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Genetic Complexity of Human Myelomeningocele

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Abstract

Myelomeningocele (MM) is the most severe form of lumbosacral open neural tube defect (NTD) compatible with survival and it results in various degrees of disability. Approximately 4 in 10,000 live births are affected in the United States (US). Mexican Americans, the fastest growing ethnic population in the US, have the highest prevalence rate for NTDs follows by Caucasian Americans. Occurrence of MM represents a significant economic and public health burden in the US. Both genetic and environmental factors contribute to the development of MM. The genetic contribution to NTD is complex. Over 205 genetic loci are implicated in mouse mutants and strains. Majority of mouse NTDs affect the rostral CNS and only a handful present only spina bifida. In contrast, the genetic variability and molecular mechanism(s) in the development of MM in humans are largely unknown. The contribution of de novo mutations to MM in humans is unknown and can be determined by characterizing the variations present in the exomes of MM affected individuals. Whole exome sequencing is a cost-effective and efficient tool to discover rare variants of large effect as well as the entire site frequency spectrum of variations in the exomes to establish disease association. In addition, sequencing of the regulatory elements in the genomes of MM affected individuals can discover rare variants that may affect expression of genes critical to the normal development of the neural tube.

Keywords: Neural tube defects; Myelomeningocele; Genetic association; Whole exome sequencing

Neural tube defect (NTD) is a general term for a congenital malformation of the central nervous system (CNS) occurring secondary to lack of closure of the neural tube and the worldwide incidence is ranging from 1 to 10 per 1,000 live births. The majority of cases are categorized as either anencephaly (lack of closure in the region of the head) or spina bifida (lack of closure below the head; SB) that occur in approximately equal frequencies at birth [1,2]. Individuals with anencephaly usually die within days of birth. Since the 1960s, advances in medical care have led to the survival of the majority of individuals affected by SB. Spina bifida encompasses several subgroups of defects including myelomeningocele (MM), meningocele, and lipomeningocele. Among these, MM (protrusion of the nervous tissue and its covering through a defect in the vertebrae) is by far the most common, accounting for greater than 90% of SB cases. Mexican Americans, the fastest growing segment of the US population, have the highest prevalence rate of SB at 4.17 per 10,000 [3]. Caucasian Americans have a slightly lower rate of MM at 3.22 per 10,000 and African-Americans have a rate of 2.64 per 10,000. An estimated lifetime of medical care costs for an individual who lives to 65 years of age and is affected with MM is ~ $806,000 in 2007 dollars [4]. MM contributes a huge economic and public health burden in the US.

Environmental risk factors for NTDs NTD formation have long been associated with environmental factors. Epidemiological studies have examined multiple factors, some possessing more potential for interaction with the genetic background of the child and mother than others. Among potential risk factors with genetic influences are maternal folate deficiency, diet and vitamin use, maternal glucose status as dictated by either obesity and/or diabetes, hyperthermia related to maternal illness or hot tub use, maternal medication use of prescribed drug particularly for epilepsy and therapies and, lastly, maternal use of recreational substances like alcohol, tobacco and illicit drugs [5]. Other nutrients and vitamins, including vitamin A, vitamin C, and zinc have also been investigated, but no consistent associations have been concluded [6-8]. A number of socio-demographic and epidemiologic findings have also been related to risk for an NTD affected pregnancy including: socioeconomic status, parental education, and maternal and paternal ages and occupations, maternal reproductive history including birth order, maternal country of birth and country of conception of NTD affected offspring [9-14].

Genetic risk factors for NTDs Numerous lines of evidence support genetic factors as important risk factors for NTDs. First, some ethnic groups/races such as Irish having a much higher *a* priori risk to have a child with an NTD [2]. In the US, Mexican Americans have the highest risk of having NTD affected offspring [3]. Second, familial recurrence of NTD provides strong support for the presence of genetic components. First-degree relatives of an affected individual have a risk of 3-5% and second-degree relatives a 1-2% risk. Further, familial recurrence is dependent on local incidence rates, with higher local risk rates predicting higher familial recurrence risk. Third, there are over 245 mutant mouse models that exhibit various types of NTDs as all/part of the phenotype with majority of the models having lesions in the head regions with spina bifida in some occasions [15]. Lastly, a number of human genetic syndromes that occur as a result of chromosomal imbalance or single gene mutation have NTDs as part of the phenotype [16]. Even though genetic factors definitely play an important role, it is difficult to discern exactly how this happens. No clear-cut mode of inheritance for NTDs can be determined. Numerous studies have concluded that for NTDs, there is only a 5-10% incidence of a positive family history making it difficult to use conventional methods to search for genes that contribute to risk for NTDs.

From human association studies, candidate genes examined were selected based on knowledge gained from epidemiologic studies such as those that identified maternal folic acid deficiency and maternal derangement of glucose metabolism as associated with increased risk for NTD-affected offspring. The strategies used are limited by prior knowledge. Over 130 studies attempting to find association of selected genes with NTDs were published between 1994 and 2010 [5]. These studies included approximately 132 candidate genes with known

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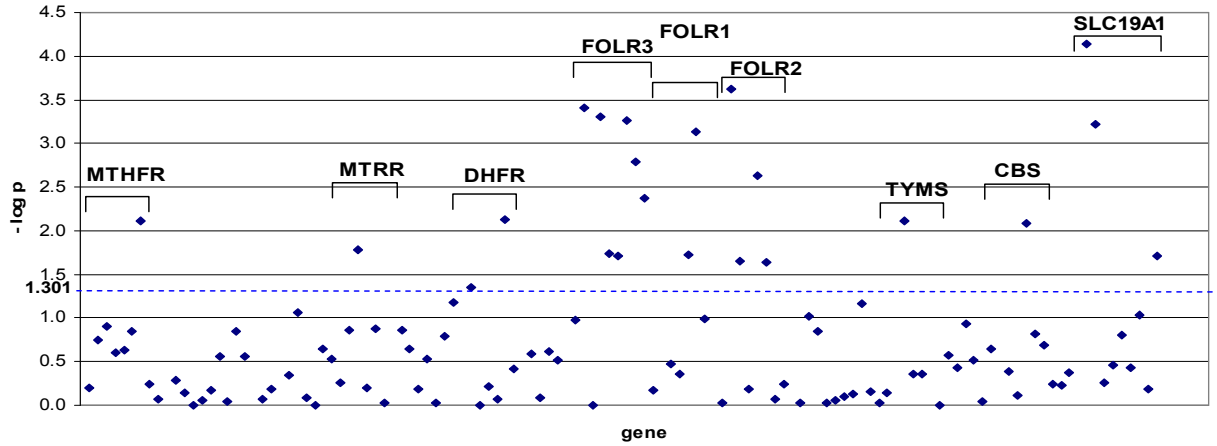
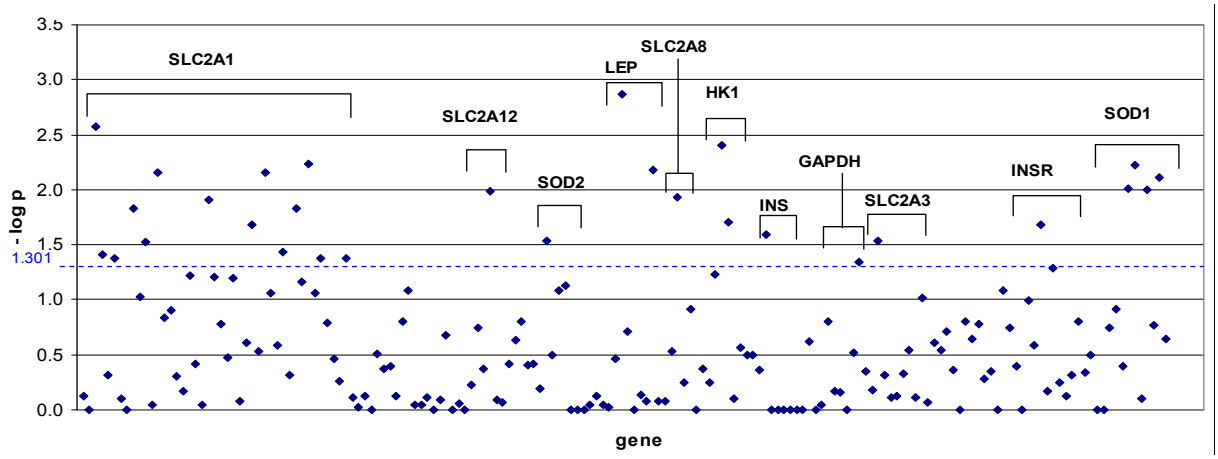
functions involving various aspects of developmental biological activity [17,18]. The association studies of human NTDs implicated that 42 of the 132 candidate genes tested has significant nominal association with NTD risk [5]. Our group has demonstrated significant association of several genes functioning in metabolism of folate as shown in figure 1 and metabolism of glucose as shown in figure 2 to susceptibility of MM development [19-21]. Another popular research strategy has been to select candidate genes identified from mutant mouse models affected with NTDs [22]. The number of mouse mutants with NTDs grew quickly from ~180 in 2007 to >240 in 2010 with identification of causative genes involved in both structural and functional roles that are vital in multiple important cell growth and development pathways [15]. Conversely, many genes with demonstrated association to MM in humans did not cause NTDs in mouse when knocked out while some mouse mutants present NTDs only with digenic or multi-genic defects [15,22]. A phenomenon suggesting gene-gene interactions, dosage effects and gene-environment interactions are involved in causing NTDs.

The involvement of approximately 205 genes with a wide range of functions in cellular and developmental pathways in animal models NTDs are well described in several recently published reviews [5,15,18,22-27]. Functional categories of these genes include but are not limited to metabolism of folate, glucose, and retinoic acids; cell cycle, cell migration and recognition, planar cell polarity, cell signaling, methylation, micro-RNAs and many others as shown in figure 3. More importantly, the number of NTD associated genes and NTD causing genes continues to increase steadily as illustrated by the growing number of NTD mouse mutants suggesting that the search for genetic etiology of human NTDs requires a comprehensive and global approach not limited by our current knowledge of the disease. These global and hypothesis-free approaches include genome-wide association (GWA) study or whole exome capture and sequencing (WES) or whole genome sequencing (WGS). Genome-wide association (GWA) studies have successfully identified genetic variants of small and modest effect for common human diseases such as hypertension and type II diabetes where thousands of subjects can be recruited for these studies [28].



Note: Each diamond represent the –log P value of PLINK TDT association analysis result of single nucleotide polymorphism (SNP) tested in the study. Dotted line represents the –log P value of 0.05.
Name of genes with SNP having –log P values above 1.301 are shown.

Figure 1: PLINK TDT identifies SNPs of folate metabolism related genes associated with myelomeningocele.



Note: Each diamond represent the –log P value of PLINK TDT association analysis result of single nucleotide polymorphism (SNP) tested in the study. Dotted line represents the –log P value of 0.05.
Name of genes with SNP having –log P values above 1.301 are shown.

Figure 2: PLINK TDT identifies SNPs of glucose metabolism related genes associated with myelomeningocele.

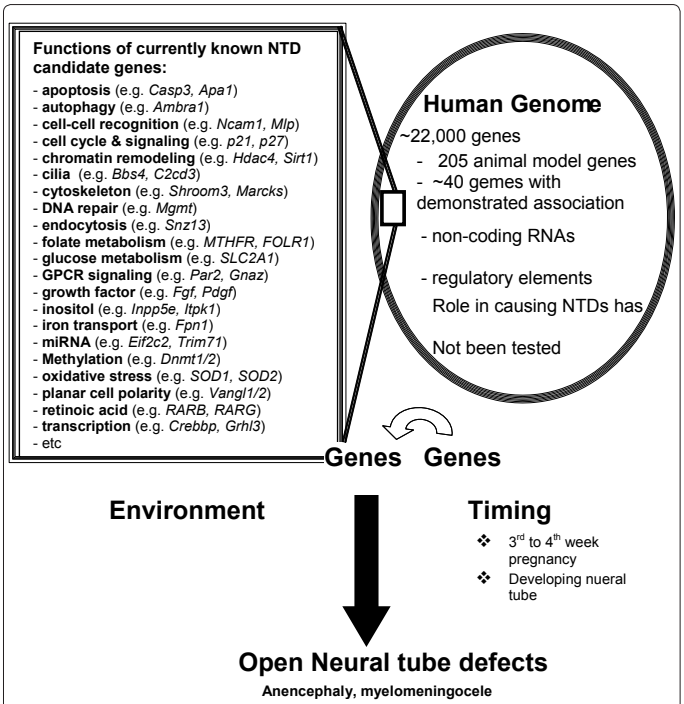


Figure 3: Complex etiology of neural tube defects development.

Because human NTDs are rare disease makes enrolling thousands of subjects for GWA studies not feasible for individual research groups or with consortium of research groups who could pool sample sets because the number of subjects still would not reach the level of those available for common, complex adult-onset diseases like hypertension and type II diabetes. Further, an NTD consortium would pool NTD sample sets of subjects with diverse genetic and socio-economic backgrounds, complicating the statistical analyses. More importantly, the majority of GWA studies are not able to detect rare variants of larger effect in all genes.

Approaches to identify genetic etiology of NTDs thus far, a paradigm that a significant proportion of MM could be caused by rare and de novo mutations in genes has not been tested. Studies on single gene disorders such as tuberous sclerosis complex [29] and complex traits neuropsychiatric disorders such as autism [30,31] have concluded gene disrupting de novo copy number variation (CNV) mutations likely contribute to the diseases between 5 and 10% of the affected population. Consistent with the observation, we and others have demonstrated gene disrupting CNVs may contribute up to 8% of all MM [32] arguing for the need to examine the paradigm that rare and de novo mutation of genes as a potentially major disease causing mechanism of MM. Results on association studies using common single nucleotide polymorphisms have been mixed [5] and the trend to examine the rare/de novo mutation paradigm as etiology for MM has been picking up. Recent review suggested the rare and de novo variants of six genes (CELSR1, FUZ, FZD6, PRICKLE1, VANGL1 and VANGL2) in the PCP pathway together may contribute to ~6% of all MM [27]. It also appears the presence of ethnic-specific risk signature of rare mutations among genes in sub-pathways of folate-related genes between Hispanic subjects and Caucasian subjects [33]. Our group has demonstrated associations between several genes relate to folate [19] and we are also the first group to demonstrated genetic association of several glucose metabolism genes to MM [21,34]. Sequencing of the exons and adjacent intronic regions of these genes (i.e. FOLR1, MTHFR and CBS) among MM subjects in our cohort found rare variants present between 1 to 3% [20,35,36]. Similar results were observed from sequencing genes relate to glucose metabolism such as SLC2A1 [34], SOD1 and SOD2 Kase et al. [37].

The development of next generation sequencing (NGS) and whole exome capturing platforms in the past few years provides the golden opportunity to find rare variants in genes that are highly likely to be important in causation of common diseases [38-41]. Up to now, approximately 80% of disease-causing mutations that have been discovered are located in the exome. WES allows an unbiased investigation of the complete protein-coding regions in the genome in relation to the disease of interest. Multiple laboratories have begun using selective re-sequencing of exons of genes with demonstrated association to spina bifida with the goal of discovering potential disease causing variants. However, the current Sanger sequencing approach is costly, has very limited throughput and is labor intensive. Using the high throughput NGS approach coupled with whole exome capture for a representative subset of subjects with defined phenotypes has proven to greatly facilitate the discovery of disease associating mutations [40,41]. In addition, rare variants within the exome of affected subjects identified by WES serve as valuable tools to facilitate follow up of association studies and functional studies to discover the genetic mechanisms leading to human complex traits development such as myelomeningocele.

Both genetics and environmental factors contribute to the development of complex traits such as myelomeningocele. While WES is a powerful hypothesis free tool in discovering rare variants in and near exonic regions of genes, mutations in "non-exonic" regions of genomes harboring gene expression regulatory elements most likely to be affected by environmental factors will be missed. It is necessary to develop tools to capture all the gene regulatory elements for NGS to identify regulatory elements mutation in the genome relevant to human diseases. Mutations and/or tissue specific methylation at gene expression regulatory regions is expected to play certain important roles in complex traits development. A comprehensive overview of the mutations and rare functional variants in the gene coding regions and the gene regulatory elements can advance our understanding of how human myelomeningocele develop.

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