Genetic Therapy for Spina Bifida

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Spina bifida (SB) is the most common congenital defect of the central nervous system. Despite family history being a risk factor for SB development, recurrence patterns are not attributed to a single genetic locus. Instead, SB is a complex trait caused by a combination of variants at multiple loci and involving multiple genes.

Keywords: Spina bifida ; Genetics

1. Neural Tube Defects in Animal Models

Over 240 mutants and strains have been identified for NTDs in mice [1]. Of the mice NTDs, some cellular processes have been identified, including planer cell polarity (PCP) pathway, cell apoptosis, DNA transcription, and the canonical Wnt/beta-catenin pathway.

Apical-basal polarization of cells is critical for the morphogenesis and function of mature tissues. *VANGL1* and *VANGL2* are mammalian homologs of a Drosophila gene required for PCP to develop eye, wing, and leg tissues [2][3]. The genes *VANGL1* and *VANGl2* are expressed at the dorsal portions of the neural tube and are essential for vertebrate morphogenesis [4]. Song et al. demonstrated that PCP is crucial in linking the anterior–posterior patterning information to the left–right asymmetry of the notochord prior to leftward nodal flow across the posterior notochord [4]. Therefore, notochord cells lacking the expression of *VANGL1* and *VANG2* have random cilia distribution, leading to turbulent nodal flow and disrupted symmetry [5]. Torban et al. also demonstrated that only mice heterozygous for *VANGL1* and *VANGL2* mutations showed profound developmental defects, including severe craniorachischisis [6].

The establishment of PCP involves the Wnt/Frizzled-PCP pathway. In the drosophila model, the proteins include cadherin Flamingo, transmembrane protein Frizzled (Fz), and cytoplasmic proteins Dishevelled (Dsh), Diego (Dgo), and Prickle (Pk). Mutations in these proteins lead to loss of polarity and cytoskeletal rearrangement [7]. Furthermore, the mouse homologs of Flamingo (CELSR), Dishevelled (DVL), Frizzled (FZD), and Prickle (Pk) have all been shown to contribute to the etiology of NTDs [8]. Non-core PCP tissue specific effectors also play an important role in the development of NTDs. The Fuzzy planar cell polarity (FUZ) gene, a tissue-specific effector, is involved in directional cell movement and is essential for neural tube formation in mice and humans [5]. A study demonstrated that Fuzzy knockout mice exhibited NTDs and proposed that mutations in the FUZ gene may account for human NTDs. The mechanisms of PCP mutations in NTDs mentioned above are further studied by Humphries et al.; the group introduced the mammalian mutation into Drosophila and revealed different defective phenotypic and functional behaviors, suggesting a causative relationship between PCP and the NTDs [9].

Other mutations leading to SB in mice involve DNA transcription and apoptosis. Specifically, the Foxc2 (MFH-1) transcription factor is suggested to mediate the extensive skeletogenesis in cells derived from neural crest and the mesoderm [10]. The Traf4 gene is involved in apoptosis. Mutations in this gene lead to the inability of nucleosome assembly proteins to bind to condensing chromatin in apoptosis and dysregulation of neuronal cell proliferation [11].

Lastly, genetic mutations in the canonical Wnt/beta-catenin pathway have been studied in the SB mouse model and shown to be implicated in human SB. Regulators for bone formation during skeletal growth and remodeling include the lipoprotein-receptor-related protein 6 (LRP6) in the canonical Wnt/beta-catenin pathway. It is implicated in the growth of axial skeleton and neural tube structures [12]. Lei et al. have found that several single nucleotide variants in LRP6 predispose embryos to NTDs [13]. The mediator complex (MED) is also known to have significant regulatory effects on WNT signaling and is associated with neural tube closures [14]. Recent studies using CRISPR/Cas9 technology to generate knock-in mice models provided strong evidence between functional variants of MED genes and some NTD etiologies [15]. The canonical Wnt signaling is also implicated in the regulation of PAX3 expression and as a target of PAX3. Mutation of the PAX3 transcription factor leads to defects in neural tube closure and is preventable by folic acid [16].

Recent studies show beta catenin gain of function and PAX3 loss of function produces additive effects on NTDs due to their interactions [17].

2. Human Genetics

Depending on the affected gene, SB inheritance patterns vary in humans. Studies have indicated autosomal dominant inheritance of SB and other NTDs at multiple loci, including the *VANGL1* gene, *VANGL2* gene, *FUZ* gene, the *CELSR1* gene, and the *TBXT* gene [13][18][19][20]. Despite the identification of the single genes, it is important to note that more and more recent evidence points toward an omnigenic model of spina bifida, suggesting that human spina bifida is caused by a series of genetic variants and their interaction with environmental factors [21][22].

Various heterozygous missense mutations of the *VANGL1* gene have been identified and associated with various NTDs in humans [20]. In a review by Marello et al., a strong association between the rare variants of *VANGL1* and the NTDs was suggested. In addition, Lei et al. and Kibar et al. sequenced the *VANGL2* gene in populations with various forms of NTDs and suggested that the mutations in *VANGL2* gene may predispose human fetuses to NTDs [13][18]. Other studies proposed indirect mutations affecting VANGL2 function associated with NTDs. The heterozygous frameshift mutation of the *CELSR1* gene is found to be associated with SB in a study of 192 patients in California [13]. Its mechanism is based on the interaction between the CELSR1 protein and the *VANGL2* gene, since the mutation results in less recruitment of VANGL2 for cell-to-cell contact. Other heterozygous missense mutations in the *FUZ* gene have been identified and linked to NTDs in the Italian population; the research team found five missense mutations in the *FUZ* gene that interfere with cilia generation, cell directional movement, or both [23].

Other mutant genes that are risk factors for spina bifida include the *CCL2* gene. The *CCL2* gene controls the monocyte chemotactic protein-1 (MCP) export levels after treatment with interleukin-1-beta in vitro [24]. Research studies have shown that first-trimester hyperthermia is associated with a twofold increase in spina bifida [25]. Thus, inflammation and elevated body temperature, especially signaling by molecules such as chemokines, may be involved in the causation of spina bifida. Jensen et al. found that polymorphism of the CCL2 A(-2518)G promoter is associated with spina bifida and it is speculated that this is due to the alleles' less intense systemic and local response to infection [26].

Furthermore, a *TBXT* mutant gene, specifically the transmission allelic variant TIVS7-2 [19], has also been linked to meningomyelocele. TBXT is encoded by the T gene and is vital for the formation and differentiation of posterior mesoderm and axial development in vertebrates. Although further studies showed that the mutant TIVS7-2 is associated with an increased risk of SB, the pathogenic mechanism remains unclear [27][28].

Since folate supplementation has been shown to prevent up to 70% of NTDs in humans, the gene mutations of enzymes involved in homocysteine-folate metabolism have also been studied extensively in humans [29][30]. These metabolic enzymes include methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MS), cobalamin coenzyme synthesis, and cystathionine b-synthase (CBS). The SNP R653Q polymorphism in the MTHFR1 gene has been observed in NTDs in Irish and Italian populations [31][32][33]. Other studies have shown that MTHFR 1298 A-C combined with MTHFR 677 C-T alternation increases the risk of spina bifida [34]. Similar effects have been observed in the polymorphism of the MS and 5-Methyltetrahydrofolate-Homocysteine Methyltransferase Reductase (MTRR). Doolin et al. concluded that variants in MS 2756A-G polymorphism and MTRR 66A-G polymorphism increase the risk of spina bifida by maternal genotype [35]. Genetic–nutrient interaction has also been considered. Christensen et al. found that MTHFR polymorphism and low folate status combined is associated with a greater risk for NTDs than either variable alone [36]. It was also shown that the 66A-G polymorphism in the MTRR gene combined with low levels of serum B12 in mothers and children increases the risk of spina bifida [37].

Next-generation whole-exome sequencing (NGS) allowed the identification of variants in new candidate genes that were previously not implicated in SB in humans. Through NGS, loss-of-function de novo variants of *SHROOM3*, *PAX3*, *GRHL3*, and *MYO1E* genes are linked to the development of NTDs; their proposed mechanisms include generation of protein-truncating variant and transcription factor defects [38][39][40]. Recently, rare and novel de novo variants in *RXRγ*, *DTX1*, and *COL15A1* genes and X-linked recessive variants *ARHGAP36*, *TKTL1*, *AMOT*, *GPR50*, and *NKRF* were also found to contribute to NTDs [41]. Furthermore, with exome sequencing, more evidence links the genes of the PCP pathway to the development of SB and craniorachischisis. The identified genes include *CELSR1*, *PRICKLE1*, *FZD6*, *SCRIB*, *PTK7*, *VANGL1*, and novel genes *FREM2* and *DISP2* [42][43][44][45][46]. Due to the genetic complexities and omnigenic nature of NTDs, clinically significant genetic targets will vary from individual to

individual. Therefore, genome sequencing is a crucial step towards improved understanding of the genetic implications in NTDs and precision medicine for SB.

References

1. Harris, M.J.; Juriloff, D.M. An Update to the List of Mouse Mutants with Neural Tube Closure Defects and Advances toward a Complete Genetic Perspective of Neural Tube Closure. Birth Defects Res. Part Clin. Mol. Teratol. 2010, 88, 653–669.
2. Kibar, Z.; Vogan, K.J.; Groulx, N.; Justice, M.J.; Underhill, D.A.; Gros, P. Ltap, a Mammalian Homolog of Drosophila Strabismus/Van Gogh, Is Altered in the Mouse Neural Tube Mutant Loop-Tail. Nat. Genet. 2001, 28, 251–255.
3. Murdoch, J.N.; Doudney, K.; Paternotte, C.; Copp, A.J.; Stanier, P. 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. 2001, 10, 2593–2601.
4. Song, H.; Hu, J.; Chen, W.; Elliott, G.; Andre, P.; Gao, B.; Yang, Y. Planar Cell Polarity Breaks the Bilateral Symmetry by Controlling Ciliary Positioning. Nature 2010, 466, 378–382.
5. Gray, R.S.; Abitua, P.B.; Wlodarczyk, B.J.; Szabo-Rogers, H.L.; Blanchard, O.; Lee, I.; Weiss, G.S.; Liu, K.J.; Marcotte, E.M.; Wallingford, J.B.; et al. The Planar Cell Polarity Effector Fuz Is Essential for Targeted Membrane Trafficking, Ciliogenesis and Mouse Embryonic Development. Nat. Cell Biol. 2009, 11, 1225–1232.
6. Torban, E.; Patenaude, A.-M.; Leclerc, S.; Rakowiecki, S.; Gauthier, S.; Andelfinger, G.; Epstein, D.J.; Gros, P. Genetic Interaction between Members of the Vangl Family Causes Neural Tube Defects in Mice. Proc. Natl. Acad. Sci. USA 2008, 105, 3449–3454.
7. Humphries, A.C.; Mlodzik, M. From Instruction to Output: Wnt/PCP Signaling in Development and Cancer. Curr. Opin. Cell Biol. 2018, 51, 110–116.
8. Juriloff, D.; Harris, M. A Consideration of the Evidence That Genetic Defects in Planar Cell Polarity Contribute to the Etiology of Human Neural Tube Defects. Birth Defects Res. Part A Clin. Mol. Teratol. 2012, 94, 824–840.
9. Humphries, A.C.; Narang, S.; Mlodzik, M. Mutations Associated with Human Neural Tube Defects Display Disrupted Planar Cell Polarity in Drosophila. Elife 2020, 9, e53532.
10. Iida, K.; Koseki, H.; Kakinuma, H.; Kato, N.; Mizutani-Koseki, Y.; Ohuchi, H.; Yoshioka, H.; Noji, S.; Kawamura, K.; Kataoka, Y.; et al. Essential Roles of the Winged Helix Transcription Factor MFH-1 in Aortic Arch Patterning and Skeletogenesis. Development 1997, 124, 4627–4638.
11. Régnier, C.H.; Masson, R.; Kedinger, V.; Textoris, J.; Stoll, I.; Chenard, M.-P.; Dierich, A.; Tomasetto, C.; Rio, M.-C. Impaired Neural Tube Closure, Axial Skeleton Malformations, and Tracheal Ring Disruption in TRAF4-Deficient Mice. Proc. Natl. Acad. Sci. USA 2002, 99, 5585–5590.
12. Kokubu, C.; Heinzmann, U.; Kokubu, T.; Sakai, N.; Kubota, T.; Kawai, M.; Wahl, M.B.; Galceran, J.; Grosschedl, R.; Ozono, K.; et al. Skeletal Defects in Ringelschwanz Mutant Mice Reveal That Lrp6 Is Required for Proper Somitogenesis and Osteogenesis. Development 2004, 131, 5469–5480.
13. Lei, Y.; Zhu, H.; Yang, W.; Ross, M.E.; Shaw, G.M.; Finnell, R.H. Identification of Novel CELSR1 Mutations in Spina Bifida. PLoS ONE 2014, 9, e92207.
14. Rocha, P.P.; Bleiss, W.; Schrewe, H. Mosaic Expression of Med12 in Female Mice Leads to Exencephaly, Spina Bifida, and Craniorachischisis. Birth Defects Res. Part Clin. Mol. Teratol. 2010, 88, 626–632.
15. Tian, T.; Cao, X.; Chen, Y.; Jin, L.; Li, Z.; Han, X.; Lin, Y.; Wlodarczyk, B.J.; Finnell, R.H.; Yuan, Z.; et al. Somatic and de Novo Germline Variants of MEDs in Human Neural Tube Defects. Front. Cell Dev. Biol. 2021, 9, 641831.
16. Sudiwala, S.; Palmer, A.; Massa, V.; Burns, A.J.; Dunlevy, L.P.E.; de Castro, S.C.P.; Savery, D.; Leung, K.-Y.; Copp, A.J.; Greene, N.D.E. Cellular Mechanisms Underlying Pax3-Related Neural Tube Defects and Their Prevention by Folic Acid. Dis. Model Mech. 2019, 12, dmm042234.
17. Palmer, A.J.; Savery, D.; Massa, V.; Copp, A.J.; Greene, N.D.E. Genetic Interaction of Pax3 Mutation and Canonical Wnt Signaling Modulates Neural Tube Defects and Neural Crest Abnormalities. Genesis 2021, 59, e23445.
18. Kibar, Z.; Torban, E.; McDearmid, J.R.; Reynolds, A.; Berghout, J.; Mathieu, M.; Kirillova, I.; Marco, P.D.; Merello, E.; Hayes, J.M.; et al. Mutations in VANGL1 Associated with Neural-Tube Defects. N. Engl. J. Med. 2007, 356, 1432–1437.
19. Morrison, K.; Papapetrou, C.; Attwood, J.; Hol, F.; Lynch, S.A.; Sampath, A.; Hamel, B.; Burn, J.; Sowden, J.; Stott, D.; et al. Genetic Mapping of the Human Homologue (T) of Mouse T (Brachyury) and a Search for Allele Association between Human T and Spina Bifida. Hum. Mol. Genet. 1996, 5, 669–674.

20. Bartsch, O.; Kirmes, I.; Thiede, A.; Lechno, S.; Gocan, H.; Florian, I.S.; Haaf, T.; Zechner, U.; Sabova, L.; Horn, F. Novel VANGL1 Gene Mutations in 144 Slovakian, Romanian and German Patients with Neural Tube Defects. Mol. Syndromol. 2012, 3, 76–81.
21. Chen, Z.; Lei, Y.; Zheng, Y.; Aguiar-Pulido, V.; Ross, M.E.; Peng, R.; Jin, L.; Zhang, T.; Finnell, R.H.; Wang, H. Threshold for Neural Tube Defect Risk by Accumulated Singleton Loss-of-Function Variants. Cell Res. 2018, 28, 1039–1041.
22. Boyle, E.A.; Li, Y.I.; Pritchard, J.K. An Expanded View of Complex Traits: From Polygenic to Omnigenic. Cell 2017, 169, 1177–1186.
23. Seo, J.H.; Zilber, Y.; Babayeva, S.; Liu, J.; Kyriakopoulos, P.; Marco, P.D.; Merello, E.; Capra, V.; Gros, P.; Torban, E. Mutations in the Planar Cell Polarity Gene, Fuzzy, Are Associated with Neural Tube Defects in Humans. Hum. Mol. Genet. 2011, 20, 4324–4333.
24. Rovin, B.H.; Lu, L.; Saxena, R. A Novel Polymorphism in the MCP-1 Gene Regulatory Region That Influences MCP-1 Expression. Biochem. Biophys. Res. Commun. 1999, 259, 344–348.
25. Chambers, C.D.; Johnson, K.A.; Dick, L.M.; Felix, R.J.; Jones, K.L. Maternal Fever and Birth Outcome: A Prospective Study. Teratology 1998, 58, 251–257.
26. Jensen, L.E.; Etheredge, A.J.; Brown, K.S.; Mitchell, L.E.; Whitehead, A.S. Maternal Genotype for the Monocyte Chemoattractant Protein 1 A(-2518)G Promoter Polymorphism Is Associated with the Risk of Spina Bifida in Offspring. Am. J. Med. Genet. A 2006, 140A, 1114–1118.
27. Shields, D.C.; Ramsbottom, D.; Donoghue, C.; Pinjon, E.; Kirke, P.N.; Molloy, A.M.; Edwards, Y.H.; Mills, J.L.; Mynett-Johnson, L.; Weir, D.G.; et al. Association between Historically High Frequencies of Neural Tube Defects and the Human T Homologue of Mouse T (Brachyury). Am. J. Med. Genet. 2000, 92, 206–211.
28. Jensen, L.E.; Barbaux, S.; Hoess, K.; Fraterman, S.; Whitehead, A.S.; Mitchell, L.E. The Human T Locus and Spina Bifida Risk. Hum. Genet. 2004, 115, 475–482.
29. Blencowe, H.; Cousens, S.; Modell, B.; Lawn, J. Folic Acid to Reduce Neonatal Mortality from Neural Tube Disorders. Int. J. Epidemiol. 2010, 39, i110–i121.
30. Rosenthal, J.; Casas, J.; Taren, D.; Alverson, C.J.; Flores, A.; Frias, J. Neural Tube Defects in Latin America and the Impact of Fortification: A Literature Review. Public Health Nutr. 2014, 17, 537–550.
31. 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. 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. 2002, 71, 1207–1215.
32. de Marco, P.; Merello, E.; Calevo, M.G.; Mascelli, S.; Raso, A.; Cama, A.; Capra, V. Evaluation of a Methylenetetrahydrofolate-Dehydrogenase 1958G>A Polymorphism for Neural Tube Defect Risk. J. Hum. Genet. 2006, 51, 98–103.
33. 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. 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. 2006, 14, 768–772.
34. Akar, N.; Akar, E.; Deda, G.; Arsan, S. Spina Bifida and Common Mutations at the Homocysteine Metabolism Pathway. Clin. Genet. 2000, 57, 230–231.
35. Doolin, M.-T.; Barbaux, S.; McDonnell, M.; Hoess, K.; Whitehead, A.S.; Mitchell, L.E. Maternal Genetic Effects, Exerted by Genes Involved in Homocysteine Remethylation, Influence the Risk of Spina Bifida. Am. J. Hum. Genet. 2002, 71, 1222–1226.
36. Christensen, B.; Arbour, L.; Tran, P.; Leclerc, D.; Sabbaghian, N.; Platt, R.; Gilfix, B.M.; Rosenblatt, D.S.; Gravel, R.A.; Forbes, P.; et al. Genetic Polymorphisms in Methylenetetrahydrofolate Reductase and Methionine Synthase, Folate Levels in Red Blood Cells, and Risk of Neural Tube Defects. Am. J. Med. Genet. 1999, 84, 151–157.
37. Wilson, A.; Platt, R.; Wu, Q.; Leclerc, D.; Christensen, B.; Yang, H.; Gravel, R.A.; Rozen, R. A Common Variant in Methionine Synthase Reductase Combined with Low Cobalamin (Vitamin B12) Increases Risk for Spina Bifida. Mol. Genet. Metab. 1999, 67, 317–323.
38. Lemay, P.; Marco, P.D.; Traverso, M.; Merello, E.; Dionne-Laporte, A.; Spiegelman, D.; Henrion, É.; Diallo, O.; Audibert, F.; Michaud, J.L.; et al. Whole Exome Sequencing Identifies Novel Predisposing Genes in Neural Tube Defects. Mol. Genet. Genom. Med. 2018, 7, e00467.

39. Lemay, P.; Marco, P.D.; Emond, A.; Spiegelman, D.; Dionne-Laporte, A.; Laurent, S.; Merello, E.; Accogli, A.; Rouleau, G.A.; Capra, V.; et al. Rare Deleterious Variants in GRHL3 Are Associated with Human Spina Bifida. Hum. Mutat. 2017, 38, 716–724.
40. Lemay, P.; Guyot, M.-C.; Tremblay, É.; Dionne-Laporte, A.; Spiegelman, D.; Henrion, É.; Diallo, O.; Marco, P.D.; Merello, E.; Massicotte, C.; et al. Loss-of-Function de Novo Mutations Play an Important Role in Severe Human Neural Tube Defects. J. Med. Genet. 2015, 52, 493.
41. Azzarà, A.; Rendeli, C.; Crivello, A.M.; Brugnoletti, F.; Rumore, R.; Ausili, E.; Sangiorgi, E.; Gurrieri, F. Identification of New Candidate Genes for Spina Bifida through Exome Sequencing. Child's Nerv. Syst. 2021, 37, 2589–2596.
42. Lei, Y.; Zhu, H.; Duhon, C.; Yang, W.; Ross, M.E.; Shaw, G.M.; Finnell, R.H. Mutations in Planar Cell Polarity Gene SCRIB Are Associated with Spina Bifida. PLoS ONE 2013, 8, e69262.
43. Lei, Y.; Finnell, R.H. New Techniques for the Study of Neural Tube Defects. Adv. Tech. Biol. Med. 2016, 4, 157.
44. Lei, Y.; Kim, S.; Chen, Z.; Cao, X.; Zhu, H.; Yang, W.; Shaw, G.M.; Zheng, Y.; Zhang, T.; Wang, H.; et al. Variants Identified in PTK7 Associated with Neural Tube Defects. Mol. Genet. Genom. Med. 2019, 7, e00584.
45. Robinson, A.; Escuin, S.; Doudney, K.; Vekemans, M.; Stevenson, R.E.; Greene, N.D.E.; Copp, A.J.; Stanier, P. Mutations in the Planar Cell Polarity Genes CELSR1 and SCRIB Are Associated with the Severe Neural Tube Defect Craniorachischisis. Hum. Mutat. 2012, 33, 440–447.
46. Beaumont, M.; Akloul, L.; Carré, W.; Quélin, C.; Journel, H.; Pasquier, L.; Fradin, M.; Odent, S.; Hamdi-Rozé, H.; Watrin, E.; et al. Targeted Panel Sequencing Establishes the Implication of Planar Cell Polarity Pathway and Involves New Candidate Genes in Neural Tube Defect Disorders. Hum. Genet. 2019, 138, 363–374.