

叶酸缺乏及相关基因SNP对神经管缺陷的影响

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摘要: 神经管缺陷(neural tube defects, NTDs)是由多重复杂因素影响的神经管形成及分化过程紊乱而产生的严重先天畸形。叶酸缺乏及代谢酶所携带的单核苷酸多态(single nucleotide polymorphism, SNP)与NTDs存在高度相关性。叶酸代谢关键酶SNP在人群中分布广泛, 是NTDs的重要潜在危险因素。叶酸代谢关键酶SNP会影响叶酸的代谢过程, 进而致使DNA甲基化、DNA合成、同型半胱氨酸代谢等的异常, 最终引发NTDs。本综述旨在通过分析总结体内叶酸缺乏及相关基因SNP对神经管缺陷的影响来为更好地防治神经管缺陷提供新思路。

关键词: 叶酸; 神经管缺陷; 单核苷酸多态

The effect of folate deficiency and related gene SNP on neural tube defects

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Abstract: Neural tube defects (NTDs) are serious congenital malformations caused by the disorder of neural tube formation and differentiation that are affected by multiple complex factors. Single nucleotide polymorphism (SNP) carried by metabolic enzymes and folate deficiency is highly correlated with the incidence of NTDs. In particular, SNP, a key enzyme of folate metabolism, which is widely distributed in people, is an important potential risk factor of NTDs. It can affect the metabolic process of folate, which further leads to the abnormalities of DNA methylation, DNA synthesis, homocysteine metabolism and other exceptions, eventually triggering NTDs. This review aims to provide new ideas for better prevention and treatment of neural tube defects by analyzing and summarizing the effects of folate deficiency and related gene SNP on neural tube defects.

Key Words: folate; neural tube defects; Single nucleotide polymorphism

神经管缺陷(neural tube defects, NTDs)的全球患病率在0.12%~1.24%之间^[1]。神经管是神经系统的主要始基, 其参与脑与脊髓的发育。神经管发育缺陷导致的无脑儿、脑膨出、唇裂、腭裂等对

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个体成长有致命影响。研究表明,叶酸经代谢转化后在神经管的发育过程中有重要作用,神经管形成期间,基因的正确时空表达是至关重要的^[2],此过程涉及甲硫氨酸合成酶(methionine synthase, MTR)^[3]、甲硫氨酸合酶还原酶(methionine synthase reductase, MTRR)^[4]、亚甲基四氢叶酸还原酶(methylenetetrahydrofolate reductase, MTHFR)、胱硫醚-β合酶(cystathionine β-synthase, CBS)^[5]等多种关键酶。在人群中,编码关键酶的基因序列存在单核苷酸多态(single nucleotide polymorphism, SNP),某些位点碱基的改变可以影响酶的活性从而使叶酸代谢过程发生改变^[6],叶酸的代谢产物对基因的表达调控有关键作用,一旦叶酸缺乏或代谢失调会致使DNA甲基化异常^[7]、DNA的合成异常^[8]、血浆同型半胱氨酸(homocysteine, Hcy)浓度升高^[9],进而使神经管发育过程中的基因表达异常,导致神经管在个体发育过程中延闭或中断不闭,最终引发NTDs。因此,预测叶酸代谢障碍关键酶基因的SNP亚型,适当调整补充叶酸剂量,能够有效降低NTDs的患病率。

1 叶酸代谢途径概述

蔬果、乳类、谷物等普遍含有叶酸,但Vidmar等^[10]表示,食物中的叶酸作为人体必须的微量营养素在体内不能被直接利用,可在MTHFR的帮助下由5,10-亚甲基四氢叶酸结构转化为5-甲基四氢叶酸形式才能被完全利用,转运参与一碳单位的代谢。而Fernández-Arroyo等^[11]证实,5-甲基四氢叶酸在有甲基载体运输作用的MTRR、MTR及维生素B12催化下,为Hcy再次甲基化为甲硫氨酸(methionine, Met)供给原料。Met是S-腺苷甲硫氨酸(s-adenosylmethionine, SAM)的前体, SAM在多种甲基转移酶如DNA甲基转移酶3α(DNA methyltransferase 3 alpha, Dnmt3a)、DNA甲基转移酶3β(DNA methyltransferase 3 beta, Dnmt3b)等的作用下为DNA提供甲基供体,参与DNA甲基化反应。SAM下游产物S-腺苷高半胱氨酸(s-adenosyl homocysteine, SAH)可逆地分解为Hcy再次进行循环利用, Hcy作为Met循环的中间体,既是SAM、SAH的下游产物,又在CBS作用下为同型半胱氨酸胸内酯(homocysteine thiolactone, HTL)提供原

料,其中血红素及5'-磷酸吡哆醛(pyridoxal-5'-phosphate, PLP)作为CBS的辅助因子,主要影响CBS活性^[12]。另外,据相关资料证实,核苷酸的形成受叶酸代谢影响。叶酸经体内转化的活化的四氢叶酸可在MTHFR作用下经10-甲酰四氢叶酸、5,10-甲基四氢叶酸间接转化为5,10-亚甲基四氢叶酸,又可经丝氨酸羟甲基转移酶(serine hydroxymethyl transferase, SHMT)直接转化,5,10-亚甲基四氢叶酸依赖胸苷酸转移酶(thymidylate synthase, TYMS)催化形成二氢叶酸,二氢叶酸再转化为四氢叶酸进行循环利用, TYMS还参与了脱氧尿苷酸单磷酸脂向胸苷酸单磷酸脂的转化,进而参与核苷酸的合成^[13](图1)。由此可见,叶酸代谢途径是人体将摄取的叶酸转化为有活性的四氢叶酸吸收利用,或由中间产物5-甲基四氢叶酸产生下游产物SAM、SAH、Hcy等,并在MTHFR、MTRR、CBS等关键酶的作用下,维持稳定的DNA甲基化、DNA合成、Hcy循环的过程。

2 叶酸缺乏对NTDs产生的影响

叶酸作为参与体内新陈代谢的必需微量元素,孕期摄入不足易产生NTDs的胎儿。叶酸缺乏引发的NTDs主要经以下3个重要的代谢过程调节。

2.1 叶酸缺乏致使DNA甲基化稳态破坏引发NTDs

无论是摄入减少还是吸收障碍,当红细胞内和血浆中的叶酸水平明显下降时,下游5-甲基四氢叶酸代谢途径受阻,其直接表现为Met水平的降低。Dunlevy等^[14]定量测定胚胎中SAM、SAH的浓度,发现增加同剂量Met处理后二者均升高,但后者较前者升高更明显,使SAM/SAH比值下调,从而抑制甲基转移酶活性,尤其是会改变Dnmt3a/3b等甲基转移酶的活性,破坏DNA甲基化稳态从而导致神经管的闭合延迟,最后形成NTDs。因此, Met的水平与NTDs的发生有直接关系。而Sobczyska-Malefora等^[15]验证当叶酸缺少时,给予高剂量Met可使NTDs发生率下降40%~70%。Okano等^[16]证实, Dnmt3b对胚胎干细胞及早期胚胎中着丝粒小卫星重复序列产生的甲基化具有特异性,并在敲除DNA甲基化转移酶Dnmt3b的造模鼠中发现,以SAM为甲基供体的DNA甲基化稳态被破坏。说明5-甲基四氢叶酸是DNA甲基化过程中生

Hcy介导的神经管形成过程中多种基因表达异常。Kobus等^[24]证实,与对照发育第6天的鸡胚相比,在经Hcy处理后具有开放脊柱裂的鸡胚中发现神经管轴向间充质细胞中发育调控基因配对框1(paired box 1, *Pax1*)、配对框9(paired box 9, *Pax9*)和性别决定区Y框9(SRY-box transcription factor 9, *Sox9*)的基因产物表达下调,而给予叶酸预处理可以逆转由Hcy诱导的基因表达异常产生的NTDs。Han等^[25]采用禽类模型,将鸡胚胎暴露于Hcy(50 μ M) 24 h后,发现其神经管不闭合,前肠门区域显示弥漫的 β -N-乙酰氨基己糖苷酶(β -N-acetylhexosaminidase, *Hex*)表达,给予叶酸可以逆转这种现象,证实叶酸缺乏可以导致Hcy浓度升高,高浓度的Hcy靶向Wnt- β -catenin通路中的调节基因*Hex*,从而诱发NTDs。

2.3 叶酸缺乏致使核苷酸生物合成减少引发NTDs

Splootch小鼠胚胎常用来研究由叶酸缺乏致使核苷酸合成减少引发NTDs的发病机制。Wlodarczyk等^[26]使用脱氧胸苷抑制测定法显示,Splootch胚胎中*Sp/Sp*纯合型胚胎合成胸苷的能力下降,当在培养基中添加叶酸或胸苷后,可以减轻*Sp/Sp*胚胎的代谢障碍,进而起到预防NTDs的作用。又有研究显示,*Sp/+*和*Sp/Sp*胚胎中SHMT蛋白浓度升高,而TYMS蛋白浓度降低。经PCR检测发现,二者无任何基因型差异,表明Splootch突变对两种蛋白质浓度的影响发生在转录后^[13]。Laks等^[27]在引起核苷酸合成减少的离体模型dT+DI-39中发现,转录因子配对框3(paired box 3, *Pax3*)激活。Sudiwala等^[28]进一步证实,叶酸缺乏使*Pax3*发生突变进而导致杂合小鼠神经棘细胞缺陷,表现出纯合斑点(*Sp/Sp*)小鼠皮毛呈白色斑点状。由此可见,叶酸缺乏使转录因子*Pax3*被激活,致使SHMT、TYMS蛋白质浓度异常,SHMT影响胸苷酸的从头合成,致使尿嘧啶的错误掺入增加,使核苷酸可用性降低,而TYMS是产生二氢叶酸的关键酶,二氢叶酸是胸腺嘧啶的必需前体分子,胸腺嘧啶又是DNA合成所必需的,表明叶酸缺乏后二者浓度异常导致的核苷酸合成减少,降低了细胞增殖速率并促使基因组易变,致使NTDs的发病率升高。

3 叶酸代谢障碍相关基因SNP对NTDs产生的影响

叶酸代谢过程中的多种关键酶基因SNP主要通过影响酶活性使下游代谢通路受阻,导致DNA低甲基化或高Hcy引起的甲基化转运异常,延迟或中断神经管的闭合,形成NTDs。

3.1 *MTHFR* C677T基因的SNP引发NTDs

目前关于*MTHFR*基因的SNP导致NTDs的探究是最多的,*MTHFR*基因常作为引发NTDs的独立危险因素,主要存在C677T、A1298C两种突变类型,其中对A1298C的争议较大,而C677T较为常见。该酶位于甲硫氨酸和DNA甲基化循环的交点,SNP的出现会导致酶活性降低,Met循环受阻,限制转甲基能力。Misselbeck等^[29]建立了微积分方程和随机模拟的计算模型,探究*MTHFR* C677T多态性是否会致使MTHFR活性降低,证实将*MTHFR* C677T的TT型与CC型进行比较时,TT型中SAM/SAH的比率随着MTHFR活性的降低而降低,SAM/SAH作为DNA甲基化的标志物,其比率降低表明*MTHFR*基因的SNP会通过致使DNA甲基化异常降低其本身活性而引发NTDs。与此同时,SAM/SAH比率失衡还会导致二者下游产物Hcy水平的波动。有研究显示,在*MTHFR*突变基因样本中,纯合母亲TT基因型突变的子代受NTDs影响的风险显著高于CC基因型,如果母亲为杂合子,则观察到后代患NTDs的风险增加,且TT型患者Hcy水平明显高于CT、CC两型^[30]。Nasri等^[31]发现,胎儿患NTDs的风险还与男性*MTHFR* C677T多态性有关。他们通过PCR-RFLP及统计学对照分析了父母组中*MTHFR* C677T的SNP等位基因频率及基因型分布,意外发现只有在父亲组中出现的T等位基因和TT基因型增加了NTDs的发生率,并表现出低甲基化状态。Yu等^[32]研究了3例具有NTDs生殖史的男性病例,发现在夫妻染色体均没有异常的情况下,父系的外周血Hcy水平平均明显升高并伴有*MTHFR* C677T的CT、TT基因型的改变,经过降低Hcy水平治疗后,自发分娩足月健康婴儿。以上研究表明,无论父亲还是母亲*MTHFR*基因的SNP都会通过升高Hcy水平,影响甲基转运过程,致使DNA甲基化异常进而引发NTDs。此外,与叶酸缺

乏结果一致的是,因MTHFR参与了四氢叶酸与5,10-亚甲基四氢叶酸之间的循环,SNP的发生会降低体内有活性的四氢叶酸含量影响叶酸的吸收利用^[27]。

3.2 MTRR A66G基因的SNP引发NTDs

MTRR A66G基因的SNP与NTDs的形成高度相关。MTRR作为Hcy再甲基化的关键酶,能促进甲基转运。Wang等^[33]选取1 407例NTDs病例和2 416例对照进行meta分析,证实MTRR A66G的GG基因型与其他基因型相比,受试者的Hcy水平明显升高。与MTHFR不同的是,MTRR的致病突变基因型主要是纯合GG型。Cai等^[30]采用SIFT软件观察比较61例患有NTDs的后代和61例健康者的基因型和等位基因的分布,显示NTDs患儿母亲的等位基因的频率G型高于A型。Gaughan等^[34]提供证据表明,NTDs患儿母亲MTRR A66G基因的SNP位点频率仅29%是AA基因型,其余均是GG型,同时测定显示血浆总Hcy含量明显增多。上述结果表明,MTRR A66G的GG基因型主要降低酶活性引起的Hcy在体内的蓄积,使Met循环受阻,进而影响甲基转运过程。

3.3 CBS基因的SNP引发的NTDs

目前关于CBS基因存在的SNP引发的NTDs,最深入的研究对象是T833C、G919A两种突变型,这两种SNP的突变位点均位于第8外显子^[35,36]。CBS 833位核苷酸由T突变为C后,肽链第287位苏氨酸被异亮氨酸取代^[35],通过改变CBS活性中心的空间构象,造成CBS结构域异常,影响CBS与PLP的结合,最终降低了酶本身的活性^[12]。CBS 919位核苷酸由G突变为A后,肽链第307位丝氨酸被甘氨酸替代,与T833C不同的是,G919A虽然酶活性降低,但CBS与PLP结合不受影响,引发其酶活性降低的机制尚不清楚^[36]。活性降低的CBS无法催化Hcy转硫化为半胱氨酸以进入细胞吸收利用,从而导致Hcy在体内蓄积^[37],已发现的NTDs患儿母亲血浆Hcy多处于高水平状态^[31]。T833C型突变出现同型半胱氨酸尿的患者对维生素B6的治疗有反应性,而G919A型则无反应性,这与CBS活性还依赖血红素有关,血红素参与了PLP结合区域氨基酸序列的折叠,两种SNP突变位点的不同导致对血红素的需求有所差别,进而间接影响CBS与PLP结合后的

活性^[38]。

3.4 MTR A2756G基因的SNP引发的NTDs

MTR基因可编码维生素B12(钴胺素)依赖性酶,随着反应的进行,钴胺素 I 因子被氧化形成 II 因子,导致MTR蛋白失活,正常代谢情况下需要MTRR将氧化的 II 因子还原成 III 因子以恢复MTR的活性^[39]。当MTRR A66G时,无法维持MTR的正常活性,会使Hcy在体内蓄积且Met合成减少。此外,Wang等^[33]获取3 809例样本(1 492例病例和2 317例对照),发现MTR A2756G的AA、AG两种基因型产生了与正常情况下MTR失活一致的结果,而GG基因型则会降低Hcy水平,但不会造成Met水平的升高,反而造成5-甲基四氢叶酸的积累,减少了胸苷酸的合成。由此可见,MTR基因本身的SNP位点改变及MTRR基因SNP位点变化间接影响都会使MTR蛋白活性丧失,从而使Met水平降低。Met合成的减少会抑制经典Wnt- β -catenin信号转导^[40]。腭裂是NTDs的表现形式之一,MTR活性的降低会增加腭裂患儿风险^[41]。Machado等^[42]在3 360个腭裂患儿的相关基因中筛选出38个相关基因的SNP位点,在这些相关基因形成的网络结构中,MTR和低密度脂蛋白受体相关蛋白6是最重要的两个连接点,而低密度脂蛋白受体相关蛋白6是Wnt信号转导不可或缺的辅助受体,二者之间的连接作用加强了其相互联系致使腭裂的风险增高。由此可见,MTR基因的SNP和NTDs的发生有密切的联系。

3.5 多种酶基因SNP共同作用

有研究认为,MTHFR基因的SNP既可作为独立危险因素又可与其他酶基因SNP共同作用,且多种酶基因SNP共同作用会增加NTDs的危险性^[29,34]。而Gaughan等^[34]利用方差分析了MTRR、MTHFR、MTR基因型组合的血浆总Hcy,发现MTR A2756G基因的SNP位点的AA型、MTHFR C677T基因的SNP位点的TT型与MTRR A66G基因的SNP位点的AA型均会引发NTDs,并且组合作用更加显著。此外,Yadav等^[43]也表示,如果儿童同时携带MTRR A66G的GG基因型和MTHFR C677T的TT基因型,使血浆Hcy水平明显增加而导致的酶活性降低引发患NTDs的风险,比这两种酶基因SNP单独存在时患NTDs的风险增加近5倍。

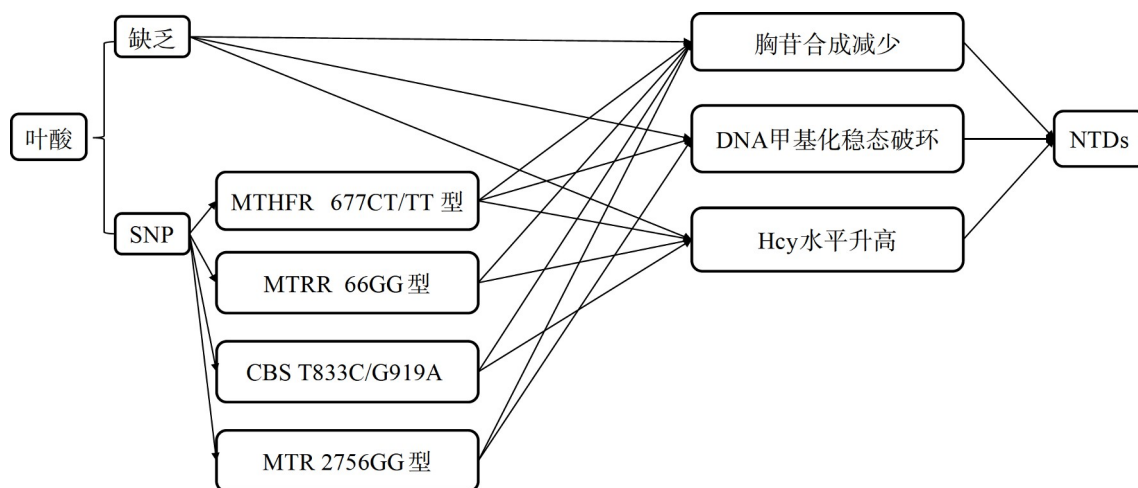


图2 叶酸缺乏叶酸代谢障碍关键酶基因SNP引发NTDs的过程示意图

4 总结

个体生长发育通常由多种因素共同决定,同样,NTDs的产生也不是由单一因素决定,而是多因素共同作用的结果。综上所述,叶酸作为Met循环必不可少的原料,其缺乏会降低Met水平、下调SAM/SAH比率,以此影响*Dnmt3b*活性和*Cecr2*等基因突变,导致DNA甲基化稳态被破坏, Met循环中的关键酶*MTHFR*发生C677T突变时,通过改变酶活性,同样会出现DNA低甲基化现象,以致甲基转运异常及参与神经管形成的钙、mTOR等信号通路无法完成(图2)。另一致病重要因素则是高Hcy水平, Hcy作为Met循环的中间体,由于叶酸缺乏时SAM/SAH比率失衡,或*MTRR* A66G、*MTHFR* C677T、*CBS* T833C、G919A、*MTR* A2756G等突变致使酶活性降低,均会使Hcy在体内蓄积,导致再甲基化的转运异常及半胱氨酸无法进入细胞被吸收利用。同时由Hcy介导的*Hex*等基因产物表达会上调, *Pax1*、*Pax9*和*Sox9*等基因产物表达会下调,这可能是未来深入研究具体调控机制的方向之一。此外,叶酸有助于胸苷酸的合成,叶酸缺乏时*Pax3*等转录因子突变影响*TYMS*、*SHMT*的水平,致使胸苷合成不足。*MTHFR*基因的SNP会降低有活性的四氢叶酸含量,影响叶酸的吸收利用。叶酸代谢途径异常通过以上因素相互作用,上调NTDs发病率的危险性。但由于环境和遗传的多重复杂因素,叶酸代谢异常与NTDs的确切产生

机制仍需继续研究。因此,叶酸代谢异常可作为孕期合理营养补充建议及基因水平检查的研讨方向,以减少NTDs患儿的产生。此外, *MTRR* A66G、*MTHFR* C677T、*CBS* T833C、G919A协同作用时明确表明会增加NTDs的患病率,因此,同时检测叶酸代谢途径多个关键酶基因的SNP会大大提高筛查准确率,避免漏检导致的NTDs患儿产生。伴随着基因测序技术的不断优化和测序成本的降低,叶酸代谢通路多基因SNP位点检测较之前更易实现,结合血浆叶酸及其代谢产物的检测,可精准地对叶酸代谢能力进行判断,提供个性化叶酸营养干预及NTDs防控。因此,研发多基因SNP位点检测试剂盒可能是未来的研究方向。

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