, H., Yang, W., Lu, W., Shaw, G.M., Lammer, E.J. and Finnell, R.H. (2006) CHKA and PCYT1A gene polymorphisms, choline intake and spina bifida risk in a California population. *BMC Med.*, **4**, 36.
119. Volcik, K.A., Zhu, H., Finnell, R.H., Shaw, G.M., Canfield, M. and Lammer, E.J. (2004) Evaluation of the Cited2 gene and risk for spina bifida and congenital heart defects. *Am. J. Med. Genet.*, **126A**, 324–325.
120. Johnson, W.G., Stenroos, E.S., Sychala, J.R., Chatkupt, S., Ming, S.X. and Buyske, S. (2004) New 19 bp deletion polymorphism in intron-1 of dihydrofolate reductase (DHFR): a risk factor for spina bifida acting in mothers during pregnancy? *Am. J. Med. Genet.*, **124A**, 339–345.
121. Parle-McDermott, A., Pangilinan, F., Mills, J.L., Kirke, P.N., Gibney, E.R., Troendle, J., O'Leary, V.B., Molloy, A.M., Conley, M., Scott, J.M. *et al.* (2007) The 19-bp deletion polymorphism in intron-1 of dihydrofolate reductase (DHFR) may decrease rather than increase risk for spina bifida in the Irish population. *Am. J. Med. Genet. A*, **143A**, 1174–1180.
122. Van der Linden, I., Nguyen, U., Heil, S.G., Franke, B., Vloet, S., Gellekink, H., den, H.M. and Blom, H.J. (2007) Variation and expression of dihydrofolate reductase (DHFR) in relation to spina bifida. *Mol. Genet. Metab.*, **91**, 98–103.
123. Gos, M., Sliwerska, E. and Szepecht-Potocka, A. (2004) Mutation incidence in folate metabolism genes and regulatory genes in Polish families with neural tube defects. *J. Appl. Genet.*, **45**, 363–368.
124. O'Leary, V.B., Mills, J.L., Parle-McDermott, A., Pangilinan, F., Molloy, A.M., Cox, C., Weiler, A., Conley, M., Kirke, P.N., Scott, J.M. *et al.* (2005) Screening for new MTHFR polymorphisms and NTD risk. *Am. J. Med. Genet. A*, **138A**, 99–106.
125. Vieira, A.R., Murray, J.C., Trembath, D., Orioli, I.M., Castilla, E.E., Cooper, M.E., Marazita, M.L., Lennon-Graham, F. and Speer, M. (2005) Studies of reduced folate carrier 1 (RFC1) A80G and 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms with neural tube and orofacial cleft defects. *Am. J. Med. Genet. A*, **135**, 220–223.
126. Grandone, E., Corrao, A.M., Colaizzo, D., Vecchione, G., Di, G.C., Paladini, D., Sardella, L., Pellegrino, M., Zelante, L., Martinelli, P. *et al.* (2006) Homocysteine metabolism in families from southern Italy with neural tube defects: role of genetic and nutritional determinants. *Prenat. Diagn.*, **26**, 1–5.
127. Munoz, J.B., Lacasana, M., Cavazos, R.G., Borja-Aburto, V.H., Galaviz-Hernandez, C. and Garduno, C.A. (2007) Methylenetetrahydrofolate reductase gene polymorphisms and the risk of anencephaly in Mexico. *Mol. Hum. Reprod.*, **13**, 419–424.
128. Dalal, A., Pradhan, M., Tiwari, D., Behari, S., Singh, U., Mallik, G.K., Das, V. and Agarwal, S. (2007) MTHFR 677C→T and 1298A→C polymorphisms: evaluation of maternal genetic risk and association with level of neural tube defect. *Gynecol. Obstet. Invest.*, **63**, 146–150.
129. Gonzalez-Herrera, L., Castillo-Zapata, I., Garcia-Escalante, G. and Pinto-Escalante, D. (2007) A1298C polymorphism of the MTHFR gene and neural tube defects in the state of Yucatan, Mexico. *Birth Defects Res. A Clin. Mol. Teratol.*, **79**, 622–626.
130. Candito, M., Rivet, R., Herbeth, B., Boisson, C., Rudigoz, R.C., Luton, D., Journel, H., Oury, J.F., Roux, F., Saura, R. *et al.* (2008) Nutritional and genetic determinants of vitamin B and homocysteine metabolisms in neural tube defects: a multicenter Case-control study. *Am. J. Med. Genet. A*, **146A**, 1128–1133.
131. Shang, Y., Zhao, H., Niu, B., Li, W.I., Zhou, R., Zhang, T. and Xie, J. (2008) Correlation of polymorphism of MTHFRs and RFC-1 genes with neural tube defects in China. *Birth Defects Res. A Clin. Mol. Teratol.*, **82**, 3–7.
132. O'Leary, V.B., Mills, J.L., Pangilinan, F., Kirke, P.N., Cox, C., Conley, M., Weiler, A., Peng, K., Shane, B., Scott, J.M. *et al.* (2005) Analysis of methionine synthase reductase polymorphisms for neural tube defects risk association. *Mol. Genet. Metab.*, **85**, 220–227.
133. Van der Linden, I., Den Heijer, M., Afman, L.A., Gellekink, H., Vermeulen, S.H., Kluijtmans, L.A. and Blom, H.J. (2006) The methionine synthase reductase 66A>G polymorphism is a maternal risk factor for spina bifida. *J. Mol. Med.*, **84**, 1047–1054.

134. Parle-McDermott, A., McManus, E.J., Mills, J.L., O'Leary, V.B., Pangilinan, F., Cox, C., Weiler, A., Molloy, A.M., Conley, M., Watson, D. *et al.* (2003) Polymorphisms within the vitamin B12 dependent methylmalonyl-coA mutase are not risk factors for neural tube defects. *Mol. Genet. Metab.*, **80**, 463–468.
135. Jensen, L.E., Hoess, K., Whitehead, A.S. and Mitchell, L.E. (2005) The NAT1 C1095A polymorphism, maternal multivitamin use and smoking, and the risk of spina bifida. *Birth Defects Res. A Clin. Mol. Teratol.*, **73**, 512–516.
136. Jensen, L.E., Hoess, K., Mitchell, L.E. and Whitehead, A.S. (2006) Loss of function polymorphisms in NAT1 protect against spina bifida. *Hum. Genet.*, **120**, 52–57.
137. Lu, W., Zhu, H., Wen, S., Yang, W., Shaw, G.M., Lammer, E.J. and Finnell, R.H. (2008) Nicotinamide N-methyl transferase (NNMT) gene polymorphisms and risk for spina bifida. *Birth Defects Res. A Clin. Mol. Teratol.*, **82**, 670–675.
138. Deak, K.L., Boyles, A.L., Etchevers, H.C., Melvin, E.C., Siegel, D.G., Graham, F.L., Slifer, S.H., Enterline, D.S., George, T.M., Vekemans, M. *et al.* (2005) SNPs in the neural cell adhesion molecule 1 gene (NCAM1) may be associated with human neural tube defects. *Hum. Genet.*, **117**, 133–142.
139. Brown, K.S., Cook, M., Hoess, K., Whitehead, A.S. and Mitchell, L.E. (2004) Evidence that the risk of spina bifida is influenced by genetic variation at the NOS3 locus. *Birth Defects Res. Part A Clin. Mol. Teratol.*, **70**, 101–106.
140. Van der Linden, I.J., Heil, S.G., den, H.M. and Blom, H.J. (2007) The 894G>T variant in the endothelial nitric oxide synthase gene and spina bifida risk. *J. Hum. Genet.*, **52**, 516–520.
141. Zhu, H., Yang, W., Lu, W., Zhang, J., Shaw, G.M., Lammer, E.J. and Finnell, R.H. (2006) A known functional polymorphism (Ile120Val) of the human PCMT1 gene and risk of spina bifida. *Mol. Genet. Metab.*, **87**, 66–70.
142. Zhu, H., Wicker, N.J., Volcik, K., Zhang, J., Shaw, G.M., Lammer, E.J., Suarez, L., Canfield, M. and Finnell, R.H. (2004) Promoter haplotype combinations for the human PDGFRA gene are associated with risk of neural tube defects. *Mol. Genet. Metab.*, **81**, 127–132.
143. Au, K.S., Northrup, H., Kirkpatrick, T.J., Volcik, K.A., Fletcher, J.M., Townsend, I.T., Blanton, S.H., Tyerman, G.H., Villarreal, G. and King, T.M. (2005) Promotor genotype of the platelet-derived growth factor receptor-alpha gene shows population stratification but not association with spina bifida meningomyelocele. *Am. J. Med. Genet. A*, **139**, 194–198.
144. Zhang, J., Zhu, H., Yang, W., Shaw, G.M., Lammer, E.J. and Finnell, R.H. (2006) Phosphatidylethanolamine N-methyltransferase (PEMT) gene polymorphisms and risk of spina bifida. *Am. J. Med. Genet. A*, **140**, 785–789.
145. Zhu, H.P., Lu, W., Laurent, C., Shaw, G.M., Lammer, E.J. and Finnell, R.H. (2005) Genes encoding catalytic subunits of protein kinase A and risk of spina bifida. *Birth Defects Res. A Clin. Mol. Teratol.*, **73**, 591–596.
146. Relton, C.L., Wilding, C.S., Laffling, A.J., Jonas, P.A., Burgess, T., Binks, K., Tawn, E.J. and Burn, J. (2004) Low erythrocyte folate status and polymorphic variation in folate-related genes are associated with risk of neural tube defect pregnancy. *Mol. Genet. Metab.*, **81**, 273–281.
147. O'Leary, V.B., Pangilinan, F., Cox, C., Parle-McDermott, A., Conley, M., Molloy, A.M., Kirke, P.N., Mills, J.L., Brody, L.C. and Scott, J.M. (2006) Reduced folate carrier polymorphisms and neural tube defect risk. *Mol. Genet. Metab.*, **87**, 364–369.
148. Pei, L., Liu, J., Zhang, Y., Zhu, H. and Ren, A. (2008) Association of reduced folate carrier gene polymorphism and maternal folic acid use with neural tube defects. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*; Epub.
149. Jensen, L.E., Barbaux, S., Hoess, K., Fraterman, S., Whitehead, A.S. and Mitchell, L.E. (2004) The human *T* locus and spina bifida risk. *Hum. Genet.*, **115**, 475–482.
150. Swanson, D.A., Pangilinan, F., Mills, J.L., Kirke, P.N., Conley, M., Weiler, A., Frey, T., Parle-McDermott, A., O'Leary, V.B., Seltzer, R.R. *et al.* (2005) Evaluation of transcobalamin II polymorphisms as neural tube defect risk factors in an Irish population. *Birth Defects Res. A Clin. Mol. Teratol.*, **73**, 239–244.
151. Wen, S., Lu, W., Zhu, H., Yang, W., Shaw, G.M., Lammer, E.J., Islam, A. and Finnell, R.H. (2009) Genetic polymorphisms in the thioredoxin 2 (TXN2) gene and risk for spina bifida. *Am. J. Med. Genet. A*, **149A**, 155–160.
152. Pangilinan, F., Geiler, K., Dolle, J., Troendle, J., Swanson, D.A., Molloy, A.M., Sutton, M., Conley, M., Kirke, P.N., Scott, J.M. *et al.* (2008) Construction of a high resolution linkage disequilibrium map to evaluate common genetic variation in TP53 and neural tube defect risk in an Irish population. *Am. J. Med. Genet. A*, **146A**, 2617–2625.
153. Wilding, C.S., Relton, C.L., Sutton, M.J., Jonas, P.A., Lynch, S.A., Tawn, E.J. and Burn, J. (2004) Thymidylate synthase repeat polymorphisms and risk of neural tube defects in a population from the northern United Kingdom. *Birth Defects Res. Part A Clin. Mol. Teratol.*, **70**, 483–485.
154. Mitchell, A., Pangilinan, F., Van der, M.J., Molloy, A.M., Troendle, J., Conley, M., Kirke, P.N., Scott, J.M., Brody, L.C. and Mills, J.L. (2009) Uncoupling protein 2 polymorphisms as risk factors for NTDs. *Birth Defects Res. A Clin. Mol. Teratol.*, **85**, 156–160.
155. Klootwijk, R., Groenen, P., Schijvenaars, M., Hol, F., Hamel, B., Straatman, H., Steegers-Theunissen, R., Mariman, E. and Franke, B. (2004) Genetic variants in ZIC1, ZIC2 and ZIC3 are not major risk factors for neural tube defects in humans. *Am. J. Med. Genet.*, **124A**, 40–47.
156. Copp, A.J., Checiu, I. and Henson, J.N. (1994) Developmental basis of severe neural tube defects in the *loop-tail* (*Lp*) mutant mouse: use of microsatellite DNA markers to identify embryonic genotype. *Dev. Biol.*, **165**, 20–29.
157. Kibar, Z., Underhill, D.A., Canonne-Hergaux, F., Gauthier, S., Justice, M.J. and Gros, P. (2001) Identification of a new chemically induced allele (Lp(m1Jus)) at the loop-tail locus: morphology, histology, and genetic mapping. *Genomics*, **72**, 331–337.
158. Curtin, J.A., Quint, E., Tshipouri, V., Arkell, R.M., Cattanach, B., Copp, A.J., Fisher, E.M., Nolan, P.M., Steel, K.P., Brown, S.D.M. *et al.* (2003) Mutation of *Celsr1* disrupts planar polarity of inner ear hair cells and causes severe neural tube defects in the mouse. *Curr. Biol.*, **13**, 1–20.
159. Wang, J., Hamblet, N.S., Mark, S., Dickinson, M.E., Brinkman, B.C., Segil, N., Fraser, S.E., Chen, P., Wallingford, J.B. and Wynshaw-Boris, A. (2006) Dishevelled genes mediate a conserved mammalian PCP pathway to regulate convergent extension during neurulation. *Development*, **133**, 1767–1778.
160. Wang, Y., Guo, N. and Nathans, J. (2006) The role of Frizzled3 and Frizzled6 in neural tube closure and in the planar polarity of inner-ear sensory hair cells. *J. Neurosci.*, **26**, 2147–2156.
161. Qian, D., Jones, C., Rzadzinska, A., Mark, S., Zhang, X., Steel, K.P., Dai, X. and Chen, P. (2007) Wnt5a functions in planar cell polarity regulation in mice. *Dev. Biol.*, **306**, 121–133.
162. Satoh, W., Matsuyama, M., Takemura, H., Aizawa, S. and Shimono, A. (2008) Sfrp1, Sfrp2 and Sfrp5 regulate the Wnt/beta-catenin and the planar cell polarity pathways during early trunk formation in mouse. *Genesis*, **46**, spcone.
163. Stiefel, D., Shibata, T., Meuli, M., Duffy, P. and Copp, A.J. (2003) Tethering of the spinal cord in mouse fetuses and neonates with spina bifida. *J. Neurosurg. (Spine)*, **99**, 206–213.