Neural Tube Defects: A Review on Maternal Genetic Determinants

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ABSTRACT: Neural Tube Defects, resulting from the failure in the closure of the neural tube, have been found to be one of the major causes of mortality and morbidity in the infants and have a variable worldwide incidence rate. Various debatable theories suggest different models for the process of the formation of the neural tube or neurulation, resulting in different types of NTDs. NTDs are an outcome of the association of environmental, nutritional and genetic factors and their various interactions. The environmental and nutritional factors are mainly associated with maternal risk, while genetic factors can be both maternal and embryonic origin. Studies have shown strong association of genetic alterations in the metabolic pathway genes, embryonic transcription factor genes and developmental pathway genes with NTDs. Very few studies have focused on the genetic risk predisposed in the mothers of the affected pregnancies. The candidate genes studied as maternal risk have been derived from mainly one carbon metabolism or mouse mutants for NTDs.

INTRODUCTION

One of the major causes of infant mortality and morbidity is the congenital abnormalities which have adverse consequences on the society as well as on the families of affected individuals. The malformation of central nervous system, known as Neural-tube defects (NTDs), affects nearly 0.5-2 per 1,000 pregnancies worldwide (Green et al., 2009); however the incidence varies widely depending on geographical and ethnic background. NTDs result from failure of the neural tube (that gives rise to brain and spinal cord) to close spontaneously between the 3rd and 4th week of in-vitro development. The ‘open’ NTDs being the most common forms include Anencephaly and Myelomeningocele (spina bifida). Anencephaly results from failed closure at the cranial end, and is a lethal condition, whereas failed closure at the caudal end results in myelomeningocele. Multiple surgical interventions have increased the survival rate of myelomeningocele; although with lifelong disabilities. Yet the most severe form of open NTDs is craniorachischisis, characterised by the opening that extends from mid brain to low spine. Skin covered or ‘closed’ NTDs include encephalocele (mostly occurs in occipital region and less commonly in nasal and parietal region) and lipomeningocele (also known as spina bifida occult).

Neural Tube Defects may also occur as part of a syndrome or association, but represent less than 10% of all defects (Green et al., 2009). Some of the conditions which have myelomeningocele as a feature are Chairi malformations, Lehman syndrome, disorganization-like syndrome, Meckel-Gruber syndrome, PHAVER syndrome (Powell-Chandra-Saal syndrome), VATER syndrome etc, or sometimes associated with chromosomal abnormalities like Trisomy 13 and Trisomy 18. A condition known as Hydrocephalus (i.e. accumulation of cerebrospinal fluid in the ventricles of brain) is most often seen with myelomeningocele. However majority of NTDs occur in isolation. These associations indicate towards the genetic component in the etiology of NTDs.
OVERVIEW OF NEURULATION

Neurulation (formation of the neural tube) is an important morphogenetic event in human development. It is the conversion of flat neural plate into the neural tube, which gives rise to the entire central nervous system. Mammalian neurulation occurs in two phases: primary and secondary neurulation (Purves and Lichtman, ‘85). Primary neurulation generates the entire neural tube from rostral to the caudal neuropore (openings), which occurs during the third and fourth week of development. Broadly the process is divided into four distinct but spatially and temporally overlapping stages: (1) formation of neural plate, (2) shaping of the neural plate, (3) bending of the neural plate to form the neural groove and (4) closure of the neural groove to form the neural tube. Then the secondary neurulation is followed, which is limited to the tailbud, beyond the caudal neuropore. The process involves the proliferation of stem cells, which form a rod-like structure. The cavitation of rod transforms it into a tube, and the lumen of this tube comes into continuity with the lumen of the tube formed during primary neurulation.

Various debatable theories exist for the initiation sites of fusion and their location. The first hypothesis, based on the NTDs affected mouse fetuses, suggested multiple sites of initiation of neural folds fusion (Sakai, ‘89, Juriloff et al., ‘91, Golden and Chernoff, ‘93). Van Allen and group (‘96; ‘93) proposed similar multiple site (five sites) closure model for the human embryos. Using light microscopy and laser scanning electron microscopy, Sulik and team (‘93) gave a zipper like fusion of neural tube from a single initiation site. The finding was corroborated by two more studies. Nakatsu et al., (2000) examined histological sections of human embryos at various stages of neurulation and gave a three site initiation model. Later, O’Rahilly and Muller (2002) gave a model of two initiation site of neurulation. However, the zipper model can explain almost all cases of myelomeningocele but fail to explain multiple NTDs. Recent triple NTDs case studies strongly support multisite closure theory of neurulation (Tekkök, 2004; Srinivas et al., 2008; Ahmad and Mahapatra, 2008). According to multisite closure theory, failure of neural tube closure at different neuropores during primary neurulation leads to different forms of ‘open’ NTDs and during secondary neurulation leads to the less common ‘closed’ forms of NTDs where the developing neural tube fails to separate from other tissues of the tail bud (Copp et al., 2003). Failure to close the posterior neuropore around day 27 results in spina bifida, failure to close the rostral neural tube, keeps the anterior neuropore open, resulting in the lethal condition of anencephaly. The failure of the entire neural tube to close over the entire body axis causes craniorachischisis.

ETIOLOGY

NTD has been categorized into multifactorial disorder, being the consequence of the association of two integral components – environment and genetic, and their interactions for the manifestation of the disorder. Various environmental components like low or high maternal age, low parity, low socio-economic status of parents, seasonal variations, maternal hyperthermia, maternal drug exposure, maternal infection, maternal diabetes & obesity, maternal diarrhea, maternal emotional stress, proximity to hazardous waste and pesticides, and low nutritional status have been implicated as risk factors for NTDs. Increased recurrence risk of NTDs among siblings, high prevalence among monozygotic twins and in consanguineous community, and association with chromosomal disorders and other genetic syndromes, are few of evidences that indicate towards the strong genetic contribution in the causation of NTDs. Differences in prevalence of Infant’s sex, sibling recurrence, and illness and drug exposure between the two sub groups (lower and upper level) of NTDs further suggest an underlying heterogeneity in genetic susceptibility factors (Park et al., ‘92, Pei et al. 2003; Pérez-Molina, 2002).

Due to scarcity of large families with multiple affected members, positional cloning strategy is difficult to carry out. Most of the genes have been elucidated using candidate gene approach, involving either case-control analysis or transmission disequilibrium test (TDT). Although both maternal and embryonic genes are expressed during embryonic development, much focus has been on the embryonic genes for NTDs. Mother provides an environment to
the developing embryo, and therefore many maternal factors are known to influence NTD risk (Steegers-Theunissen and Steegers 2003). Besides the known preventive role of maternal periconception folic acid supplementation in nearly 70% of the cases, NTDs can also be prevented by myo-inositol (Greene and Copp, ’97) or D-chiro-inositol (Cogram et al., 2002) supplementation in folic acid–resistant curly tail mouse mutants. High levels of glucose inhibits the cellular uptake of myo-inositol (Scalera et al., ’91), thereby increasing the risk of NTDs in diabetic mothers (Loeken, 2005; Hendricks et al., 2001). These data suggest that myo-inositol supplementation may also reduce NTD risk in humans (Groenen et al., 2003). Furthermore, women with a pre-pregnancy body mass index (BMI) greater than 29 kg/m² have been associated with increased risk of NTDs (Watkins et al., 2003; Mitchell, 2008).

In additional to these specific nutritional factors, maternal illness profile like hyperthermia (Li et al., 2007; Moretti et al., 2005; Suarez et al., 2004; Lynberg et al., ’94) and drug exposure to antipyretics (Li et al., 2007; Moretti et al., 2005; Suarez et al., 2004; Lynberg et al., ’94), valproic acid (Wyszynski et al., 2005; Rothenberg, 2004; Nau, 1994; Lindhout et al., ’86) and efavirenz (Jeaentils et al., 2006; Saitoh et al., 2005) are the well-known risk factors. In view of the maternal role in causation of NTDs, this review will focus on the maternal genetic risk factors studied using candidate gene approach.

MATERNAL GENETIC FACTORS OF NTDs

Candidate gene approach for neural tube defects have mainly focused on genes related to one carbon metabolism (due to protective role of folic acid) and on candidate genes of mouse model. Besides, genes involved in glucose metabolism and obesity are also studied because of the 2-fold increased risk for NTDs in obese or diabetic pregnancies (Davidson et al 2008, Reece 2008). The insight into the pathways followed during early vertebrate development provides further candidate genes for NTDs; these developmental pathways includes planer cell polarity (PCP, also known as non-canonical Wnt signalling) pathway, retinoic acid pathway, hedgehog signalling pathway (Goodrich, 2008; Zallen, 2007; Deak et al., 2005; Rat et al., 2006; Zhu et al., 2003; Zhu et al., 2005).

Majority of studies elucidating the maternal risk factors are focussed on the candidate genes of folate one-carbon metabolism and mouse mutants.

ONE-CARBON METABOLISM RELATED GENES

The primary focus of the research studies has been on genes encoding proteins that participate in one-carbon metabolism. This has been driven by the findings that the women on peri-conceptional folic acid supplementation are at 50–70% reduced risk for NTD-affected pregnancies (Vitamin Study Research Group, ’91; Czeizel and Dudas ’92). Further, it has been observed that reduced serum folate and/or elevated homocysteine increase susceptibility towards the conception of NTDs child (Mills et al., ’95; Kirke et al., ’93). In search of other modifying risk factors, Cobalamin (a cofactor) deficiency has been found to be independent risk factor for NTD-affected pregnancies (Molloy et al., 2009, Suarez et al., 2003). Cobalamin (vitamin B12), Folate (folic acid or vitamin B9) and Homocysteine metabolic cycles are closely related and involve more than 25 proteins (Fig. 1). Table 1 represents the genes of one carbon metabolic cycle studied in the mothers of NTD affected pregnancies in different parts of the world to identify specific maternal genotypes predisposing the risk of NTD conception.

Dietary folate exists predominantly in the form of polyglutamates, which must be deconjugated to monoglutamates before absorption by the enzyme folylpoly-Ö-glutamate carboxypeptidase (FGCP). The H475Y (C1561T) polymorphism of glutamate
carboxypeptidase II (GCPII) gene, encoding the enzyme, has been associated with increased plasma folate levels and decreased plasma tHcy concentrations; no association with erythrocyte folate levels and NTDs risk have been observed in any of the study (Afman et al., 2003; Relton et al., 2004). Monoglutamate folates get converted to 5 methyl tetrahydrofolate (5-methyl THF) and transported to the cell through folate receptor (FR) or reduced folate carrier (RFC). Reduced folate carrier (RFC-1) is a facilitative anion exchanger that mediates 5 methyl THF delivery into the cells of different origin; a common polymorphism (80A/G) in the gene has been implicated in modifying NTD risk rates (De Marco et al., 2001 and 2003; Shang et al., 2008; Pei et al., 2009). Other genes involved in cellular uptake of folates, folate receptor (FR) genes occur as a family: FR-α, FR-β, FR-δ and FR-ε. Mutations have been implicated in NTDs risk in animal models, but so far, there is no evidence of these mutations in human NTDs (Barber et al., ’98, 2000; O’Leary et al., 2003). Once inside the cell, 5methyl THF reduces to tetrahydrofolate (THF), by the action of methionine synthase (MTR), simultaneously remethylating homocysteine to form methionine. A SNP 2756A/G, in coding region of MTR gene have been studied widely in mothers but no positive correlation have been observed so far (Christensen et al., ’99; De Marco et al., 2002; Zhu et al., 2003; Candito et al., 2008). Methionine synthase reductase (MTRR) is required to maintain the methionine synthase cofactor, methylcobalamin, derived from vitamin B12 in an active state (Brody et al., ’99). A variant in MTRR gene, 66A/G can be considered as maternal risk factor (Pietrzyk et al., 2003; Zhu et al., 2003; van der Linden et al., 2006; Candito et al., 2008). The cofactor B12 (methylcobalamin) is delivered to the cells and tissues by transcobalaminII (TCNII); variants have been studied but found no significant association with the risk (Afman et al., 2002; Swanson et al., 2005; Candito et al., 2008). A trifunctional enzyme, MethyleneTHF dehydrogenase/formylTHF synthase/methenylTHF cyclohydrolase (MTHFD) further catalyzes the conversion of THF to the corresponding 10-formyl, 5,10-methynyl, and 5,10-methylene derivatives important for the de novo biosynthesis of purines and pyrimidines and thus DNA biosynthesis. Single nucleotide polymorphism (SNP) 1958G/A in the gene has been demonstrated as a maternal risk factor for NTDs (Carroll et al., 2009; Parle-McDermott et al., 2006; De Marco et al., 2006; Brody et al., 2002). The conversion of 5,10-methylene THF to 5-methyl THF is required for the remethylation of homocysteine, and is mediated by 5, 10-methylene-tetrahydrofolate reductase (MTHFR). The enzyme has been the principle focus of attention, for being the first and the most potential maternal risk factor for NTDs. A common single-nucleotide polymorphism (SNP) in this gene, 677C/T, has been found to be associated with reduced levels of enzyme activity, elevated levels of plasma Hcy and an increased NTD risk in some populations (van der Linden et al., 2006; Pietrzyk et al., 2003, Christensen et al., ’99); however, others suggested that the effect may be dependent on level of lesion (Dalal et al., 2007; Shang et al., 2008). It has been suggested that the increased NTDs risk results from reduced serum and red blood cell folate levels associated with the T allele of 677C/T polymorphism of MTHFR, instead of homocysteine accumulation caused via impaired methylation cycle (Beaudin and Stover, 2009). Another mutation in the MTHFR gene, 1298A/C, has been identified and found to be associated with a reduced level of enzyme activity (not as severe as with 677C/T) and an increased risk for NTDs but with no effect on Hcy plasma levels (van der Put et al., ’98; Weisberg et al., ’98; De Marco et al., 2002). Studies suggested that the coexistence of the MTHFR 677 TT genotype with the MTRR 66A3G polymorphism may exacerbate the effect of the MTHFR variant alone (Vaughn et al., 2004). The 5,10-methylene THF can also be reduced to dihydrofolates (DFH), converting dUMP to dTMP, vital for pyrimidine biosynthesis. The reaction is catalyzed by thymidylate synthase (TYMS); 28-bp repeat variant is not found to be associated with the increased risk in mothers. DFH further reduces to THF by the enzyme dihydrofolate reductase (DHFR). The 19-bp intron 1 deletion variation have been associated with NTD risk (Johnson et al., 2004) but the results are very inconsistent as other studies observed protective effect of the variation (Parle-McDermott et al., 2007).

Homocysteine (Hcy) can also be remethylated using the methyl group of dimethylglycine, mediated by betaine-homocysteine methyl transferase (BHMT). Although, a variant (742G/A) of BHMT gene have
### TABLE I

<table>
<thead>
<tr>
<th>Gene (Locus)</th>
<th>Type of Study</th>
<th>Sample Size</th>
<th>Population studied</th>
<th>Findings</th>
<th>Reference</th>
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<tr>
<td>BHMT (5q13.1-q13.2)</td>
<td>Case – Control</td>
<td>57 case mothers, 86 control mothers</td>
<td>Canadian</td>
<td>No significant difference in the G742A polymorphism among case and control mothers</td>
<td>Morin et al. 2003</td>
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<tr>
<td>CBS (21q22.3)</td>
<td>Case – Control</td>
<td>79 NTD mothers, 241 control mothers</td>
<td>Irish</td>
<td>No increased frequency of any of the gene variation, No increased risk associated with the gene</td>
<td>Ramsbottom et al. 1997</td>
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<td></td>
<td>Case – Control</td>
<td>40 NTD mothers, 36 control mothers</td>
<td>Chinese</td>
<td>No significant difference among case and control mothers for the frequencies of both variations (T833C and G919A)</td>
<td>Zhao et al. 2000</td>
</tr>
<tr>
<td></td>
<td>Case – Control</td>
<td>201 NTD mothers, 542 control mothers</td>
<td>UK</td>
<td>No association with the 844ins68 variation</td>
<td>Relton et al. 2004</td>
</tr>
<tr>
<td>DHFR (5q11.2-q13.2)</td>
<td>Case – Control</td>
<td>50 SB mothers, 219 controls</td>
<td>Mixed US</td>
<td>Significantly Increased frequency of del/del genotype of 19-bp intron 1 deletion variation among case mothers</td>
<td>Johnson et al. 2004</td>
</tr>
<tr>
<td></td>
<td>Case – Control</td>
<td>280 NTD mothers, 256 controls mothers</td>
<td>Irish</td>
<td>19-bp intron deletion shows a protective effect in case mothers</td>
<td>Parle-McDermott et al. 2007</td>
</tr>
<tr>
<td></td>
<td>Case – Control</td>
<td>221 SB mothers, 292 control mothers</td>
<td>Dutch</td>
<td>No association of 19-bp intron 1 deletion and 9-bp exon1 repeat with SB risk</td>
<td>Van der Linden et al. 2007</td>
</tr>
<tr>
<td>GCPII (11p11.2)</td>
<td>Case – Control</td>
<td>113 SB mothers, 101 controls</td>
<td>Dutch</td>
<td>No significant association found with H475Y polymorphism of the gene</td>
<td>Afman et al. 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>201 NTD mothers, 539 control mothers</td>
<td>UK</td>
<td>No significant association found with C1561T polymorphism of the gene</td>
<td>Relton et al. 2004</td>
</tr>
<tr>
<td>MTHFD1 (14q24)</td>
<td>Case – Control</td>
<td>410 NTD mothers, 997 control mothers</td>
<td>Irish</td>
<td>Significant increased frequency of Q allele of R653Q (G1958A) polymorphism among NTD mothers, with increased risk for NTD child</td>
<td>Brody et al. 2002</td>
</tr>
<tr>
<td></td>
<td>Case – Control</td>
<td>125 NTD mothers, 523 controls</td>
<td>Italian</td>
<td>Increased risk for NTD with ‘A’ allele of G1958A among NTD mothers</td>
<td>De-Marco et al. 2006</td>
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<td></td>
<td>Case – Control</td>
<td>113 SB mothers, 257 control mothers</td>
<td>Dutch</td>
<td>No association between G1958A polymorphism and SB risk</td>
<td>van der Linden et al. 2007</td>
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<td></td>
<td>Case – Control</td>
<td>485 NTD mothers, 966 controls</td>
<td>Irish</td>
<td>Promoter variant rs1076991 C &gt; T as potential risk factor when combined with G1958A polymorphism</td>
<td>Carroll et al. 2009</td>
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<tr>
<th>Study</th>
<th>Case – Control</th>
<th>Control Mothers</th>
<th>Polymorphism</th>
<th>Risk Observations</th>
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<td><strong>MTHFR (1p36.3)</strong></td>
<td>Case – Control</td>
<td>62 NTD mothers, 90 control mothers</td>
<td>Canadian</td>
<td>Increased risk for NTD associated with T allele of C677T polymorphism</td>
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<tr>
<td></td>
<td>Case – Control</td>
<td>98 NTD mothers, 210 controls</td>
<td>Italian</td>
<td>Increased risk for NTD associated with C allele of A1298C polymorphism</td>
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<td>Case – Control</td>
<td>25 NTD mothers, 75 controls</td>
<td>Brazilian</td>
<td>No significant difference in the frequencies of two polymorphisms</td>
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<td>Case – Control</td>
<td>106 SB mothers, 100 controls</td>
<td>Polish</td>
<td>Increased risk associated with the C677T polymorphism in homozygous case mothers</td>
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<tr>
<td></td>
<td>Case – Control</td>
<td>28 NTD mothers, 159 controls</td>
<td>Spanish</td>
<td>No significant difference in the frequencies of two polymorphisms</td>
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<td>Case – Control</td>
<td>186 NTD mothers, 522 control mothers</td>
<td>UK</td>
<td>No significant difference in the frequencies of two polymorphisms among case and control groups</td>
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<tr>
<td></td>
<td>Case – Control</td>
<td>57 NTD mothers, 143 controls</td>
<td>Italian</td>
<td>Increased risk of NTD with TT genotype of C677T polymorphism, no risk with A1298C polymorphism</td>
</tr>
<tr>
<td></td>
<td>Case – Control</td>
<td>118 NTD mothers, 112 control mothers</td>
<td>Mexican</td>
<td>Increased risk of lower level NTD with 677T allele, no difference in the frequency of A1298C polymorphism</td>
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<td></td>
<td>Case – Control</td>
<td>83 NTD mothers, 60 control mothers</td>
<td>Indian</td>
<td>No significant association with C677T polymorphism, reduced risk with C allele of A1298C polymorphism</td>
</tr>
<tr>
<td></td>
<td>Case – Control</td>
<td>77 NTD mothers, 61 control mothers</td>
<td>French</td>
<td>Reduced frequency of 677T allele in case mothers of lower defect group.</td>
</tr>
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<td></td>
<td>Case – Control</td>
<td>38 NTD mothers, 80 control mothers</td>
<td>Chinese</td>
<td></td>
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<tr>
<td><strong>MTR or MS (1q43)</strong></td>
<td>Case – Control</td>
<td>62 NTD mothers, 90 control mothers</td>
<td>Canadian</td>
<td>No significant difference in genotypic frequencies of A2756G polymorphism of the gene among case and control mothers</td>
</tr>
<tr>
<td></td>
<td>Case – Control</td>
<td>75 NTD mothers, 210 control</td>
<td>Italian</td>
<td>No significant difference observed in A2756G genotypic frequencies among cases and controls</td>
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*table contd.*
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Case Population</th>
<th>Control Population</th>
<th>Result</th>
<th>Reference</th>
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<tr>
<td>Zhu et al. 2003</td>
<td>Case – Control</td>
<td>122 NTD mothers, 127 control mothers</td>
<td>US Hispanic</td>
<td>No significant association of A2756G polymorphism with NTD risk</td>
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<tr>
<td>Candito et al. 2008</td>
<td>Case – Control</td>
<td>77 NTD mothers, 61 control mothers</td>
<td>French</td>
<td>No significant association of A2756G polymorphism with NTD risk.</td>
<td></td>
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<tr>
<td>Pietrzyk et al. 2003</td>
<td>Case – Control</td>
<td>106 SB mothers, 100 controls</td>
<td>Polish</td>
<td>Significantly increased risk associated with the A66G polymorphism in homozygous case mothers</td>
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<tr>
<td>Zhu et al. 2003</td>
<td>Case – Control</td>
<td>122 NTD mothers, 127 control mothers</td>
<td>US Hispanic</td>
<td>Significant association of G allele of A66G polymorphism with increased risk of NTD, additional risk if combined with MTR 2756G allele</td>
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<tr>
<td>Candito et al. 2008</td>
<td>Case – Control</td>
<td>61 controls</td>
<td>French</td>
<td>No significant association of A66G polymorphism with NTD risk</td>
<td></td>
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<tr>
<td>Pietrzyk et al. 2003</td>
<td>Case – Control</td>
<td>203 NTD mothers, 532 control mothers</td>
<td>UK</td>
<td>No significant association of three variants with risk of NTD</td>
<td></td>
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<td>O’Leary et al. 2005</td>
<td>Case – Control</td>
<td>447 NTD mothers, 476 controls</td>
<td>Irish</td>
<td>Increased risk of SB with GG genotype of A66G polymorphism</td>
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<td>van der Linden et al. 2006</td>
<td>Case – Control</td>
<td>116 SB mothers, 264 control mothers</td>
<td>Dutch</td>
<td>Marginally increased risk with G allele of A66G polymorphism</td>
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<tr>
<td>Candito et al. 2008</td>
<td>Case – Control</td>
<td>77 NTD mothers, 61 controls</td>
<td>French</td>
<td>No significant association of A66G polymorphism with NTD risk</td>
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<td>Parle Mc-Dermott et al. 2003</td>
<td>Case – Control</td>
<td>279 NTD mothers, 256 controls</td>
<td>Irish</td>
<td>No significant association of three variants with NTD risk</td>
<td></td>
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<td>Jensen et al. 2005</td>
<td>Family based association</td>
<td>354 NTD families</td>
<td>Mixed US</td>
<td>No significant association of C1095A polymorphism with NTD risk, risk may be influenced with maternal smoking.</td>
<td></td>
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<tr>
<td>Jensen et al. 2006</td>
<td>Family based association</td>
<td>374 NTD families</td>
<td>Mixed US</td>
<td>Variants of NAT1 with absent/diminished enzyme activity are associated with a decreased risk of spina bifida.</td>
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<td>De Marco et al. 2001</td>
<td>Case – Control</td>
<td>98 NTD mothers, 156 controls</td>
<td>Italian</td>
<td>Increased frequency of G allele of G80A mutation among case mothers</td>
<td></td>
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<tr>
<td>De Marco et al. 2003</td>
<td>Case – Control</td>
<td>43 NTD mothers, 156 controls</td>
<td>Italian</td>
<td>Increased risk of NTD with GG genotype in mothers</td>
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<tr>
<td>Relton et al. 2004</td>
<td>Case – Control</td>
<td>200 NTD mothers, 532 controls</td>
<td>UK</td>
<td>No association of G80A</td>
<td></td>
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</tbody>
</table>
Case – Control mothers, 539 control mothers UK with NTD risk O’Leary et al. 2006

Case – Control 437 NTD families, 852 controls Irish No significant association of G80A and 61-bp tandem repeat polymorphism with NTD risk O’Leary et al. 2006

Case – Control 38 NTD mothers, 80 control mothers Significant association of GG genotype of G80A polymorphism with NTD risk Shang et al. 2008

Case – Control 99 NTD mothers, 100 control mothers Chinese Increased risk for NTD child with GG genotype and without folic acid supplementation Pei et al. 2008

SHMT (17p11.2 and 12q13.2) Case – Control 120 SB mothers, 420 controls Dutch No association with increased risk of NTD is observed, CC genotype of C1420T mutation is associated with increased Hcy levels in mothers Heil et al. 2001

Case – Control 97 NTD mothers, 190 controls UK C1420T variation is associated with protective maternal effect Relton et al. 2004

TCNII (11q11-q12) Case – Control 42 NTD mothers, 73 controls Caucasian None of the variants found to be associated with increased risk of NTD No association of gene variants with SB risk Afman et al. 2002

Case – Control 366 NTD mothers, 724 controls Irish No association of C776G polymorphism with NTD risk Swanson et al. 2005

Case – Control 77 NTD mothers, 61 controls French No association of 28-bp repeat polymorphism with increased risk of NTD Candito et al. 2008

TYMS (18p11.32) Case – Control 194 NTD mothers, 177 control mothers UK with NTD risk Wilding et al. 2004

MOUSE MODEL BASED GENES

Naturally occurring or transgenic mouse models have provided nearly 200 candidate genes for NTDs (Greene et al., 2009). Majority of these genes are involved in the process neurulation at different steps, from neural induction to closure of the neural tube, and a few are implicated in essential cellular functions such as DNA repair and apoptosis. However, the investigation of these genes in human NTDs have not yielded much positive results (Kibar et al., 2007). This could be due to the difference in the process of neurulation or relative contribution of the gene to the NTD phenotype exhibited by the mouse and human or kind of study conducted. Most of the homozygous null mouse embryos exhibit syndromic NTDs, which...
do not resemble human NTDs closely. However, isolated NTDs in mouse can also result from the effect of hypomorphic alleles, combinations of heterozygous mutations and/or gene-environment interactions, which may resemble human NTDs more closely (Greene et al., 2009). Association studies with small sample size have provided inconsistent results. Large scale studies with detailed gene to gene and gene to environment interactions are further needed to detect the role of the mouse mutant candidate genes in human neural tube defects.

Table 2 highlights the mouse mutant genes that have been studied in relation to the maternal risk factor for NTDs. Two of the genes are tumor suppressor genes (BRCA1 and TP53), involved in maintaining a delicate balance between cell proliferation, differentiation and apoptosis, which is vital for proper neural tube closure (Gos and Szpecht-Potocka, 2002). Promoter haplotype of platelet derived growth factor receptor alpha (PDGFRA) gene related to the low activity is also studied amongst the mothers of NTDs in two of the population, but the results are inconsistent (Zhu et al. 2004, Toepoel et al. 2009). Furthermore, many mouse mutants point to the transcription factors involved in the formation of neural tube during the early embryonic development; but only few have been studied for the association of maternal genotype with the risk of NTD child. So far, only one variation in T gene (G allele of A530G) is found to be significantly high amongst British NTD

<table>
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<tr>
<th>Gene</th>
<th>Type of Study</th>
<th>Sample Size</th>
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<tr>
<td>BRCA1 (17q21)</td>
<td>Case – Control</td>
<td>62 case mothers, 142 controls</td>
<td>Dutch and British</td>
<td>Significantly Increased frequency of G4956 allele in NTD mothers with No significant increase in risk associated with the allele. Significantly Increased frequency of G1186 allele in British NTD mothers, no increase in risk associated with the allele.</td>
<td>Morrison et al. 1998</td>
</tr>
<tr>
<td>PDGFRA (4q12)</td>
<td>Case – Control</td>
<td>122 NTD mothers, 127 control mothers</td>
<td>US Hispanics</td>
<td>Significantly increased frequency of promoter haplotype with low transcription activity in NTD mothers, increased risk associated with the haplotype.</td>
<td>Zhu et al. 2004</td>
</tr>
<tr>
<td>T (Brachyury) (6q27)</td>
<td>Case – Control</td>
<td>56 SB mothers, 72 control mothers</td>
<td>Dutch</td>
<td>H1 promoter haplotype of mothers is not associated with increased risk for SB.</td>
<td>Toepoel et al. 2009</td>
</tr>
<tr>
<td>TFAP2 (6q24)</td>
<td>Family based association study</td>
<td>38 multiplex families</td>
<td>Dutch</td>
<td>No significant association of 1257C&gt;T SNP with risk of SB.</td>
<td>Klootwijk et al. 2003</td>
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<tr>
<td>TP53 (p53) (17p13.1)</td>
<td>Case – Control</td>
<td>532 NTD mothers, 999 control mothers</td>
<td>Irish</td>
<td>Increased risk of NTD child with 135 allele of intron 1 VNTR and T allele of rs1614984.</td>
<td>Pangilinan et al. 2008</td>
</tr>
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</table>
mothers (Morrison et al., 1998); however the author failed to explain the effect of maternal genotype for a gene that is expressed only during early development.

CONCLUSION

The etiology of human neural tube defects is very complex, with the involvement of different environmental, nutritional, and genetic factors. This further gets complicated with both maternal and embryonic genes participating in the process of neurulation. Besides the individual effects of these factors, coexistence of two or more factors aggravates the risk for NTDs. As maternal nutrition plays a significant role, maternal genetic predisposition and their interactions become very important. Candidate genes approach from one carbon metabolic cycle and their interactions become very important. Candidate genes approach from one carbon metabolic cycle and mouse mutants have revealed many maternal genotypes at risk in many populations worldwide, but no specific gene has been elucidated with the risk of NTDs. On the other hand, the altered effects of the genes are sometimes so small that it is insignificant if studied individually without taking into consideration the other factors, genetic or environmental. Hence, the combinatorial effect may disrupt the normal developmental process and increases the risk of defects up to many folds. Large scale studies involving the environmental, nutritional, and genetic factors in the same individual predisposing the risk for NTDs can prove beneficial to understand these interactions, and to reduce the incidence of occurrence and recurrence of the defects.

REFERENCES CITED


