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# 精子鞭毛多发形态异常的遗传因素及干预策略的研究进展

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**摘要** 精子鞭毛是精子运动的必需组件, 数以千计的蛋白参与精子鞭毛的有序组装. 精子鞭毛多发形态异常(multiple morphological abnormalities of the flagella, MMAF)以短尾、卷尾、无尾、折尾和/或不规则尾等精子尾部畸形为典型特征, 导致精子运动异常和生育障碍. 得益于高通量测序技术和基因修饰小鼠建模技术的发展, 目前40余个MMAF致病基因被揭示, 多数MMAF患者的致病基因可被精准检测与诊断. 不同MMAF致病基因对应的辅助生殖结局存在差异. 本文围绕MMAF遗传因素研究进展进行综述, 梳理精子鞭毛结构与精子运动力的关联、MMAF致病基因的筛查和验证技术、MMAF代表性致病基因的功能、MMAF目前常用和未来可能的干预方法, 希望为MMAF及类似疾病的遗传致病因素分析和干预提供参考.

**关键词** 精子鞭毛多发形态异常, 弱畸精子症, 弱精子症, 基因修饰小鼠, 精子鞭毛, 精子运动力

不孕不育指在一年或更长时间内未采取任何避孕措施常规性生活后未能怀孕[1]. 作为一个全球性的生殖健康问题, 不孕不育正影响着数百万育龄人群[1]. 据估计, 世界上大约8%~12%的育龄夫妻被不孕不育所困扰[1], 这部分不育夫妻中30%~50%与男性因素有关[2]. 男性不育一般表征为少精子症(oligozoospermia)、无精子症(azoospermia)、弱精子症(asthenozoospermia, AZS)、畸形精子症(teratozoospermia), 或存在这些缺陷的组合[1,3,4], 如少弱精子症(oligoasthenozoospermia)、弱畸精子症(asthenoteratozoospermia, ATS)或少弱畸精子症(oligoasthenoteratozoospermia, OAT). 其中, AZS是男性不育的主要原因之一[5]. 当一次射精中前向性运动精子比率低于32%, 即诊断为AZS[1,5]; 当精子前向运动率和精子形态正常率同时低于正常值(即精子前向运动率低于32%、精子形态正常率低于4%)时, 即可进一步分类为ATS[6]. 临床上, 引发AZS的因素多种多样, 包括遗传因素、环境因素(辐射、高温、环境污染物)、不良生活方式(如吸烟、饮酒)和疾病因素(如精索静脉曲张、感染)等[7]. 成千上万的基因参与男性性腺发育、睾丸功能和精子发生过程, 提示遗传因素在男性不育症中起着重要作用. 目前相当大一部分特发性AZS病例可能是由未知的遗传因素引起, 但遗传致病因素还不清楚[8,9].

精子鞭毛结构精细. 在人类精子中, 已鉴定出上

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千种蛋白与精子鞭毛的构成有关[10],一旦这些蛋白发生改变, 精子鞭毛可能会出现结构异常或功能障碍, 导致精子运动力下降, 呈现AZS[5]. 越来越多研究结果表明, 多数伴有精子鞭毛缺陷的精子运动力异常与遗传因素相关, 如MMAF和原发性纤毛运动障碍(primary ciliary dyskinesia, PCD)等[11]. 其中, MMAF是一种表现为短尾、卷尾、无尾、折尾和/或不规则尾等精子尾部畸形导致精子运动异常和生育障碍的严重AZS[12]. 由于同时存在鞭毛形态畸形和运动异常的表型, MMAF也被划分为ATS[5].

随着高通量测序技术的普及, 基于临床患者样本进行AZS候选致病基因的筛选变得更加高效, 而基因编辑技术的发展也使AZS相关基因修饰小鼠模型的构建能够快速实现. 在这些技术的支撑下, 目前已鉴定多个与精子鞭毛发育相关的基因, 如*CEP135*[13], *DZIP1*[14~16], *CFAP43*[17], *CFAP44*[17], *CFAP58*[18], *DNAH1*[19]和*DNAH2*[20]等, 这些基因缺陷会导致鞭毛中心体发育异常或轴丝及其附属结构紊乱等, 进而导致MMAF的发生. 然而MMAF是一种高遗传异质性的疾病[21,22], 部分患者的病因尚不明确, 亟需挖掘新的致病基因为未来更加准确和高效进行遗传学诊断提供可能. 此外, 这些由遗传因素导致的AZS难以通过常规治疗手段(如生活方式优化、药物靶向治疗和外科手术等)得到改善[2,23], 往往需要借助辅助生殖等方式达到生育目的. 如何找到合适的辅助生殖方案及明确预后效果也是研究人员密切关注的问题.

本文介绍代表性的AZS类型, 着重对MMAF研究进展进行梳理, 简述精子鞭毛与精子运动的相关性, 论述高通量测序技术和基因修饰小鼠建模技术在致病基因鉴定中的应用、MMAF相关基因的功能, 并总结常规和潜在干预策略.

## 1 精子鞭毛与精子运动相关性

### 1.1 精子运动

自然状态下, 睾丸中产生的精子细胞并不具备使卵子受精的能力. 当其转移至附睾中成熟, 最后在女性生殖道内完成精子获能后才具有受精潜能[24]. 精子前向性运动是其进入女性生殖系统到达输卵管完成受精的前提条件, 并且是男性生育潜力的重要预测指标[25].

当精子进入女性生殖道经过获能后, 会呈现出高度不对称的搏动模式, 表现得更为活跃, 这种状态叫作“超活化运动”[26]. 一般认为获能的精子才具备使卵子受精的能力[27], 因此对精子运动所开展的研究很多集中于其在输卵管中的运动. 而精子鞭毛中含有推动精子前进所需的机械装置[7], 精子在鞭毛的摆动下前进, 最终穿过女性生殖道, 在输卵管壶腹部与卵子结合[28].

不同于传统的2D显微镜只能观察精子鞭毛活动的局部动态, 3D显微镜能够分析精子三维游动行为, 可以为准确检测鞭毛的完整运动提供机会, 因而是研究精子运动的重要辅助工具[29]. 基于三维建模, 2023年一项研究指出, 精子能够调整其能量输出来动态适应变化的流体条件, 从而优化其在女性生殖道内的运动和航向, 提高成功受精概率[30].

尽管三百年来对精子运动方式提出种种新的观点, 但精子各部分结构完整、功能正常并相互协调配合才能使精子正常运动这一点是毋庸置疑的. 精子大体可分为三个主要部分: 头部, 其中蕴含遗传物质; 颈部, 连接头部和尾部; 尾部, 也称作鞭毛, 是精子的主要运动组件, 人类精子尾部形态均一, 长约50~60 μm, 大约为头部长度的10倍[31], 是精子的重要组分. 精子尾部出现缺陷很可能导致精子无法正常运动、活力降低, 出现AZS. 其中, MMAF就表现为精子尾部多发形态畸形, 呈现精子运动异常. 如*DNAH1*基因的缺陷导致患者出现MMAF, 精子运动力受损, 需借助卵胞浆内单精子注射来实现妊娠[32].

除鞭毛结构的完整性, 还有一些因素同样影响精子运动力. 在鞭毛摆动过程中, 微管蛋白甘氨酰化(tubulin glycylation)能调节轴突动力蛋白(axonemal dynein)运动活性, 从而调节鞭毛摆动; 甘氨酰化的缺失会造成小鼠精子运动紊乱和雄性不育, 这也可能是人类男性精子运动力降低的原因之一[33]. 睾丸中的精子必须经过附睾的管道系统才能逐步成熟, 这一过程需要附睾信号途径的调节, 而附睾的发育则也依赖睾丸来源的分泌因子的调节. 比如睾丸生殖细胞分泌的NELL2能够对ROS1通路发出信号, 调节附睾初始节段(initial segment, IS)的分化, 影响附睾蛋白酶OVCH2的分泌, 该蛋白酶又作用于精子表面跨膜蛋白ADAM3以使精子获得受精能力[34].

能量代谢对精子运动也非常重要[9]. 精子运动需要大量三磷酸腺苷(adenosine triphosphate, ATP)为供

能基础. 精子ATP主要通过糖酵解(glycolysis)和氧化磷酸化(oxidative phosphorylation, OXPHOS)产生, 而脂肪酸氧化(fatty acid oxidation, FAO)也参与附睾精子成熟过程中的能量产生[35~38]. 研究发现, SLC22A14是一种位于精子线粒体内膜上的核黄素转运蛋白, 促进FAO能量代谢, 其缺陷可引起精子运动力下降和雄性不育[39]. *AK9*基因缺陷会扰乱核苷酸代谢稳态和抑制糖酵解, 从而导致人类和小鼠的AZS[40]. 精子运动是一个十分耗能的过程, 尽管越来越多的证据提示, 精子能量代谢的异常会引起AZS, 但目前在不育患者中被鉴定出来的这类基因还较少[9], 需要进行更深入的研究. 此外, 高脂饮食引起的代谢障碍[41]、肠道微生物失调[42]、线粒体异常[43]、氧化应激异常[44]等也均有可能导致男性精子运动力下降, 造成不育.

### 1.2 精子鞭毛结构

精子鞭毛主要由轴丝和轴丝外周附属结构组成. 轴丝由“9+2”微管结构组成, 中心为2条中央微管形成的中央微管蛋白复合体(central apparatus complex, CPC), 外侧环绕着9对外周二联微管(double-microtubule, DMT). 连接蛋白-动力蛋白调节复合物(nexin-dynein regulatory complex, N-DRC)这一结构将相邻的DMT连接. 内动力蛋白臂(inner dynein arm, IDA)、外动力蛋白臂(outer dynein arm, ODA)连接在9对DMT上, 产生鞭毛的动力. 径向辐条(radial spoke, RS)向CPC呈放射状分布. 按照轴丝外周附属结构的差异, 人类精子鞭毛分为中段、主段和末段[45]. 中段的轴丝外依次被外周致密纤维(outer dense fiber, ODF)、线粒体鞘(mitochondrial sheath, MS)和质膜(plasma membrane)包裹; 主段的轴丝外被ODF、纤维鞘(fibrous sheath, FS)和质膜包裹; FS和ODF均在主段尾端逐渐变细消失, 因此鞭毛末段只有由质膜包围的轴丝[45]. ODF, MS, FS等轴丝外周附属结构参与鞭毛内结构衔接、能量调节和细胞信号转导[46]. 在鞭毛中段和主段的连接处还存在终环(annulus/terminal ring)这一重要致密环形板状结构, 它被质膜附着, 能防止线粒体在精子运动时发生移位, 确保中段正确组装, 其缺失可以造成精子线粒体异常和尾部严重弯曲等[47]. *SCL26A8*[48]或*SEPT12*[49]基因缺陷被证实会导致精子annulus缺失, 男性患者出现严重弱精子症. 此外, 精子头尾部之间具有精子头尾耦合装置(head-tail coupling apparatus, HTCA), 这是一种基于中心体的结构, 由一对中心粒和相关元件组成[50]. HTCA组装失败会导致精子头尾分离, 造成无头精子症(acephalic spermatozoa syndrome, ASS)和男性不育, 如SPATA6, BAG5或CCDC188等蛋白的缺失[50,51].

目前的研究已经证实, 鞭毛的超微结构对于精子运动非常重要, 各结构及其承担的功能之间的紧密配合让精子能够突破女性生殖道的黏稠环境, 成功获能使卵子受精.

## 2 精子鞭毛缺陷导致弱精子症

AZS可分为两种类型, 即以多种并发症状为特征且具有不同表型的“综合型AZS”和无其余并发症的“孤立型AZS”. 前者包括由纤毛功能障碍引起的综合型纤毛病变, 以原发性纤毛运动障碍(PCD)为代表[5]; 后者包括MMAF和其他AZS等[5]. PCD是运动纤毛相关疾病; MMAF则为鞭毛多种形态缺陷组合导致的畸形[52], 通常被归类为一种特殊的ATS[5]或AZS[53].

运动纤毛与精子鞭毛都以微管及相关蛋白组装而成的轴丝为基本结构, 所以男性PCD患者除纤毛相关器官疾病表型外可能还并发精子鞭毛结构异常, 造成不育. 同时, 相较于运动纤毛, 精子鞭毛的组装过程有其独特性, 涉及一些特有的附属结构, 如ODF和FS[54], 因此, 部分MMAF临床患者只有精子鞭毛异常的表型, 而无肺部等其他器官的异常表型[5].

### 2.1 原发性纤毛运动障碍

PCD是一种常染色体隐性或X-连锁遗传病[55], 会引起呼吸道疾病和内脏异位. 男性PCD患者若精子鞭毛异常则出现不育, 而女性患者若输卵管处纤毛异常则出现不孕或宫外孕[55,56]. PCD的患病率约为0.0025%~0.0050%[55], 该病导致的不孕不育症状使它亦受到生殖医学领域研究人员的重视[57,58], 目前已鉴定的PCD致病基因中, 少数基因具有纤毛功能特异性, 不引起精子鞭毛结构异常, 如*CCDC114*[59]和*DNAH5*[60]; 部分致病基因缺陷患者同时出现男性不育表型和运动纤毛功能障碍, 如*CFAP65*[61,62]和*SPEF2*[63,64].

### 2.2 精子鞭毛多发形态异常

1987年, 研究人员在对五名原发性不育患者的研

究中报道, 精子鞭毛形态异常导致的AZS, 并且由于精子的纤维鞘存在明显异常而引入纤维鞘发育不良(dysplasia of the fibrous sheath, DFS)这一概念[65,66]. 随后的研究发现该类精子的鞭毛所表现的是多种类型的鞭毛结构异常, 因此, 2014年一项研究将这种鞭毛缺陷重新命名为MMAF[12], 可以更为准确地描述该精子鞭毛缺陷的表型.

MMAF患者一般无不育外的特殊临床症状, 90%以上精子不活动, 且存在常规精液分析难观察到的鞭毛异常[67]. 精子超微结构分析发现CPC缺失、轴丝紊乱、动力蛋白臂缺失、FS异常和/或MS异常等. MMAF属于高度遗传异质性弱精子症, 目前已知的致病基因主要包括*AKAP*, *DNAH*, *CCDC*, *CFAP*, *TTC*等家族基因, 40余个致病基因已被鉴定[21,22]. *DNAH1*基因编码内动力蛋白重链, 并在睾丸中表达, 是第一个被报道的人类MMAF致病基因[12].

## 3 弱精子症相关基因的筛查及基因修饰小鼠模型的构建

哺乳动物中有超过两千个基因主要在睾丸中表达[68,69]. 精子从发生、成熟到获能并最终使卵子受精是一个复杂的过程, 涉及全身多组织器官正常运作与协调配合, 该过程的许多环节容易受到遗传变异的影响. 总结过去的研究发现, 鉴定AZS相关致病基因主要用到两种思路: 一种是对AZS患者进行高通量测序, 筛选出疑似致病基因, 而后建立基因修饰小鼠模型进行验证; 另一种则是从小鼠出发, 分析基因修饰小鼠模型的精子运动力和生育表型, 从中找到候选基因, 为临床AZS患者的遗传变异筛查提供指导.

目前针对男性不育的遗传因素筛查常用的有全外显子组测序(whole exome sequencing, WES)、比较基因组杂交技术(array-based comparative genomic hybridization, aCGH)等. WES利用序列捕获技术将全基因组的外显子区域DNA捕获富集后进行高通量测序, 检测准确性高且成本较低[70]. WES可以有效地鉴定出插入/缺失突变、点突变, 因此其在MMAF的致病基因鉴定中被应用得十分成功[71~73]. 但WES对于大范围的基因组变异并不太适用, aCGH则对于鉴定基因组拷贝数变异(copy number variation, CNV)这类大范围的基因组变异非常有效. 因此, 利用WES和CNV的联合分析能较为全面地筛选可能的致病因素. 通过该联合分析策略, 研究人员发现CNV也是MMAF的危险因素之一[17,74].

致病基因的缺陷会导致患者无法生育, 这些突变在人群中出现的频率往往较低, 加之男性不育的高度遗传异质性, 致使某个特定基因的缺陷可能仅出现于少数患者. 此外, 部分患者可能存在多个候选基因缺陷. 那么, 确认真正的致病基因往往需要结合动物或细胞模型来进行全面分析和验证. 目前尚无人类精子鞭毛形成的体外培养体系, 因此, 研究人员常用小鼠、果蝇, 甚至一些有鞭毛和/或纤毛的单细胞生物(如锥虫), 来作为研究鞭毛缺陷相关的男性不育基因的模式生物.

小鼠与人类共享99%同源基因[75], 参与精子生成的大多数基因在小鼠和人类间是保守的. 目前, 基因修饰小鼠模型是验证候选基因的基因型-表型关系的金标准之一[76]. 在高通量测序技术普及之前, 研究人员往往根据基因的分子功能, 通过观察基因敲除小鼠的精液、影像学、激素水平, 以及配子的形态或超微结构等确定候选基因缺陷对于男性生殖的影响[77,78]. 人类男性不育的候选致病基因可通过构建基因修饰小鼠模型进行验证[79]. 反过来, 一些已经在小鼠中被证实的不育相关基因在人类男性中发生突变后也显示出类似表型, 例如*Tex11*[80,81], *Sycp3*[82]基因, 提示可以通过基因修饰动物的研究为临床的遗传致病基因筛查提供参考[83]. 如今已有一些MMAF候选致病基因在小鼠模型中被发现(其缺陷导致雄性小鼠不育), 而尚未发现相关人类不育患者, 如*Ccdc189*[84], *Ccdc183*[85,86], *Ccdc38*[46,87], 这些基因可以为未来不育患者的致病基因筛查提供候选基因.

基因修饰小鼠模型的构建方法也随着疾病研究的深入而快速发展. 使用胚胎干细胞进行同源重组打靶, 再进行囊胚注射可获得嵌合体小鼠. 嵌合体小鼠与野生型小鼠进行繁育, 可以获得特定基因敲除小鼠用以功能研究[88]. 近年来CRISPR/Cas9技术被应用于基因修饰小鼠模型的制备[89]. 通过设计候选基因的gRNA载体, 并将CRISPR/Cas9系统注射至小鼠受精卵中, 再在胚胎移植后进行小鼠繁育, 即可对候选基因进行体内基因功能验证[90]. 研究人员基于CRISPR/Cas9系统已构建*Ccdc189*[84], *Ak9*[40], *Tsks*[91]等基因突变小鼠以研究男性生殖领域基因相关功能. 该技术相对于胚胎

干细胞同源重组打靶的方式，可更为简便高效地产生基因修饰小鼠.

尽管目前CRISPR/Cas9系统在基因修饰小鼠建模中应用广泛, 但易造成首建者基因型嵌合性[92], 往往需要两代繁育才能得到纯合子[93]. iSTOP小鼠建模技术通过胞嘧啶碱基编辑系统，将靶位点的C:G转化为T:A, 从而使四个密码子(CAA, CAG, CGA, TGG)转化成STOP终止密码子，提前终止mRNA翻译来敲除基因；该技术被证明可在首建者小鼠的生殖细胞中直接实现表型分析, 适用于快速建立生殖疾病小鼠模型[85]. 与常规CRISPR/Cas9相比, iSTOP小鼠建模技术的可控性更强、嵌合性现象更少、副作用更小，且绕过额外两代育种的时间(约六个月)[85,94]. 研究人员成功利用iSTOP小鼠建模技术鉴定出*Ccdc183*[85], *Dnah3*[6], *Dnali1*[95], *Ccdc38*[46]等精子运动力异常相关的雄性不育基因. 由于两细胞胚胎具有全能性, 对两细胞的一个卵裂球进行基因编辑，可以获得嵌合体小鼠用于表型分析, 并可通过繁育建立基因突变小鼠品系[96,97]. 该技术有望与iSTOP技术进行结合，进一步助力候选基因的快速建模和功能验证.

单基因遗传模式被认为是MMAF的常见遗传致病模式. 但通过比较携带MMAF相关基因的单突变到四突变雄性小鼠的生育力和精子参数，研究人员发现, 随着杂合突变数量的增加，精子形态和运动力逐渐恶化[98]，提示寡基因遗传模式可能也是MMAF的重要遗传致病模式之一. 对于多基因突变小鼠的建模，受精卵建模技术会面临多基因突变后代繁育周期长、阳性率低等问题[99]. 类精子干细胞来源于精子, 只具有一套染色体, 可在体外长期培养, 并且能通过卵母细胞注射一步获得多基因突变的小鼠个体[99]. 通过类精子干细胞结合基因编辑技术，有望为多基因突变小鼠的建模和寡基因遗传模式的分析提供技术支撑[99~101].

蛋白质是细胞生物功能的执行者[102]. 但是，抗体的不特异性与不稳定性影响诸如免疫印迹、免疫荧光、免疫共沉淀等实验的结果与准确性，限制蛋白功能的研究. 此外, 对于一些在人类蛋白质组计划(human proteome project, HPP)的人类蛋白质蓝图中缺少明确实验证据的蛋白(约9.6%)，研究人员需要进行高严格性分析[103]. 通过构建标签化小鼠模型可以帮助许多缺乏合适抗体的目标蛋白质的功能研究[104]. 研究人员目前已构建*CCDC176*-HA[105], *CCDC183*-HA[85], *SSH2*-HA[106]等HA标签化小鼠用于生殖相关表型研究. 敲入HA标签后的小鼠即能使用通用型标签抗体检测目标蛋白的动态表达情况和精准定位(如*SSH2*-HA小鼠[106]、*CCDC183*-HA小鼠[85])、快速探究分子互作网络(如*CCDC176*-HA小鼠[105]、*CCDC183*-HA小鼠[85]). 以上技术对于相关基因的功能机制研究具有重要意义.

## 4 精子鞭毛缺陷相关的弱精子症代表性基因及表型

小鼠精子变形(spermiogenesis)的具体过程可划分为16步(steps 1~16)[3,107,108]. 第1~3步中，单倍体圆形精子细胞轴丝伸出，FS前体也开始形成；第4~7步，FS从轴丝远端向近端开始组装；第8步，ODF从轴丝近端向远端方向组装，ODF的组装直到第16步才全部完成；第9步, 细胞核拉长凝聚, annulus开始形成; 第15步, 细胞质中的线粒体向轴丝和ODF处迁移，形成MS；第16步, 精子细胞基本发育完成, 多余细胞质、细胞器等脱落形成胞质小滴(cytoplasmic droplets)释放[109,110]. 精子变形期间会形成一个暂时性的结构——精子领(manchette)围绕精子头部远端，该结构在精子头部和鞭毛的形成中发挥重要作用[111]. 精子变形伴随着精子的顶体发生(acrosome biogenesis), 顶体发生通常分为4个主要阶段：高尔基期(1~3步)、顶体帽期(4~7步)、顶体(伸长)期(8~12步)和成熟期(13~16步)[3,112~114]，可与精子变形步骤相对应.

精子的变形及各部位的组装是一个极其复杂精密的过程，任何一个步骤出现差错都可能导致精子异常，导致出现运动能力低下甚至无活力的弱畸精子. 目前领域内已鉴定出多种与MMAF相关的基因，按照精子尾部的发育和超微结构，此处总结了轴丝微管发育、轴丝外周附属结构发育、中心体发育、鞭毛内运输(intra-flagellar transport, IFT)和精子领内运输(intra-manchette transport, IMT)相关基因.

### 4.1 轴丝微管发育相关基因

轴丝在真核生物纤毛和鞭毛运动中占据重要地位, 精子轴丝主要包括CPC, DMT, N-DRC, RS, IDA和ODA几部分. 目前研究发现参与轴丝微管发育的基因主要有*CFAP*家族基因、*CCDC*家族基因、*DHC*家族

基因及*DRC*家族基因等.

纤毛和鞭毛相关蛋白的超家族CFAP (cilia and flagella associated protein)家族参与鞭毛重要结构的组成. 据研究报道许多*CFAP*家族基因缺陷均会导致MMAF[111,115], 包括*CFAP43*[17,52,116], *CFAP44*[17,52], *CFAP47*[117~119], *CFAP54*[120], *CFAP57*[121,122], *CFAP58*[18,123], *CFAP65*[62,124,125], *CFAP69*[126,127], *CFAP70*[128~130]. CFAP43与CFAP44主要参与鞭毛的生成与组装, *CFAP43*或*CFAP44*基因缺陷MMAF患者精子表现出CPC缺失[17]. 携带*CFAP47*半合子有害突变的男性和雄性小鼠表现出精子运动力降低和不育[74,119]. *CFAP57*[121]缺陷破坏精子鞭毛的IDA组装, 导致患者出现MMAF. *CAFP58*[18]也参与轴丝组装, *CFAP58*缺陷的患者出现精子运动障碍和畸形. *CFAP65*[124]定位于人的成熟精子的鞭毛中段和顶体区域, 在精子变形晚期通过参与MS以及轴丝形成来参与鞭毛的组装, *CFAP65*基因缺陷患者会出现MMAF以及运动纤毛相关的PCD样表型. *CFAP69*[131]参与鞭毛的组装, 一旦缺失, 鞭毛会出现轴丝缺陷最终出现MMAF.

CCDC (coiled-coil domain containing)蛋白参与多种生理和病理过程[87]. *CCDC42*[132], *CCDC38*[46,87]等基因缺陷会导致小鼠精子鞭毛畸形, 出现MMAF表型. 在睾丸中同样高表达的CCDC146能与CCDC42, CCDC38两种蛋白相互作用, 促进某些蛋白在鞭毛形成过程中通过IFT途径被转运; *Ccdc146*基因缺陷的小鼠出现精子头部畸形和精子领结构缺陷[133]. CCDC176在精子轴丝中非对称性分布并结合微管及RS相关蛋白, 在精子成熟过程中特异性维持1号和9号DMT的稳定; *Ccdc176*基因缺陷小鼠的精子运动力显著降低, 产生弱精子症表型和雄鼠不育[105]. CCDC181定位于精子鞭毛基部[134], 与LRRC46互相作用并调节该蛋白在精子鞭毛中的定位, 其缺失导致精子领和鞭毛形成缺陷[135]. *Ccdc181*敲除小鼠精子数量极低、精子形态异常且几乎无活力, 呈现典型的MMAF[135]. CCDC183, 也称为KIAA1984, 是一种睾丸特异性蛋白, 能够调节ODA的组装和参与IFT, 对精子变形起着重要作用, CCDC183缺失的小鼠会出现MMAF和雄性不育[85,86]. CCDC189蛋白位于第一个DMT的RS处, 通过与CABCOCO1、鞭毛内转运蛋白的相互作用参与精子鞭毛的形成, 其敲除导致小鼠出现MMAF表型和雄性不育, 表明其对雄性小鼠精子变形和生育至关重要[84].

动力蛋白臂由重链、中间链和轻链构成, 对精子鞭毛的摆动至关重要. 根据结构特点不同, 又将其分为ODA和IDA. *DNAH*家族基因编码动力蛋白轴丝重链蛋白. *DNAH1*基因[19]缺陷引起精子IDA结构异常、CPC缺失等鞭毛缺陷, 患者出现MMAF. *DNAH3*[6]基因缺陷引起IDA的部分异常. *DNAH8*基因缺陷患者精子运动力降低、鞭毛呈现典型MMAF表型, 该基因敲除小鼠也同样表现出MMAF表型以及生育障碍[136]. *DNAH10*[137]缺陷导致鞭毛轴丝IDA缺失, 精子运动力明显降低, 表现出MMAF特征. *DNAH17*缺陷的精子ODA出现缺失, 精子运动力异常, 患者和小鼠出现不育症状[53,138~142].

连接相邻DMT的N-DRC对鞭毛或纤毛摆动起到调节作用. 近年来发现N-DRC的各功能组分对哺乳动物精子鞭毛也存在影响. *DRC1*缺陷后精子鞭毛轴丝结构崩塌[143], 导致男性患者表现MMAF; *DRC3*缺陷的男性患者鞭毛轴丝组装过程发生装配紊乱, 并伴有精子领发育异常, 表现出MMAF症状[144]; *DRC5*缺陷导致精子鞭毛运动力显著下降, 男性不育, 出现MMAF[145].

### 4.2 轴丝外周附属结构发育相关基因

轴丝外周附属结构主要包括MS, FS及ODF, 其中MS螺旋排列在精子鞭毛中段, 为鞭毛运动提供能量; FS包围主段, 维持精子结构稳定、调节精子运动力[146]; ODF则作为精子尾的骨架存在[147], 影响精子的结构与功能稳定, 其主要成员ODF1的缺失导致小鼠无头精子的发生从而造成男性不育[148,149].

来自*CFAP*家族的*CFAP58*基因缺陷造成患者和小鼠精子轴丝组装异常, 该缺陷亦引起中段MS发育异常[18], 呈现典型MMAF. CFAP61通过与钙调素-径向辐条相关复合体(calmodulin- and spoke-associated complex, CSC)等结构相互作用, 在轴丝稳定中发挥作用, 其缺失会导致MS, FS和CPC丢失等; *Cfap61*敲除导致小鼠精子运动力下降, 引起雄性小鼠不育[150~153], 再现和人类患者相似的MMAF表型. 在*Cfap65*基因敲除的小鼠中也观察到MS组装异常, 此外还存在顶体和精子领形成异常[154]. *CFAP65*基因缺陷的MMAF患者精子存在严重缺陷, 不仅出现鞭毛结构异常如异常MS破裂和CPC缺失等, 而且出现精子头部畸形[61,62].

1700030J22RIK缺失会导致MS紊乱和轴丝结构异常, 造成严重的生育力低下和MMAF[155].

FS主要由AKAP家族蛋白(A-kinase anchoring proteins, AKAPs)组成, 包括AKAP3, AKAP4和AKAP14[146]. AKAP4在FS组装和精子运动中具有重要作用, 是精子FS中表达最丰富的蛋白[156]. *AKAP4*缺陷导致人和小鼠精子数量减少、运动能力降低、鞭毛FS发育异常, 最终造成雄性不育[157]. AKAP3在圆形精子时期参与FS基本结构的组成, 并且*AKAP3*基因缺陷会引起男性患者出现类似MMAF的不育症状[158]. FSIP2位于FS中, 与AKAP4直接相互作用, *FSIP2*缺陷导致FS紊乱、CPC以及轴丝的IDA/ODA异常等[159,160].

DNALI1富含于精母细胞、变形期精子细胞和鞭毛[161]. 通过构建基因敲除小鼠模型, 研究人员发现, *Dnali1*缺陷导致精子鞭毛FS两个纵向柱(longitudinal columns, LCs)的不对称性分布和FS结构紊乱, 小鼠和人类精子运动力受损导致不育, 证实*DNALI1*在男性生育力中具有重要作用[95,161~163].

### 4.3 中心体发育相关基因

中心体存在于大多数真核细胞中, 在细胞分裂、运动和纤毛形成等细胞过程中起着重要作用, 鞭毛轴丝即起源于位于基底的远端中心粒[164]. 早在1996年, 研究人员就发现一些临床试管婴儿失败的案例可能是精子中心体缺陷导致[165], 此后围绕中心体功能与人类男性不育又开展一系列研究, 如*CEP78*[166], *CEP135*[13]以及*DZIP1*[14~16]等基因缺陷被证实会造成中心体异常, 引起男性不育.

中心体蛋白(centrosome protein, CEP)是中心体的活性成分, 早期研究发现*CEP*基因缺陷与小头畸形、神经发育障碍有关, 最近几年的研究证实*CEP*缺陷与男性不育的关联. CEP78是一种中心体蛋白, 定位于成熟的中心粒, 参与调节中心体复制, *Cep78*敲除会导致雄性小鼠出现与人类*CEP78*缺陷患者类似的症状, 如精子近段中心粒特有的三重微管[166]. CEP135定位于中心体车轮结构, 是中心粒生物发生所必需的. 研究发现, *CEP135*过表达导致中心体和细胞质中异常纤维聚合物的积累, 而*CEP135*缺陷又导致中心体和鞭毛中形成蛋白质聚集体, 使微管紊乱, 出现MMAF[13].

DZIP1在哺乳动物细胞中介导BBSome-DZIP1-PCM1复合物在中心体中的组装, 并在细胞周期中调节BBSome蛋白的中心体定位, 而BBSome和PCM1都直接参与中心体维持和纤毛形成[15,16]. *DZIP1*缺陷引起精子中心粒功能障碍和鞭毛缺陷[14], 表明DZIP1在精子鞭毛和中心体形成中的必需性.

### 4.4 IFT和IMT相关基因

精子鞭毛形成过程中需要运输系统为鞭毛的组装输送结构蛋白, 即IFT和IMT[167]. IFT复合体A (IFT-A)和B (IFT-B)在驱动蛋白或马达蛋白驱动下能形成双向运输系统IFT[159], 负责在发育的纤毛/鞭毛中运输蛋白[168,169]. 此外, 在精子形成时会暂时存在主要由肌动蛋白、细胞骨架成分微管组成的精子领这一特殊结构. IMT即借助精子领进行物质运输, 在鞭毛组装和精子头部塑形过程中起重要作用[170]. 随着鞭毛延伸的逐步完成, 精子领迅速减少至消失, IMT途径也不再存在[167].

IFT20, IFT25, IFT27, IFT74, IFT81, IFT140和IFT172参与小鼠精子鞭毛发育, 其异常导致生育能力丧失或降低[167]. *Ift74*是一种编码必需IFT成分的基因, 被证明与IFT81在纤毛内的微管蛋白转运相关. 在小鼠中, *Ift74*缺陷引起雄性不育, 出现MMAF; 在人类中, *IFT74*缺陷导致鞭毛形成受损造成不育[171]. IFT144/WDR19是IFT-A的核心成分, 对维持IFT的结构和功能完整性至关重要, *WDR19*缺陷造成精子活力下降和形态畸形[172].

多个精子领相关基因的缺陷被发现与男性不育有关, 主要表型为ATS, 包括*SPEF2*, *SPAG6*, *CFAP43*等[111]. HOOK1与精子领微管结合, 对该结构的形成和维持都起着重要作用[134]. 小鼠*Hook1*基因缺陷导致精子领异常, 精子出现头部或鞭毛形态畸形, 引起雄性不育[173]. *TTC29*是一个在睾丸中高表达的基因, 编码一种与IFT相关的含四肽重复序列的蛋白, 是鞭毛结构和摆动所需的保守轴丝蛋白, 其缺陷会导致患者MMAF和不育[174]. CFAP43则与IMT相关, 对精子头部和鞭毛形成至关重要. *Cfap43*基因缺陷小鼠在变形期的精子中出现异常的精子领以及MMAF表型[116].

## 5 干预方案

如今针对AZS患者的干预方案选择往往以临床诊断为主要依据, 辅以遗传检查而开展[175]. 对于一些遗

传或其他因素导致的AZS患者，如接受药物等治疗后仍不能实现自然妊娠，则需要采取辅助生殖技术(assisted reproductive technology, ART)[1,44,176]，包括人工授精(artificial insemination, AI)、体外受精-胚胎移植(*in vitro* fertilization and embryo transfer, IVF-ET)及其衍生技术. 为解决一部分不育夫妇在IVF-ET介入后仍难以妊娠的问题，研究人员自1992年引入卵胞浆内单精子注射技术(intracytoplasmic sperm injection, ICSI)[177]. 该技术将单个精子通过显微注射针注入卵母细胞，使精卵被动结合形成受精卵. 部分研究认为，与IVF-ET相比，ICSI可降低完全受精失败的风险，仅需更少量的卵母细胞便可获得临床可用的胚胎.

ICSI可帮助一部分严重AZS患者获得后代，但同时也存在技术安全隐患. ICSI可能对配子造成潜在损伤[178,179]，同时该操作可能导致与自然受孕所不同的卵母细胞活化与受精模式[180,181]. 针对MMAF的辅助生殖研究发现，一些基因的缺陷会导致中心体异常和精子头部发育异常，使得患者或小鼠难以通过ICSI获得后代, 如*CEP135*[13], *CEP78*[166,182]和*CFAP65*[62,124,154]. 少数轴丝微管结构相关基因缺陷也难以通过ICSI干预，如*DNAH17*[138]. 而大部分MMAF基因缺陷精子的ICSI预后效果则相对良好[11]. 此外，导致MMAF的遗传缺陷可能会通过ICSI垂直传递给下一代，这也是需要事先进行遗传咨询的注意事项.

利用睾丸来源的圆形精子细胞注射(round spermatid injection, ROSI)技术可以获得后代小鼠[183,184]. 一部分男性不育患者缺少良好的成熟精子但存在圆形精子细胞，理论上使用ROSI有可能可以获得后代. 但ROSI面临一些问题, 限制该技术在临床上的应用: 如胚胎发育效率低下、表观修饰异常、肉眼难以精确挑选单倍体圆形精子细胞等[185~188]. 单倍体圆形精子细胞仍然处于核未浓缩阶段，其表观遗传状态与成熟精子存在差异，如DNA甲基化、组蛋白修饰和染色质结构的重编程等[186,189]. 胚胎发育受到严格的表观遗传调控，以保障发育并建立长效的表观遗传状态[190,191]. 一些表观遗传异常可能会影响受精、早期胚胎发育和ART的成功率[192]. 在人类ROSI胎儿的一项两年随访研究中发现, 90例ROSI子代中3例存在先天畸形，而其余婴儿的身体和智力发育则与自然受孕婴儿无明显差异[193]. 对ROSI小鼠E11.5胎儿和胎盘进行转录组分析, 研究人员发现, 与ICSI组和自然受孕组相比, ROSI组存在一些异常激活的重复DNA序列和差异表达基因[194].

小分子抑制剂A-366是一种肽竞争性常染色质组蛋白赖氨酸N-甲基转移酶2 (EHMT2，又称G9A)抑制剂, 能抑制EHMT2表达, 减少H3K9me2沉积[188,195]. 为解决ROSI存在的表观遗传重编程缺陷，研究人员利用EHMT2 (G9A)的小分子抑制剂A366修改表观遗传状态, 提高ROSI胚胎的囊胚发育率和活产率[188]. 为克服肉眼难以精确挑选单倍体圆形精子细胞的问题，研究人员通过荧光激活细胞分选(fluorescence-activated cell sorting, FACS)精确筛选*Tent5d*缺陷的少弱畸精子症小鼠的圆形精子细胞用于卵母细胞注射，有效获得子代(“FACS+ROSI”策略)[196]; 这种策略较之肉眼挑选圆形精子细胞, 增加准确度和可靠性. 即便如此, ROSI的安全性目前仍有待商榷, 需要更深入的研究和验证.

除上述辅助生殖技术外，研究人员也在针对男性不育探索其他干预方法. 将多能干细胞体外诱导为生殖细胞[197,198]有望为男性不育提供单倍体配子，尤其是对于减数分裂异常[199,200]引起的男性不育. 在精原干细胞或睾丸间质干细胞中，纠正遗传缺陷，再将其移植到受体小鼠睾丸中可以恢复精子发生[201~204]. 将靶基因过表达或基因编辑系统递送到小鼠睾丸，亦有助于挽救不育[205~207]. 目前，上述方法虽因技术和伦理等原因未正式应用到临床，但有望为一些ICSI失败的MMAF基因的干预提供思路(图1).

## 6 总结与展望

近几年的研究表明，通过WES等测序技术可以有效地对MMAF等AZS患者进行候选基因筛查；利用基因修饰小鼠模型可以有效地验证候选基因，揭示其功能和致病机制. 同时，一些基于小鼠模型发现的AZS致病基因也可能在人类中具有类似致病贡献. 基于基因修饰小鼠模型的干预策略探索，可以为临床辅助生殖方案选择提供指导，也为辅助生殖失败案例寻找其他干预方法提供参考(图1).

尽管AZS的致病基因鉴定和干预研究已经取得一些进展，但仍然还有许多问题需要研究人员共同解决. (ⅰ) 目前筛选AZS候选基因的常用技术为成本较低的WES. 但由于该技术不涵盖非编码区，未来仍需要借助全基因组测序(whole-genome sequencing, WGS)等技术来探索非编码区遗传变异对AZS的贡献. 与此同

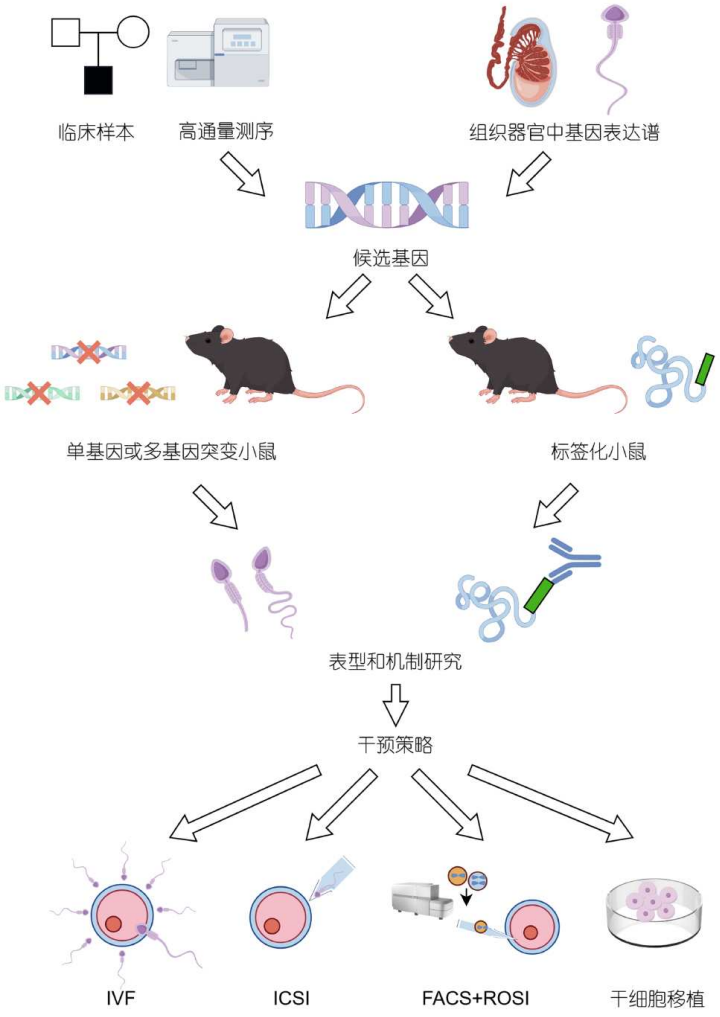


**图 1** 精子鞭毛缺陷相关的弱精子症致病基因的研究. 通过WES等高通量测序技术对病例样本进行候选基因筛查, 或从组织器官的基因表达谱中筛选出候选基因; 根据每个基因的特点, 选择合适的小鼠建模技术构建基因突变和标签敲入等多种模型; 通过多种实验技术分析患者和基因修饰小鼠的表型, 研究基因的功能和机制; 一部分致病基因可通过IVF或ICSI常规辅助生殖技术获得后代, 另一部分基因待开发其他干预方法. 本图通过Figdraw绘制, ID: UAUWI831bb

**Figure 1** A workflow for identifying genetic causes for asthenozoospermia with sperm flagellar defects. Screen candidate genes in infertile subjects using high-throughput sequencing techniques such as WES, or select candidate genes based on the gene expression patterns in reproductive tissues; based on the characteristics of each gene and its variants, adopt appropriate mouse modeling techniques to construct gene-modified mouse models such as gene mutant mice, and HA-tag knock-in mice; analyze the phenotypes of infertile subjects and gene-modified mice through various experimental techniques, as well as the function and mechanism of the pathogenic genes; conventional assisted reproductive technologies including IVF and ICSI can be used for overcoming the infertility caused by certain genes, while other intervention strategies are yet to be further optimized or developed for clinical use. This figure is drawn by Figdraw, ID: UAUWI831bb

时, 未来针对更多患者分析CNV, 亦有可能寻找到新的遗传致病因素. (ⅱ) 除单基因致病模式外, 目前已有小鼠模型的相关线索提示MMAF寡基因致病模式. 未来通过在不育症患者中深入分析寡基因致病模式, 有望为更多病例明确遗传致病因素. 而类精子干细胞小鼠建模技术等方法则可以为寡基因遗传因素的功能研究快速提供小鼠模型. (ⅲ) 临床上不同MMAF致病基因的患者的临床妊娠结局并不一致. 患者群体面临着两大问题: 一是基因缺陷的垂直传递风险, 提示需要完善遗传咨询, 二是针对辅助生殖失败的患者, 目前缺乏有效的干预方案, 提示需要探索新方法. 如何在保证伦理道德原则的同时, 满足患者孕育健康后代愿望, 更高效地改善患者生殖健康, 也是研究人员及医疗行业工作者亟待考虑的议题.

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# The genetic basis of male infertility with "multiple morphological abnormalities of the flagella" and its intervention strategies

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The sperm flagellum is essential for sperm motility. Thousands of proteins are assumed to be involved in the biogenesis and assembly of sperm flagella. "Multiple morphological abnormalities of the flagella" (MMAF) is featured with a mosaic of morphological defects of sperm flagella (incuding short, coiled, absent, bent, and/or irregular flagella), and causes abnormal sperm motility and male infertility. Owing to the development of the high-throughput sequencing and gene editing techniques, over 40 MMAF pathogenic genes have been identified, and the pathogenic genes of most MMAF patients have been identified. However, the outcomes of assisted reproduction vary among different MMAF pathogenic genes. In this review, we summarize the progress on the study of the genetic basis for MMAF, including the relationship between flagellar structure and sperm motility, the techniques for identifying MMAF pathogenic genes, the functions of representative MMAF genes, and the current and potential strategies to overcome male infertility. We hope to provide an insight into the the analysis and intervention of the MMAF and similar diseases.

**multiple morphological abnormalities of the flagella, asthenoteratozoospermia, asthenozoospermia, gene-modified mouse, sperm flagella, sperm motility**

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