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在线全文

精子嵌合变异及其对后代的影响

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【摘要】 基因组嵌合变异会导致同一个体生殖细胞和体细胞基因组不同。嵌合变异包含不同变异类型，其中新发变异指患者父母中无法检测但患者可以检测的变异，大规模人类家系基因组和遗传分析表明80%的子代新发变异来源于父方染色单体，即来源于精子嵌合变异。本文综述了精子嵌合变异的类型和已有的检测策略，同时讨论了近期有关父亲精子嵌合变异导致后代遗传疾病的原因。笔者团队的前期研究结果表明，多达5%～20%的临床表型相关的新生变异可在父亲的精子中检测为早期胚胎发育或生殖干细胞嵌合变异，并且可以作为罕见遗传病和复杂遗传病的重要预测指标。基于这些已有的研究结果，笔者认为在未来的研究中，进行大规模新发变异检测和人口水平的遗传筛查将大幅度提升对子代的遗传风险判断，并且可以有效提高人口遗传健康。在临床推广新生变异的父亲精子检测将显著提高疾病人群分层的效率并提高筛查再生育风险的效率。环境、生活因素等通过精子对后代健康的影响、对变异特征的塑造，以及实验室条件下对精子变异的定向控制将是本领域新的研究热点。

【关键词】 嵌合现象 精子嵌合变异 等位基因比例 新发变异 综述

Sperm Mosaic Variants and Their Influence on the Offspring

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【**Abstract**】 Genomic mosaicism arising from mosaic variants is a phenomenon that describes the presence of a cell or cell populations with different genome compositions from the germline cells of an individual. It comprises all types of genetic variants. A large proportion of childhood genetic disorders are defined as being *de novo*, meaning that the disease-causing mutations are only detected in the proband, not in any of the parents. Population studies show that 80% of the *de novo* mutations arise from the paternal haplotype, that is, from paternal sperm mosaicism. This review provides a summary of the types and detection strategies of sperm mosaicism. In addition, it provides discussions on how recent studies demonstrated that genomic mosaic mutations in parents, especially those in the paternal sperms, could be inherited by the offspring and cause childhood disorders. According to the previous findings of the author's research team, sperm mosaicism derived from early embryogenesis and primordial germ cell stages can explain 5% to 20% of the *de novo* mutations related to clinical phenotypes and can serve as an important predictor of both rare and complex disorders. Sperm mosaicism shows great potential for clinical genetic diagnosis and consultations. Based on the published literature, the author suggests that, large-scale screening for *de novo* sperm mosaic mutations and population-based genetic screening should be conducted in future studies, which will greatly enhance the risk assessment in the offspring and effectively improve the genetic health at the population level. Implementation of direct sperm detection for *de novo* mutations will significantly increase the efficiency of the stratification of patient cohorts and improve recurrence risk assessment for future births. Future research in the field should be focused on the impact of environmental and lifestyle factors on the health of the offspring through sperms and their modeling of mutation signatures. In addition, targeted *in vitro* modeling of sperm mutations will also be a promising direction.

【**Key words**】 Mosaicism Sperm mosaicism Allelic fraction *de novo* variant Review

1 精子嵌合变异与后代新生变异的关系

嵌合现象指同一个体不同细胞群体、不同组织和器官表现不同的差异[1]，这些差异长期以来便被不同研究者报道，被认为由遗传或表观遗传因素决定[2-3]。遗传嵌合现象按照个体内的嵌合细胞是否来源于同一受精卵可分为同源嵌合（mosaicism）和异源嵌合（chimerism）[4]，如无特殊说明，本文所述的精子嵌合主要指同源嵌合现象。导致嵌合现象的遗传变异被称为嵌合变异（mosaic variants）。嵌合变异在类型上包含单核苷酸变异[5]、插入缺失[6]等影响少数核苷酸序列的变异和结构变异[7]、拷贝数目变异[8]、转座原件[9]、染色体变异[10]等影响大量核苷酸和基因组片段的变异。

新生或新发（*de novo*）变异指通过常规临床检测方法，在父母中未发现有遗传性变异（germline variant）但能在后代中检测出的变异[11-12]。新发变异是很多人类遗传疾病的遗传病因，例如Marfan综合征（先天性中胚层发育不良）[13]，Alport综合征（遗传性肾炎）[14]，结节性硬化症[15]，

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Dravet综合征(婴儿严重肌阵挛性癫痫)[16]等孟德尔疾病和智力障碍[17]、孤独症谱系障碍[18]、先天性心脏病[19]等复杂疾病。新发变异与人类遗传学上传统认知的杂合和纯合变异不同,被认为主要存在于配子或在胚胎发生时产生[20]。笔者等最近提出,理论上所有物种的新生遗传变异最初都以嵌合变异的形式出现,这些变异是物种演化的重要驱动力[21]。

随着高通量测序以及其他检测手段的普及[22],大规模人类家系基因组和遗传分析发现子代中高达80%的新生单碱基变异和短插入缺失变异来源于父方的染色单体[23],并且变异数量与父亲生育时年龄呈显著正相关[24-26]。作为男性唯一能够传递给后代的遗传物质[27],精子被认为是新生变异的主要来源[28],虽然有学者认为母方的修复也可能是此现象的来源[29]。很多新生变异未在双亲体细胞样本中检测到[30],因此精子嵌合变异理应是这些致病新生变异最主要的来源[21]。

2 按发生时间分类的精子嵌合变异

精子嵌合变异存在于精子的基因组中,但其发生时间可能处于合子生成前、个体极早期胚胎发育、生殖干细胞、到最终精子形成、储存的任何时期中(表1)。

表1 按发生时间分类的精子嵌合变异

Table 1 Sperm mosaic variant categorized by the time of origin

Time of variant	Detectable tissue	Age-related	Natural selection
Prezygotic	Sperm and soma	No	No
Early embryonic	Sperm and soma	No	No
SSC development	Sperm and germline cells	Partially related	Yes
Sperm formation and storage	Sperm	Yes	Yes

2.1 来源于合子生成前的精子嵌合变异

虽然已有研究报道嵌合现象主要发生在受精之后,但仍有少数的研究表明在特定组织中有嵌合变异发生在合子生成前[31-32],笔者等提出自然选择和回复变异可以导致相应变异存在于包括大量精子群体在内的特定组织或不同的体细胞、生殖细胞中[31,33]。

2.2 来源于极早期胚胎发育的精子嵌合变异

胚胎干细胞在原始生殖细胞决定前产生的嵌合变异最终会同时存在于精子和体细胞中[21]。当携带变异的细胞在各个组织中比例足够高时,常规外周血检测即可检测出,对多胚层来源组织的检测如果均发现嵌合变异,则精子中就有可能存在这些变异[34]。笔者在小范围人群利用全基因组直接检测结果表明,这些变异不会随年龄增长而增多[30,35]。

2.3 来源于原始生殖细胞发育时期的精子嵌合变异

原始生殖腺细胞(PGC)及其母细胞、精原干细胞(SSC)、或精原细胞在原始生殖细胞决定后产生的变异,因最初的原始生殖细胞数目较少,虽然仍存在较高比例可检测出的嵌合变异,但不能在除精子外的其他样本中检测到。我们的前期研究证实了早期原始生殖细胞数目至少为6个,在精子中嵌合变异比例可高达15%[35]。随干细胞所产生的子代细胞逐渐减少,这些变异影响的精子数目呈指数下降,或受到选择作用因而比例上升[36],这些变异中的一部分会因年龄增长或环境暴露、生活 习惯而增加[25,37]。

2.4 来源于精子形成和储存过程中产生的精子嵌合变异

次级精母细胞完成分裂后到精子排出前产生的变异主要是在精子变形和移动时的机械损伤、微环境变化等产生的,如甲基化水平变化相关的转座现象[38]或生活压力导致的DNA损伤[39]等。虽然有一定的变异模式、随年龄增长,这些变异往往只在单个精子范围内产生影响[40],利用子代变异特征进行的家系变异分析[41]和睾丸组织中检测[42]结果提示,这些变异中的一部分会随年龄增长而增加。

3 精子嵌合变异的检测

由于不同类型的精子嵌合变异的影响范围和比例不同,对精子嵌合变异的检测方法依据检测精子类群的大小和精子所含变异的比例有所不同。笔者等提出,对精子DNA嵌合变异的检测类似于普通嵌合变异检测[1],但是由于精子DNA包装结构差异,在检测前处理和检测操作上与其他样本有一定的差异,其中一些操作步骤更加轻柔以免引入额外变异[43]。每个精子携带单倍体基因组,可以检测的精子细胞比例即为携带变异等位基因所占的比例。

对于大量精子的高比例嵌合变异可以使用PCR和传统一代测序(自动Sanger法测序)[44]、基于染料的高分辨率熔解曲线[45]进行定性或半定量检测,这些法一般适用于变异比例高于20%的样品,且要求较高的样本量。这些方法可以检测多数合子前、极早期胚胎、原始生殖细胞发育时期产生的精子嵌合变异。

对于大量精子的不同变异比例也可以采用单链构象多态性-双链分析[46]、变性高效液相色谱法(DHPLC)[47]、或基于探针的荧光定量PCR法[48]进行定点检测。本研究组建立的通过高深度全基因组[35]、全外显子组[49-50]、捕获测序[34]等进行多目标嵌合变异检测方法,对全面揭示嵌合变异频谱和基因组分布信息提供了新的研究策

略。这些方法精度较高，可检测低至1%水平的低频变异，依据所应用的实验策略、样本来源和分析流程，笔者发现这些方法可以检测出合子前、不同胚胎时期的变异[51-52]。

对于少量精子，本研究组在国际上率先建立了用基于微流控技术的数字PCR法[53-54]或基于扩增子的深度测序[54-55]，对精子嵌合变异进行定量检测，依据液滴数目或单分子标记校正[56]可以在少量精子中准确检测出低于1%的变异。后续配合显微切割技术[57]或免疫染色标记[36]，这些方法还可以检测到更晚期发生的变异。

对于单个精子，可以采用基于单细胞全基因组扩增的单细胞全基因组测序[58]或单细胞定点重测序[59]检测次级精母细胞后产生的变异，但由于全基因组扩增的准确度较低，即使经过分子条码校正，仍有一定可能产生假阳性结果[60]。

对于源于单个精子的单条DNA分子，可以使用双链校正的分子标记法[61]或者单链重复校正的单分子测序法[62]进行单分子层面的变异检测，其检测灵敏度接近理论极限（每单倍体基因组错误率$1/10^{10}$ ~ $1/10^{9}$），但每条分子需要额外测序量进行校正[40]。

4　利用精子嵌合变异追溯遗传变异来源

大量研究报道表明，当一个家庭中两名或以上后代携带相同的*COL1A2*[63]、*HUMARA*[64]、*ACTN4*[65]、*CHD7*[66]、*NOD2*[67]、*NFIX*[68]等上百个基因上的新生变异时，这些变异大概率来源于父方染色体，即来源于精子嵌合变异。本研究组和其他研究者在多种不同的疾病中发现，多达5% ~ 20%的临床表型相关的新生变异可在父亲的精子中检测为嵌合变异，其中多数是极早期胚胎发育期产生的变异[33-34, 69-80]，变异等位基因所占比例可高达40%。此比例远高于这些疾病在人群中的随机发病率，显示出精子嵌合变异对人群中变异来源的追溯能力和对人群的分层效应。

针对复杂疾病的特定单基因亚型如孤独症，本研究团队发现高达20%的新生变异可能源于可检测的早期胚胎发育或生殖干细胞来源的精子嵌合变异[30]。我们建立的首个非癌症嵌合变异数据库提示，理论上，所有可以由新生变异导致的疾病中都有一定比例来源于精子嵌合变异[81]，目前已经有一些大规模研究在不同人群中针对不同变异研究其来源于双亲嵌合变异的比例[49, 82-83]，其中大部分来源于精子嵌合变异。对精子嵌合变异的定量和建模有助于理解导致人类疾病变异变的分布模式，推进遗传诊断的进步[28, 84]。

5　利用精子嵌合变异预测再发风险

再发风险指同样的（疾病）患儿表型在健康父母家系中再次出现的比例，例如，对于常染色体隐性遗传病，假设外显率100%，家系中出现一位先证者后，家系内再发风险为25%（50%×50%）[85]。基于临床的大规模经验的数据表明，在携带新生变异的患儿家庭中约有1% ~ 2%的再发风险[76, 86-88]。但在上述父亲精子嵌合变异导致新生变异的家系中，家庭内的变异再发风险由变异等位基因所占的比例决定，我们的研究发现该比例可能会远远大于1%[30, 34]，变异可能产生于极早期胚胎干细胞或原始生殖干细胞中[35]。

我们的前期研究表明大部分精子嵌合变异为中性变异[35]，但如果这些干细胞类群中获得了一些特殊类型的受正选择的变异〔如RAS-MAPK通路相关疾病（RASopathies）变异[89]〕，会形成增殖优势，进而增加携带这些变异的精子的比例，在人群水平导致变异携带者的睾丸癌患病率升高[90]、后代先天性肿瘤或相关疾病患病率增加[91]，称为精原选择/自私选择[37]。目前国际上已经有一些在人群中通过精子嵌合变异研究新生变异再发风险的报道[86]，为临床检测和遗传咨询提供了新的思路。

除在已经检测到新发变异的疾病家系中预测再发风险外，精子嵌合变异也可以直接预测子代的遗传风险，如笔者利用全基因组深度测序中可以预测约15%的先天性心脏病和孤独症遗传风险[35]。笔者在人工授精人类胚胎中的研究表明，精子嵌合变异可以以对应比例传递给胚胎，因而对精子嵌合变异的直接检测可以在几十年的时间尺度上稳定预测高频致病变异对后代的遗传影响[92]。

6　精子嵌合变异的非直接遗传影响

除上述讨论的精子嵌合变异直接影响以外，精子中的变异也可以通过非直接遗传的方式影响后代的表型，其作用原理包括但不限于通过变异影响表观遗传学信号，即通过变异影响印记基因，改变后代的基因表达[93]。另一方面，精子中可以传递的非遗传信息如各种小RNA和蛋白，在不同的精子嵌合变异影响下也可能间接影响从胚胎开始的下一代表型[94]。

7　讨论与展望

在当下，生殖健康的重要性逐渐提升。众多系统研究表明，精子中可检测到的嵌合变异可发生于不同胚胎发育时期，其变异类型和对应检测手法对变异比例、检测结果有重要影响。

基于这些研究结果,笔者认为在由新生变异导致的遗传疾病群体中,精子嵌合变异在其中起着非常重要的作用,至少10%的疾病风险可由精子嵌合变异解释。未来的研究中,在人口水平进行大规模新发变异检测和人口水平的遗传筛查将大幅度提升对子代的遗传风险判断,并且可以有效提高人口遗传健康。

笔者也认为,目前的遗传研究尤其是突变来源研究主要利用外周血和唾液样本,但至少50%的本可以在现有技术条件下检测到的精子嵌合变异完全不存在于外周血中,形成高比例的假阴性。与此类似,随着年龄的增长,由克隆造血造成的淋巴来源假阳性变异占外周血和唾液可检测变异的70%以上。因此,为正确检测疾病再发风险和理解遗传模式,笔者认为应在临床推广新生变异的父亲精子检测。此举将显著提高疾病人群分层的效率并提高筛查再生育风险的效率。

在已有的研究中,来源于精子形成和储存过程中产生的嵌合变异因其检测成本高昂而鲜有研究,已发表的研究主要着重于结构变异、非整倍体等影响范围较大的变异。随着高通量测序成本的显著降低和单分子、单细胞纠错技术的提高,单精子高精度编译检测成为可能。笔者认为,环境、生活因素等通过精子对后代健康的影响、对变异特征的塑造,以及实验室条件下对精子变异的定向控制将是本领域新的研究热点,也将对遗传健康带来更大的指导意义。

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