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Review Article

Zoology For Better Disease Understanding

Genetic Regulation of Neural Tube Defects: A Contemporary Review.

Somenath Ghosh*

Assistant Professor, Department of Zoology, Rajendra P.G. College, Jai Prakash University, Chapra-841301, Bihar, India.

Abstract: Neural tube defects (NTDs), including spina bifida and anencephaly, are severe birth defects of the central nervous system that originate during embryonic development when the neural tube fails to close completely. It results from failure of the morphogenetic process of neural tube closure (see sidebar). In higher vertebrates, the neural tube is generated by the processes that shape, bend, and fuse the neural plate, and fusion in the dorsal midline progressively seals the neural tube as it forms. If closure is not completed, the neuroepithelium remains exposed to the environment and consequently subject to degeneration and neuronal deficit. Although the unifying feature of open NTDs is incomplete neural tube closure, evidence points to many different possible causes, both genetic and environmental. In humans, it appears that most NTDs are multifactorial, resulting from an additive contribution of several risk factors, which are each individually insufficient to disrupt neural tube closure (the multifactorial threshold model). The type and severity of these open NTDs vary with the level of the body axis affected. Thus, failure of closure in the prospective brain and spinal cord results in anencephaly and open spina bifida (myelomeningocele), respectively. Human NTDs are multifactorial, with contributions from both genetic and environmental factors. The genetic basis is not yet well understood, but several nongenetic risk factors have been identified as having possibilities for prevention by maternal folic acid supplementation. Mechanisms underlying neural tube closure and NTDs may be informed by experimental models, which have revealed numerous genes whose abnormal function causes NTDs and have provided details of cellular and morphological events whose regulation is essential for closure. Such models also provide an opportunity to investigate potential risk factors and to develop novel preventive therapies

Keywords: Differential expression, Embryogenesis, Factors, Genes, Neurulation, Neural Tube Disorder.

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***Corresponding Author**

Dr. Somenath Ghosh , Assistant Professor, Department of Zoology, Rajendra P.G. College, Jai Prakash University, Chapra-841301, Bihar, India.

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1. INTRODUCTION

It is a well known fact that there is a strict correlation between folate deficiency and impaired neural tube development in human[1]. Under each and every case, numerous hereditary and environmental risk elements are recognized from that point forward, despite the fact of potent relationship between the folate and Neural tube defects (NTD) risk**.** Early clinical perceptions during the mid-1960s prompted an understanding that diminished maternal folate level was connected with enhanced NTD risk [1-3]. Consequent studies distinguished enhanced maternal homocysteine, which is a biomarker of diminished folate level and metabolism, as a risk element for NTDs [4-6]. Later, randomized control trials and supplementation of folic acid in population [7-9] confirmed its viability in decreasing both NTD event and recurrence up to 70 % [10] however the metabolic mechanism of folate-responsive NTDs pathogenesis is still unidentified. Furthermore, previous studies are in partial and multiple information are scattered. Thus, the lacunae of the previous studies are identified and the present review is designed to accumulate all of the previous knowledge and/information under one roof to provide a preliminary/basic platform for future researches.

1.1 RISK FACTORS AND MATERNAL FOLATE LEVEL

Maternal folate level is connected to NTD risk, however certain individuals are at severe risk than others which are independent of folate level, showing that only folate deficiency is not enough to cause NTD [11]. A hereditary segment of NTDs has been perceived for a long time ago but family history is one of the most potent risk factors for NTDs [12, 13]. Discrepancies in folate metabolism and its status adds to NTD risk, examination of hereditary risk element in people has concentrated on variation within genes that encode proteins which bind, transport, prepare and metabolize folate, with the presumption that genetically-induced adjustments in folate level or metabolism are prone to add NTD pathogenesis [14-17]. In spite of the fact that polymorphisms have been distinguished in folate-related genes to NTD risk, the aggregate genetic variation recognized till date that imparts to NTD risk don't represent the overall genetic commitment to NTD rate in human population [18]. The NTD pathogenesis is rising with the association between predisposing genetic factors and lack of essential or auxiliary nutrients. In spite of the fact that maternalfolate supplementation lowers the danger for NTDs by adjusting an essential folate deficiency, it is obvious that this clarification may not represent numerous instances of NTD counteractive action. Moreover, folic acid supplementation may forestall NTDs even without clear maternal folate deficiency, in light of the fact that most women with a NTD-influenced pregnancy are not folate-deficient [19-21]. Expanded folate consumption, as folic acid supplements or strengthened food, may adjust for genetically connected disabilities in folate usage and auxiliary supplement insufficiencies not rectify fundamentally lack of folate[11] Along with these examinations concerning gene-nutrient interactions that leave a solution in NTD pathogenesis now, recognize among:

(1) Single gene variations that influence folate level alone,
(2) Single gene variations that influence folate use and metabolism, and
(3) Single gene variations that influence both utilization of folate level and metabolism. Impairments in folate level can be the outcome of lack of dietary folate, however it can also be obtained from genetic variation that affects the accumulation of cellular folate, including its assimilation, preparation, transport, maintenance and degradation [22]. Unwinding the relative contribution of the genetic and nutritional parts of NTD risk will be obliged to recognize the particular pathways that prompt NTD pathogenesis, which will empower the configuration of better focused and effective interventions for NTD prevention.

1.2 FOLATE-MEDIATED ONE-CARBON METABOLISM

Folate's capacity as a group of metabolic cofactors that contain and chemically trigger single carbons, known as "one-carbon units" for a different anabolic and catabolic responses together with known as "folate-mediated one-carbon digestion system"(OCM). Folate-initiated one-carbons are conveyed by tetrahydrofolate (THF). Tetrahydrofolate contains one-carbons at three different oxidation states, drifting from formaldehyde to methanol, and the one-carbon types of folate can be exchanged enzymatically [23-26]. Folate cofactors in cells additionally contain a poly-γ-glutamate peptide that changes long in cells from three to nine glutamate residues. FolateMonoglutamates, predominantly as 5-methyl-THF, are available in serum and transported into cells [27-29]. Folate-mediated OCM is a metabolic system of related pathways that is compartmentalized in mitochondria, cytoplasm and the nucleus. In mitochondria folate metabolism is needed for the generation of formate, glycine and ^{fmet}tRNA from the catabolism of choline, serine and glycine [30-32]. After the formation, folate is transported from mitochondria to the cytoplasm, where it acts as primary source of one-carbon units for cytoplasmic OCM. Fundamentally, folate-mediated OCM in the cytoplasm is important for

(1) Purine biosynthesis,
(2) Thymidylate biosynthesis and
(3) Remethylation of homocysteine for the production of methionine.

Methionine is needed for the biosynthesis of cofactor S-adenosylmethionine (AdoMet, [33], which serves as the universal one-carbon donor for cellular methylation responses including chromatin, proteins, lipids, and other particles [34]. The enzymes serine hydroxymethyltransferase, thymidylate synthase and dihydrofolatereductase establishes *de novo* thymidylate biosynthesis cycle that are modified by the small ubiquitin-like modifier (SUMO) and transported to the nucleus for thymidylate biosynthesis [35, 36]. Weaknesses in folate-mediated OCM can result from decreased folate level, polymorphisms in genes that encode folate-metabolizing compounds, or lack of micronutrient which adjust folate level, including vitamins B complex [37-39]. Biomarkers of impaired OCM incorporate decreased ability to combine thymidylate prompting expanded uracil content into DNA [40], enhanced serum homocysteine[41] and DNA hypomethylation[42, 43].

1.3 FOLATE TRANSPORT

Food folates, which contain a polyglutamatepeptide, and folic acid are sources of dietary folates, a synthetic dietary supplement and fortificant. Folic acid is chemically stable mono glutamic and oxidized form of folate differentiating ~~it~~

from natural reduced folates. In enterocytes, it is difficult to distinguish them from natural food folate as most folic acid is readily absorbed and converted to THF [44]. Firstly, the enzyme folylpoly-γ-glutamate carboxypeptidase II (gene name *GCPII*) in the gut converts polyglutamated food folate derivatives into monoglutamate derivatives before it's absorption through the intestine [45] thereafter, accomplished through a recently identified intestinal folate receptor (*PCFT*) [46]. Cell surface takes up serum folates in the form of monoglutamate 5-methyl-THF, through various ways, which includes the reduced folate carrier (RFC) [47], a facilitative anion-exchange carrier, or an endocytotic process mediated by one of two folate receptors, FRα and FRβ [48, 49]. Glycosyl-phosphatidyl-inositol moieties showing high affinity for 5-methyl-THF are present with the membrane-anchored folate receptor [48]. The enzyme folylpoly-γ-glutamate synthetasecatalysespolyglutamation of folate cofactors and results in the sequestration of folates within the cell. The affinity of THF cofactors towards folate-dependent enzymes is also increased by the polyglutamate peptide [23, 25] and 5-methyl-THF is the most abundant folate derivative inside the cell. At present, there is no conclusive evidence that common polymorphisms within genes encoding proteins, which mediate folate transport and absorption, affect folate level. No variants are identified within the coding regions of the folate receptor genes, and investigations of polymorphisms in noncoding regions of FRα and FRβ have not shown an association with NTD risk [50-53]. Embryonic lethality occurs due to coding variants within genes that impair folate transport and accumulation may not be compatible with life [54]. Folate deficiency can possibly influence up regulation of folate transporter expression that could mask deleterious genetic variation. Yet, risk for NTDs in humans is not deliberated by genetic variation within the folate receptor genes. However, under conditions of folate deficiency, a common single nucleotide polymorphism (SNP) in the *RFC1* gene has indicated moderate association with NTD risk [55-56], even though with low penetrance. No polymorphisms have been detected within the gene encoding folylpoly-γ-glutamate synthetase that confers risk for NTDs in human populations regarding genes encoding proteins that convey folate processing. In the same way, a single variant identified in *GCPII* does not affect NTD risk in humans [57]. Thus, it is evidenced that folate transport, absorption, processing, and retention are interrupted by genetic variation to a degree that independently contributes to NTD risk in human populations. However, it is mentioned that genetic alterations of NTDs that are complex traits, might aggravate nutritional deficiencies or metabolic impairments, thus, subgroups of a population are concerned with genetically-induced impairments in OCM.

1.4 GENETIC RISK FACTORS

Genetic deletion of the gene encoding FRα has been observed to result in NTDs in mice and also demonstrated a definitive and causal role of embryonic folate deficiency in NTD pathogenesis [58, 59]. There are no differences observed in homocysteine levels in FolR1$^{+/-}$ dams maintained on a normal diet [60], nor are there any differences in global DNA methylation in FolR1$^{+/-}$ embryos or FolR1$^{-/-}$ embryos rescued to gestation day 15.5 with folic acid [61]. Further support for the concept that genetic disruption of folate transport affects folate status in the absence of alterations in folate utilization is provided by rescuing the NTDs in nullizygous FolR1 knockout embryos by maternal supplementation with folic acid [60], because reductions in transport capacity can be surmounted by maternal vitamin supplementation. Deletion of the gene encoding RFC in mice also reveals the same finding. Deletion of RFC results in early embryonic lethality; however, maternal folic acid supplementation rescues up embryonic survival until gestation day 12, [62, 63]. Collectively, these data suggest that embryonic development and neural tube closure requires adequate folate status mediated by cellular folate uptake. However, these models do not provide further information about specific causes that lie behind human folate-responsive NTDs in the absence of an association between human genes involved in folate uptakes and NTD risk.

1.5 FOLATE METABOLISM IN THE MITOCHONDRIA

Generation of formate and glycine from the enzymatic cleavage of serine is considered as the primary role of folate metabolism in mitochondria. In some tissues, formate can also be generated through mitochondrial folate metabolism by metabolising glycine [64]. The mitochondrial isoform of serine hydroxymethyltransferase (mSHMT; gene name *Shmt2*), which catalyzes the conversion of serine and THF to form glycine and methylene-THF initiates this pathway. Methylene-THF can also be produced from glycine either by the glycine cleavage system [65] or the catabolism of sarcosine and dimethylglycine[66]. Methenyl-THF is produced by the oxidation of Methylene-THF in a reaction catalyzed by methylenetetrahydrofolate dehydrogenase (MTHFD), and is subsequently hydrolyzed to 10-formyl THF by the enzyme methenyltetrahydrofolatecyclohydrolase (MTHFC). Free formate and THF are generated by hydrolysis of the formyl group of 10-formyl-THF to complete the cycle, in a reaction catalyzed by formyltetrahydrofolatesynthetase (FTHFS) [67]. Format then traverses into the cytoplasm and serves as a major source of one-carbon units for cytoplasmic OCM. Some of the genes encoding the enzymes that catalyze the generation of formate from 5,10-methylene-THF in the mitochondria have yet to be identified [64]. Until now, we have few reports regarding the role of mitochondrial folate metabolism in NTD pathogenesis [68-70]; this includes investigations of human genetic susceptibility and genetically-manipulated mouse models [71, 72]. The paucity of knowledge regarding the identity of the genes and enzymes that regulate OCM in the mitochondria and the degree to which the capacity of mitochondrial OCM, including formate production, affects cytoplasmic OCM is one of the limitations of this model. Furthermore, mitochondrial OCM in different tissues and cell types plays different metabolic roles. Whereas it has been shown in certain cell types that mitochondrial OCM is an essential source of glycine [64], it remains to be established definitively that formate derived from mitochondrial OCM is essential for cytoplasmic OCM. Recently, a viable and fertile mouse knockout model of the gene encoding the cytoplasmic SHMT isoform (cSHMT; *Shmt1*) was produced [73]. One-carbon units generated in the cytoplasm by cSHMT, through the expression of *Shmt1* are not essential for growth and survival is indicated by this model. Mitochondrial OCM plays a vital role in the production of one-carbon units for folate-dependent anabolic reactions in the cytoplasm as emphasized by the mouse model. Further investigation on the role of mitochondrial OCM in regulating folate-dependent anabolic pathways in the cytoplasm is warranted. Apart from this, exploration of human polymorphisms in mitochondrial folate-dependent enzymes and the creation of mouse models with disruptions

in genes encoding the mitochondrial folate pathway will provide information regarding the potential contribution of mitochondrial folate metabolism to NTD pathogenesis [68, 69]

1.6 FOLATE METABOLISM IN THE CYTOPLASM

Cytoplasmic OCM is necessary for the *de novo* biosynthesis of nucleotides as well as AdoMet-dependent cellular methylation reactions[74]. The cofactor 10-formyl-THF is utilized as the one-carbon donor for carbons 2 and 8 in the purine ring by the purine biosynthesis pathway[75]. Methylene-THF is used as a cofactor for the methylation of deoxyuridine monophosphate (dUMP) by de novo thymidylate biosynthesis to form deoxythymidine monophosphate (dTMP), in a reaction catalyzed by the enzyme thymidylate synthase (TS; [76, 77]. A reaction of the pyridoxal phosphate (PLP)-dependent enzymatic conversion of serine and THF, catalyzed by the enzyme cytoplasmic SHMT (cSHMT, gene name *Shmt1*), forms Methylene-THF and also generates glycine. Catalyzed by methionine synthase, the enzyme methylene-THF reductase (*MTHFR*) irreversibly reduces Methylene-THF to form 5- methyl THF, which serves as the cofactor for the B_{12}-dependent remethylation of homocysteine to form methionine. The intermediate S-adenosylhomocysteine (AdoHcy) is formed by the transfer of the one-carbon from AdoMet, which is hydrolyzed to homocysteine and adenosine by the enzyme S-adenosylhomocysteine hydrolase [74]. In spite of having so much knowledge about OCM and its anabolic pathways in the cytoplasm, to find a solution to the causal metabolic pathway associated with NTD risk is still a challenging task. As the concentration of folate-binding proteins and enzymes far exceeds the concentration of folate cofactors within the cell, and that's why all cellular folate is protein bound [22]. This concludes the fact that folate-dependent anabolic reactions in the cytoplasm compete for a limiting pool of folate-derived one-carbon units and folate cofactors [78] These two pathways are sensitive to folate deficiency; therefore, it is difficult to determine the individual effect of either thymidylate synthesis of homocysteineremethylation (and cellular methylation reactions) on NTD risk. It has been advised by mathematical modeling [79] and experimental data [78, 80] that under normal cellular conditions, the biosynthesis of 5-methyl-THF is preferred over the biosynthesis of thymidylate.

2. CONCLUSION

From the present review, in brief we may conclude that the research in this field was initiated in the 1960s and till date it is going on. As an outcome of all of these studies, now we are aware that there are different factors responsible for Neural Tube Defects (NTD). Among different factors, some factors which are particularly associated with metabolism with some genetic disorders are of highest importance. However, further in depth studies are needed to explore the actual cause and remedy for these birth defects.

3. AUTHORS CONTRIBUTION STATEMENT

SG is solely responsible for designing the present review, survey of literature and acquiring relevant data. He himself is responsible for writing the review and all editorial correspondence.

4. CONFLICT OF INTEREST

Conflict of interest declared none.

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