

动物及医学遗传组
论文摘要

高危妇女妊娠中晚期羊水细胞SNP array技术结合传统产前筛查的回顾性研究

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摘要

目的: 针对高危妊娠妇女妊娠中晚期(大于24周)羊水细胞的单核苷酸多态性微阵列(single nucleotide polymorphism microarray, SNP array)结果进行回顾分析, 探讨羊水细胞染色体异常的检出情况。方法: 回顾性分析2015年3月至2017年11月, 湘潭市中心医院生殖与遗传中心对310例妊娠中晚期(>24周)有产前诊断指征的孕妇行羊膜腔穿刺术, 抽取20ml羊水行SNP array, 分析羊水细胞染色体异常的检出情况。结果: (1) 染色体异常24例, 异常率7.74% (24/310), 其中<5Mb的重复/缺失15例, 检出率4.84%(15/310), 其中致病性基因组拷贝数变异(copy number variations, CNVs) 7例, 检出率2.26%(7/310), 临床意义不明确CNVs 8例, 检出率2.58%(8/310)。(2) 超声指标异常、超声指标异常+血清学异常、超声指标异常+高龄、超声指标异常+不良孕产史, 超声异常+两项以上异常的染色体异常检出率分别为7.84%(12/153)、7.69%(5/65)、3.85%(1/26)、21.43%(3/14)、8.33 (1/12), 超声指标异常+不良孕产史异常检出率显著高于超声指标异常和超声指标异常+血清学异常(p<0.05)。(3) 超声单项异常中, 心血管系统异常、泌尿系统异常、消化系统异常以及脐带异常的染色体异常检出率分别为7.69%(1/13)、17.65%(3/17)、16.67%(1/6)、14.29%(1/7)。结论: (1) 与传统方法相比, SNP array可有效提高小于5Mb重复/缺失CNVs的检出率; (2) SNP array技术联合超声, 可有效提高染色体异常的检出率。

关键词

妊娠中晚期, 产前筛查, 单核苷酸多态性微阵列(SNP array), 拷贝数变异

Isolation and Functional Characterization of the Pheromone Biosynthesis Activating Neuropeptide Receptor of Chinese oak Silkworm, *Antheraea pernyi*

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Abstract

Insect pheromone biosynthesis activating neuropeptide (PBAN) controls the synthesis and actuating of sex pheromones of female adult. In the current examination, the full-length cDNA encoding the PBAN receptor was cloned from the pheromone gland (PG) of *Antheraea pernyi* (AntpePBANR). The AntpePBANR displayed the characteristic seven transmembrane areas of the G protein-coupled receptor (GPCR) and was closely related to the PBANR from *Bombyx mori* and *Manduca sexta* in the phylogenetic tree. The AntpePBANR expressed in mammalian cell lines were enacted by AntpePBAN in a concentration-dependent manner. AntpePBANR activation resulted in the calcium mobilization but did not activate the cAMP elevation pathway. Cells expressing AntpePBANR were profoundly responsive to Antpe- γ -SGNP (suboesophageal ganglion neuropeptides) and Antpe-DH (diapause hormone), different individuals from FXPRLamide (X = T, S or V) family in *A. pernyi*. Deletion of residues in the C-terminal hexapeptide (FSPRLamide) proved that P, R and L played the key parts in initiating the AntpePBANR, the amination to the last C terminal residues which can also likewise impact the activation of AntpePBAN receptor altogether. The mRNA of the AntpePBANR gene demonstrated the most noteworthy transcript levels in pheromone gland followed by fat body.

Keywords

Pheromone Biosynthesis Activating Neuropeptide, G Protein-Coupled Receptors, mRNA Expression, AntpePBANR, *Antheraea pernyi*

无创产前基因检测在性染色体非整倍体中应用研究

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摘要

目的: 探讨无创产前基因检测(non-invasive prenatal testing, NIPT)在性染色体非整倍体(sex chromosomal aneuploidy, SCA)产前检查中的临床意义。方法: 选择2016年1月1日至2018年1月31日在江西省妇幼保健院产前诊断中心行NIPT的孕妇, 建议筛查SCA阳性孕妇行羊膜腔穿刺或脐静脉穿刺获取胎儿细胞行染色体核型分析及荧光原位杂交, 并对检测结果进行分析。结果: 共检测20334例NIPT样本, 提示胎儿SCA76例, 筛查阳性率为0.37%, 经遗传咨询知情同意下, 52例胎儿SCA孕妇行介入性产前诊断, 确诊胎儿SCA病例25例(阳性预测值48%), 随其中23例已引产, 2例胎儿出生时正常; 另27例排除胎儿SCA的孕妇均继续妊娠。24例筛查胎儿SCA孕妇拒绝介入性产前诊断, 其中15例选择继续妊娠(随访出生时正常), 2例直接引产, 7例孕妇失访。结论: NIPT可用于性染色体异常检测, 但阳性预测值不高, 仍需产前诊断确诊, 故NIPT技术需进一步优化。

关键词

无创产前检测, 性染色体非整倍体, 产前诊断

一例X染色体连锁显性低磷佝偻病家系基因突变分析及产前诊断

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摘要

目的 对1个有3名女性患者的X染色体连锁显性低磷佝偻病(X-linked hypophosphatemic rickets, XLH)家系进行基因突变分析,并为患者提供产前诊断。**方法** 采用遗传性骨病基因靶向捕获测序,对发现的突变位点进行分析验证,最后通过羊水标本对胎儿行产前诊断。**结果** 在患者X染色体上的*PHEX*基因检测到一个杂合突变:c.1645+1G>A。其母亲与妹妹也为佝偻病患者,针对*PHEX*基因c.1645+1G>A突变位点对该家系进行验证,发现先证者以及其母亲和妹妹均携带该突变,为其母亲和妹妹明确了分子诊断,丈夫未检测到此突变,符合X连锁显性遗传病的发病规律。结合先证者的孕周情况,实施羊膜腔穿刺术,获取羊水标本进行产前诊断。最终诊断结果提示胎儿*PHEX*基因存在c.1645+1G>A杂合突变,为低磷佝偻病患儿。**结论** 在拟诊的患者中均检测到*PHEX*基因的突变,确诊了家系中的患者,并通过胎儿精准的产前诊断,从源头上阻断了低磷佝偻病患儿的出生,达到防控出生缺陷的目的。

关键词

X染色体连锁显性低磷佝偻病, *PHEX*基因, 突变, 产前诊断

一个先天性心脏病家系致病基因筛查

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摘要

目的: 先天性心脏病是新生儿最常见的先天性缺陷类型之一。通过对一个家族性先天性心脏病的家系成员进行致病基因筛查, 为先天性心脏病的分子遗传学机制研究、早期筛查、早期干预治疗提供重要的基础。

方法: 收集家系资料, 绘制家系图, 提取先证者及其亲属外周血基因组DNA, 对先证者进行全外显子组测序, 筛选出致病基因, 并用Sanger测序在家系其他成员中进行验证。

结果: 该先天性心脏病家系共收集3代共22人, 确认患病人数6人。全外显子组测序检测到先证者存在*TAB2* c.446 C>T (p.Ser149*) 杂合突变, 通过Sanger 测序验证, 该突变在此家系先心病患者及正常人中共分离, 符合常染色体显性遗传。

结论: 通过对这个先天性心脏病家系的研究发现, 在该家系中发现尚未报道的*TAB2* c.446 C>T (p.Ser149*) 杂合突变, 可能与该家系发病相关。为先天性心脏病研究、基因筛查和产前诊断提供新的思路。

关键词

先天性心脏病, *TAB2*, 家系分析, 基因诊断

Genome-Wide Characterization of Endogenous Retroviruses in the Galliformes

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Abstract

The endogenous viral elements (EVEs) is a complete or fragmented viral genome that has been integrated into the genome of its host and became part of the host genome. Since retroviral elements account for more than 99.99% of avian EVEs they obviously represent the most meaningful data set for explore patterns of EVE evolution. And for birds, only five viral families were been observed. These families are retroviridae, hepadnaviridae, bornaviridae, circoviridae and parvoviridae. Among them, the endogenous virus of the retroviridae family is called endogenous retroviruses (ERV). Most Galliformes are robust ground-dwelling birds, and the Galliformes order has been of considerable economical importance at all stages of the humankind development. Here, we took advantage of the availability of located in the chromosomes for Galliformes species, such as *Gallus gallus*, *Meleagris gallopavo*, *Coturnix japonica* and *Numida meleagris*, to systematically identify and analyze ERVs in above species. We mined an initial set of 260 potentially complete ERVs in the *G. gallus* genome, 42 potentially complete ERVs in the *M. gallopavo* genome, 38 potentially complete ERVs in the *Coturnix japonica* genome, 68 potentially complete ERVs in the *N. meleagris* genome. We found that all three major ERV classes distributed across all species. The detection and analysis of endogenous viruses in Galliformes genome plays an important role in the study of avian viruses and disease prevention.

Keywords

Endogenous Retroviruses, Galliformes Genome, Complete ERVs

雀形目NUMT及线粒体系统发育基因组学研究

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摘要

雀形目 (Passeriformes) 是鸟纲中最大的目 (约占总数60.4%), 大多数雀形目鸟类在早第三纪 (Paleogene) 经历了快速进化, 关于雀形目的起源及各科间的系统发育关系错综复杂, 是鸟类学研究中争论的热点。本研究通过第一代测序技术扩增并测定8种雀形目鸟类线粒体基因组, 并利用新一代高通量测序技术对5个红嘴蓝鹊 (*Urocissa erythroryncha*) 个体进行cDNA文库的构建及转录组测序。此外, 本研究还通过高通量测序技术对6种雀形目鸟类和1种鸮形目鸟类进行基因组文库的构建及序列的测定, 并结合本课题组已测定的其他鸟类转录组数据, 以及NCBI公共数据库中的相关数据, 对雀形目核线粒体假基因 (NUMT, Nuclear Mitochondrial pseudogene) 及雀形目鸟类线粒体系统发育基因组学进行研究。

本研究通过BLASTn工具对红嘴蓝鹊 (*U. erythroryncha*)、画眉 (*Garrulax canorus*)、树麻雀 (*Passer montanus*)、红嘴相思鸟 (*Leiothrix lutea*)、灰椋鸟 (*Spodiopsar cineraceus*) 转录组中的NUMT进行研究, 并通过基因组测序对探测到的NUMT进行验证, 共发现25个具有转录活性的NUMT。通过随机PCR扩增和测序, 表明组学方法探测到的NUMT可以在基因组上得以验证; RT-PCR及测序结果为NUMT具有转录活性提供了强有力的证据; 通过染色体定位分析, 本研究认为雀形目鸟类NUMT主要分布在基因密度较低的染色体上; 通过对NUMT两翼序列进行分析, 本研究认为雀形目鸟类NUMT两翼序列AT含量较高并含有大量插入序列。本研究首次同时从转录组水平和基因组水平展开对雀形目鸟类NUMT的研究。

本研究测得15种鸟类线粒体全基因组数据, 并结合NCBI公共数据库中释放相关数据进行雀形目线粒体系统发育基因组学研究。构建系统发育树所用物种共260种 (内类群), 隶属于雀形目57个科。利用IQ-tree (UBFboot) 和RAxML (bootstrap) 两种软件构建最大似然树, 结合MrBayes构建贝叶斯树, 结果认为: (1) 支持将雀形目划分为新西兰鸚鵡 (Acanthisittidae)、鸣禽 (Oscines) 和亚鸣禽 (Suboscines) 三个支系, 新西兰鸚鵡应该独立为一个类群, 位于整个雀形目的基部位置; (2) 鸦小目为并系类群, 其中基部鸦小目 (basal Corvida) 为鸣禽的基部类群, 冠部鸦小目 (crown Corvida) 为单系类群; (3) 噪眉属的黑脸噪鹛 (*Garrulax perspicillatus*) 位于蓝噪眉属内, 这两个属均为并系类群; (4) 亚鸣禽沿着地理界限可划分为2个单系类群: 新世界亚鸣禽和旧世界亚鸣禽; (5) 褐背拟地鸦和北红尾鹟 (*Phoenicurus auroreus*) 为姐妹群, 被包围在鹟科内部, 初步建议将褐背拟地鸦从山雀科移到鹟科的红尾鹟属; (6) 鹟总科为并系类群; (7) 垂耳鹟科 (Callaeidae)、缝叶吸蜜鸟科 (Notiomystidae)、啄果鸟科 (Melanocharitidae)、鹟科 (Petroicidae)、戴菊科 (Regulidae)、丛莺科 (Hylotiidae)、山雀科 (Paridae)、攀雀科 (Remizidae) 和夏威夷吸蜜鸟科 (Mohoidae) 等9个科分类学位置尚未确定。

关键词

雀形目, 核线粒体假基因, 线粒体基因组, 系统发育组学

78743例新生儿遗传代谢病串联质谱筛查的初步报告

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摘要

目的: 分析串联质谱技术在新生儿遗传代谢病筛查中的应用情况。

方法: 采用串联质谱技术(非衍生法)对湖南地区78743例新生儿进行氨基酸代谢、有机酸代谢和脂肪酸氧化代谢三类共27种遗传代谢病筛查。对可疑阳性患儿进行尿气相色谱(Gas Chromatography-Mass Spectrometer, GC-MS)、DNA质谱基因分析或高通量测序进行确诊。

结果: 初筛阳性1886例, 复筛阳性163例, 结合GC-MS及基因检测结果确诊29例, 其中24例完善基因检测。筛查阳性率2.36%, 总体阳性预测值为1.54%, 总体发病率为1/2715, 脂肪酸氧化代谢异常居首位, 有机酸代谢异常次之。原发肉碱缺乏症(primary carnitine deficiency, PCD) 10例, 母源性PCD 5例(全部突变类型中以c.51C>G及c.1400C>G多见, 此外还发现两个未见报道的新突变, c.774-775insTCG和c.1298T>C); 短链酰基辅酶A脱氢酶缺乏症4例; 丙酸血症(Propionic acidemia, PA) 3例; 肉碱/酰基肉碱移位酶缺陷(CACT) (c.199-10T>A及c.1A>T复合杂合突变)、3-甲基巴豆酰辅酶A羧化酶缺乏症、甲基丙二酸血症(Methylmalonic academia, MMA)、枫糖尿病、极长链酰基辅酶A脱氢酶缺乏症、异戊酸血症及异丁酰基辅酶A脱氢酶缺乏症各1例。除一例CACT及PA病情凶险夭折外, 其余患儿均及时干预, 随访结果显示MMA及PA预后较差。

结论: 应用串联质谱技术进行新生儿疾病筛查, 辅以GC-MS及分子遗传学检测进行确诊, 有利于新生儿遗传代谢病的早期发现及诊治, 有效保障儿童健康提高出生人口素质。新生儿串联质谱筛查结果提示, 湖南(长沙)地区原发肉碱缺乏症发生率最高, 存在地域性差异可能。

关键词

新生儿疾病筛查, 串联质谱分析, 脂肪酸氧化代谢障碍, 有机酸代谢障碍, 基因检测

5例46,XY女性表型性发育异常患者的遗传学分析

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摘要

对5例46,XY表型为女性的性发育异常患者进行鉴别诊断及遗传学分析,探讨其临床表现与基因型的相关性。回顾分析5例46,XY性发育异常患者的临床资料,应用常规染色体G显带分析患者的核型,利用Sanger测序、全基因组外显子基因测序、FISH等方法进行遗传学检测,寻找致病基因变异。常规G显带核型分析显示5名患者染色体核型均为46,XY。其中3例患者为完全型雄激素不敏感综合征(CAIS),患者1的AR基因1号外显子存在一个移码突变c.1067delC(p.A356Efs*123),是一个新发的致病突变;患者2的AR基因4号外显子存在一个错义突变c.2112C>G(p.S704R),该位点为一个已知致病突变位点;患者3的AR基因4号外显子存在一个错义突变c.2125G>T(p.G709X),该突变使对应密码子突变为终止密码子,是一个新发的致病突变;上述3例CAIS患者的突变位点均在其另外一名家系成员中检出,且3名患者的母亲均为该突变位点的携带者。1例患者临床诊断为单纯性腺发育不良,患者SRY基因缺失;1例患者临床诊断为单纯性腺发育不良, NR5A1的7号外显子存在一个移码突变c.1229delC杂合致病突变,患者的母亲为该杂合突变位点的携带者。通过综合利用多种检测方法明确了5名46,XY表型为女性的性发育异常患者致病基因,为患者的临床治疗及家族的产前诊断提供了依据。

关键词

46,XY女性, AR基因, SRY基因, NR5A1基因

湖南省56万例串联质谱技术新生儿疾病筛查及随访分析

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摘要

目的: 回顾性分析湖南地区串联质谱新生儿遗传代谢病筛查及随访情况

方法: 自2013年3月~2017年9月采集湖南地区568690例活产新生儿足底血, 制备合格干血滤纸片, 采用非衍生化串联质谱技术, 开展脂肪酸、氨基酸、有机酸代谢障碍疾病新生儿筛查。筛查阳性者召回确诊, 对确诊患儿进行治疗及随访, 平均随访时间24个月。

结果: 共确诊17种疾病91例患儿, 患病率为: 1: 6249。脂肪酸B氧化代谢障碍疾病60例(65.93%), 其中以原发肉碱缺乏症40例, 占脂肪酸B氧化代谢障碍疾病66.67%。其次为短链酰基辅酶A脱氢酶缺乏症(8例), 中链酰基辅酶A脱氢酶缺乏症、极长链酰基辅酶A脱氢酶缺乏症各3例, 肉碱酰基肉碱移位酶缺乏症、异丁酰辅酶A脱氢酶缺乏症、多种酰基辅酶A脱氢酶缺乏症各2例。氨基酸代谢障碍疾病19例(20.88%), 其中高苯丙氨酸血症9例, 最为常见。其次为希特林蛋白缺乏症7例, 枫糖尿病、蛋氨酸血症、同型半胱氨酸血症各1例。有机酸血症12例(13.18%), 其中丙酸血症、3-甲基巴豆酰辅酶A羧化酶缺乏症各4例, 甲基丙二酸血症2例, 异戊酸血症和 β -酮硫解酶缺乏症各1例。随访结果显示: 1例原发肉碱缺乏症未接受治疗夭折(上呼吸道感染、高热), 2例肉碱酰基肉碱移位酶缺乏症、2例丙酸血症、1例枫糖尿病、1例异戊酸血症患儿虽经积极治疗仍然于新生儿期夭折。2例甲基丙二酸血症患儿虽然治疗, 仍然发病, 存在一定程度发育落后。3-甲基巴豆酰辅酶A羧化酶缺乏症患者未经治疗生长发育良好。其余患儿经治疗, 发育正常。

结论: 脂肪酸氧化代谢障碍、氨基酸代谢障碍和有机酸代谢障碍疾病在湖南地区并不少见, 原发肉碱缺乏症、高苯丙氨酸血症最常见。串联质谱新生儿疾病筛查是发现此类遗传代谢病可靠而有效的方法。通过早期诊断和早期干预, 大部分患儿可以获得正常的生长发育, 提高生活质量, 避免体格和/或智能残疾。

关键词

串联质谱, 新生儿疾病筛查, 遗传代谢病

利用小鼠模型研究心肌肥厚的分子调控机制

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摘要

心血管病是威胁人类生命的第一大杀手, 研究表明几乎所有类型的心脏疾病, 包括高血压、心肌梗塞、心房室瓣疾病, 以及基因、病毒或代谢引起的不同心肌病等, 在其发生中都会经历一个共同的心肌肥厚的病理过程。因此, 研究抵制心肌肥厚的遗传机理, 是控制心脏疾病的首要环节, 但心肌肥厚发生的始发性因子及其致病机制至今不明。我们注意到心肌细胞的胚胎型转化是病理心肌肥厚的一个共同特征, 且胚胎期心脏和成体心脏心肌细胞中存在着两种近乎相反的基因表达谱。于是我们推测出生后那些被关闭的一系列胚胎期基因, 必然被成体心脏中一类内源性组成型激活的分子所抑制。在病理条件下, 这一类内源性的活性分子的失活或者是表达下调, 导致胚胎期基因的重新开启, 使得终末分化的心肌细胞得以逆向胚胎型转化而重新获得可塑性, 从而使病理性重构成为可能, 导致心肌肥厚。据此我们提出成体心脏中这类高表达的心肌肥厚抑制因子是抑制心肌肥厚疾病发生的始发性因子的假说, 并通过生物信息学分析筛选出心脏中特异性表达或高表达的候选基因, 同时研制模仿压力负荷和激素刺激诱发的心肌肥大小鼠模型, 以及体外培养的原代心肌细胞肥大刺激模型, 结合人类心衰标本, 进一步鉴定出在肥大中表达下调的心脏特异性或高表达的始发性候选基因。针对候选基因研制遗传修饰的动物模型, 最终发现Tgfr2、Hole等多个基因, 在心肌细胞病理生长中发挥着抑制作用。

关键词

基因修饰, 心肌肥厚, 抑制因子, 动物模型

利用小鼠模型研究5q35综合征发生的分子病理机制

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摘要

5q35综合征是指由人类染色体5q35.2-5q35.3区域微缺失或微重复引起的一类临床症候群, 属先天性染色体异常疾病, 其临床症状主要表现为心脏发育异常、骨骼发育异常及内分泌系统发育异常, 并伴有颅面部畸形、智力发育迟缓, 严重者可并发癌症。该综合征与Sotos综合征(一种以儿童期过度生长现象为特征的遗传病, 主要表现为巨头畸形、特殊面容、骨龄提前以及不同程度的发育迟缓。)具有相似的临床表现, 但至今尚未确定其致病基因和发病机制。为确定5q35综合征相关疾病的致病基因, 本课题组通过动物模型全基因组基因突变分析, 结合生物信息学分析、数据库查询, 筛选得到5q35综合征5号染色体微缺失与微重复区域的相关候选疾病基因DDX41、B4GALT7、ADAMTS2等基因, 正利用基因敲除与转基因技术建立人类疾病候选基因小鼠模型, 通过分析候选基因的生物学功能, 结合转录组测序技术检测小鼠全转录组表达水平的变化, 进一步通过RNA-seq、报道基因、Western blot等技术检测5q35综合征5号染色体微缺失与微重复区域相关候选疾病基因对下游基因的调控作用进行分析, 以研究相关候选疾病基因的调控网络, 探索5q35综合征发生的分子病理机制。

关键词

5q35综合征, 微缺失与微重复, 候选疾病基因, 疾病基因小鼠模型, 分子病理机制

利用小鼠模型研究TCF25调控基因的表达

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摘要

心脏发育的过程是十分复杂的,对于心脏发育相关发育基因表达机制的研究是解开心脏发育复杂发育过程的重要步骤。TCF25是本实验室筛选出的人类心脏发育候选基因,已知其在小鼠胚胎心脏等组织发育过程中广泛表达。TCF25是一个含有bHLH结构域的转录因子,其C端还含有一个功能未知的DUF654结构域。根据TCF25广泛的表达模式和包含的转录激活结构域,推测其可能在心脏等各种器官发育过程中发挥重要的调控作用。小鼠作为模式生物其发育和调控机制与人类相似,因此被广泛用于疾病发生及调控机制的研究。本课题通过构建TCF25小鼠突变品系,利用原位杂交技术分析小鼠TCF25突变对其下游基因表达的影响。胚胎原位杂交具有特异性强、敏感性高、定位精确、定量、可完整地保持组织细胞的形态,能准确地反映组织细胞的相互关系及功能代谢状态的特点。我们首先进行TCF25突变胚胎心脏的全转录组表达谱分析,进一步选取小鼠TCF25突变系胚胎E7.5、E8.5、E9.5、E10.5、E11.5时期检测心脏发育标志基因Bmp4、GATA4、NKx2.5、Mesp1、Tbx5等基因表达情况,另分析TCF25的下游调控基因,以研究出该基因的调控信号与调控网络,为进一步研究TCF25基因在心脏发育中的具体调控机制打下基础。

关键词

TCF25, 小鼠模型, 心脏发育, 胚胎原位杂交, 分子机制

利用小鼠敲除模型研究GEFT对肌肉损伤后修复的影响

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摘要

GEFT是一种膜锚定蛋白,是Rho家族鸟嘌呤核苷酸转换因子(GEFs),在小鼠心脏骨骼肌大脑等可兴奋组织有较强的表达。有研究表明,肌肉损伤后GEFT表达上调,并且利用腺病毒外源性过表达GEFT能加快肌肉损伤的修复,通过RhoA, Rac1和Cdc42介导肌细胞生成。在体外C2C12细胞系中,GEFT能促使C2C12向肌细胞分化,并抑制其向脂肪细胞分化。尽管如此,由于体内基因敲除小鼠模型的缺乏,GEFT在体内肌肉再生和分化中的功能仍不清楚。我们利用Cre/loxP系统,设计构建同源臂共13K的打靶载体,将loxP锚定在GEFT第5-17个外显子的上下游,得到GEFT全敲小鼠。通过PCR与Southern检测,都显示GEFT全敲小鼠中的GEFT表达沉默。但是全敲小鼠与野生型小鼠的生活状况无明显差异,而且心、肌肉、胃和肠的组织切片对比,观察发现两者无明显区别。我们利用注射心脏毒素(CTX)成功诱导了一个肌肉损伤和修复的模型。我们向小鼠骨骼肌注射50 μ l的10 μ g/ml的心脏毒素,通过检测c-myc、MyoD和myogenin因子发现,在注射CTX5天后,肌肉卫星细胞增殖和向肌细胞分化达到顶峰。而且我们通过对全敲小鼠和野生型小鼠分别注射心脏毒素5天,10天后,取肌肉损伤部位进行组织切片对比,观察发现GEFT全基因敲除鼠,肌肉损伤恢复状况较野生型小鼠明显延迟。我们的结果首次在体内证明,GEFT在调节肌肉损伤修复中的重要作用。

关键词

GEFT, 肌肉修复, GEFT敲除小鼠模型

维生素D水平以及其受体基因多态性与女童性早熟关系的研究*

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摘要

目的: 研究中枢性性早熟女童的维生素D浓度变化及VDR基因多态性表达的差异, 了解维生素D影响女童性早熟的部分机制。方法: 将40例中枢性性早熟女童、40例健康对照组女童作为研究对象。运用化学发光法检测其血清中Vit D 的浓度, 同时应用Snapshot检测技术进行VDR多态性位点ApaI(rs7975232)、BsmI(rs1544410)、TaqI(rs731236)、FokI(rs2228570)的检测。结果: 1. 中枢性性早熟患儿25-OH Vit D水平高于健康对照儿童, 两组的差别有统计学意义($P < 0.05$)。2. (1) BsmI, FokI和TaqI三个位点分别对应的基因型CC、CT, AA、GA、GG, 和AA、GA 在两组中的分布差异都无统计学意义(分别 $X^2=0.721, P=0.396$; $X^2=3.414, P=0.181$; $X^2=0.000, P=1.000$)。 (2) ApaI检测到的三种基因型CC,CA,AA在两组中的分布差异有统计学意义($X^2=9.833, P=0.007$)。结论: 1、性早熟女童维生素D的水平低于健康女童, 提示血清维生素D水平可能对性早熟发病有影响。2、ApaI(rs7975232) 的不同基因型在两组中的分布差异有统计学意义, 提示该基因序列的多态性可能在性早熟的发病机制中发挥作用。BsmI(rs1544410)、TaqI(rs731236)、FokI(rs2228570)的不同基因型以及等位基因在两组中的分布差异无统计学意义, 提示其多态性可能与性早熟的发病无关。3、维生素D对女童性早熟的影响可能是通过VDR基因位点ApaI(rs7975232)的多态性来起作用的。

关键词

维生素D, VDR, 基因多态性, 性早熟

CERKL Regulates Autophagy via the NAD-Dependent Deacetylase SIRT1

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Abstract

Autophagy is an important intracellular mechanism for the maintenance of cellular homeostasis. Here we show that ceramide kinase-like gene (CERKL) a retinal degeneration pathogenic gene plays a critical role in regulating autophagy by stabilizing SIRT1. In vitro and in vivo, suppressing CERKL results in impaired autophagy. SIRT1 is one of the main regulator of acetylation/deacetylation in autophagy. In CERKL-depleted retinas and cells, SIRT1 is downregulated. ATG5 and ATG7, two essential components of autophagy show the higher degree of acetylation in CERKL-depleted cells. Overexpression of SIRT1 rescues autophagy in CERKL depleted cells. Whereas CERKL losses its function of regulating autophagy in SIRT1-depleted cells. And overexpression of CERKL up-regulates SIRT1. Finally, we show that CERKL directly interacts with SIRT1, and may regulate its phosphorylation at Ser27 to stabilize SIRT1. These results show that CERKL is an important regulator of autophagy and it plays this role by stabilizing a deacetylase SIRT1.

Keywords

Retinitis Pigmentosa, CERKL, Autophagy, Photoreceptors, Zebrafish

Pathophysiological Mechanisms Shaping Schizophrenic Brain: From Genetics to Neural Circuit

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Abstract

Schizophrenia is a chronic and severe neuropsychiatric disease affecting 1% of human population worldwide. The symptoms of schizophrenia usually appear from adolescence, and last for the rest of human life, thus the disease leads to a great burden for the society and family. Genetic factors contribute largely to the etiology of schizophrenia. Currently, GWAS study has identified numerous risk loci for schizophrenia. However, the questions about how the genetic mutations regulate the gene expression and function, and how these risk genes contribute to the pathophysiology of schizophrenia remain largely unresolved. The development of neural synapse and establishment of neural circuitry are important processes for the brain development and function. The disturbances of these processes underly the behavioral disorders of many neuropsychiatric diseases including schizophrenia. Hence understanding the role of schizophrenia risk genes on synapse development and circuit connectivity will help to elucidate the mechanism underlying the etiology and pathophysiology of schizophrenia. *Dtnbp1* has been reported as a schizophrenia risk gene; its expression is decreased in hippocampus of schizophrenia patients. By using a genetic mouse model in which *Dtnbp1* is deleted, we find that *Dtnbp1* mutation causes a decrease in density of hippocampal dendritic spine, hyperactivation of hippocampal spine dynamics, a dysconnection in entorhinal-hippocampal circuit, and impaired working memory. *Kcnh2-3.1* is another schizophrenia risk gene whose expression is increased in hippocampus of individuals with schizophrenia. We observe that overexpression of *Kcnh2-3.1* in mice decreases the synapse number and synaptic transmission, as well as synchronized activity between ventral hippocampus and medial prefrontal cortex. Together, these results uncover the mechanisms of how genetic mutations change the establishment of neural circuitry in schizophrenic brain.

Keywords

Schizophrenia, Risk Gene, Synapse Development, Neural Circuit

染色体微阵列芯片(CMA)在复杂染色体异常诊断中应用

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摘要

染色体微阵列芯片(CMA)是一种新型的分子细胞遗传分析技术, 具有高分辨率、高通量、高敏感性和高准确性等优点, 在对染色体拷贝数异常(copy number variation, CNVs)即染色体的数目异常、片段缺失和重复的检测方面比传统的染色体核型分析具有明显的优越性。本研究中我们应用CMA对9例染色体核型异常患者进行了染色体微阵列芯片(CMA)分析, 探讨细胞遗传学与分子遗传学技术相结合进行诊断的可行性及优越性。

2例47, XY+mar中一例经四种方法(染色体核型分析、FISH, CMA, QPCR-Y微缺失检测)互相验证, 确诊为Y染色体部分扩增综合征和AZFb+AZFc微缺失综合征; 1例CMA确定为dup(5)(q11.2q13.3)综合征, 与临床症状相符。4例染色体相互易位中, 1例t(7;22), CMA确定为2q31.1微缺失综合征, 与临床症状相符; 1例t(1;2;11)CMA确定为17q21.3微缺失综合征; 该2例通过CMA可确定染色体的相互易位为平衡易位, 而发现其它染色体存在微缺失, 弥补了核型分析的不足; 另2例t(9;16)和t(2;7) CMA均未发现与患者疾病相关基因组不平衡现象, 临床运动发育障碍未找到原因。2例45,X/46,X,+mar, 芯片均为缺失整条X染色体, 检测未发现其它大片段的重复, Marek染色体不是来源于常染色质区, 而是来自异染色质区, SRY阴性, 确定为Turner综合征。1例有明显精神运动发育障碍伴有腭裂, 染色体核型正常, CMA未发现异常, 该患儿有出生缺氧窒息史, 可解释临床症状。

结论 传统的细胞遗传学分析技术与染色体微阵列芯片(CMA)技术相结合, 有利于检测结果的相互补充与验证, 从而获得更为精确的遗传学数据, 为进一步研究染色体畸变、基因组重排与临床表型的关系奠定了基础。

关键词

染色体微阵列芯片, 微缺失 / 重复综合征, 标记染色体

Sam68 Confers Protection Against Genotoxic Stress to Healthy and Cancerous Colon

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Abstract

The nuclear factor kappa B (NF- κ B) signaling pathway remains one of the most attractive avenues for pharmacological intervention, given its crucial function in human health and disease. NF- κ B-mediated gene transcription is one of the most important mediators for cells to sense genomic DNA damage and initiate appropriate cellular responses. Genotoxic agents trigger a “nuclear-to-cytoplasmic” signaling pathway leading to NF- κ B activation. In spite of the widespread use of chemotherapy and radiotherapy in cancer treatments that can cause DNA damage and initiate this signaling pathway, the early nuclear signaling cascade linking DNA damage and NF- κ B activation, is still not well understood. My recent studies demonstrate that Sam68 (Src-associated-substrate-during-mitosis-of-68kDa) is an important regulator of the genotoxic stress-initiated NF- κ B signaling pathway. Sam68 deficiency abolishes DNA damage-stimulated polymers of ADP-ribose (PAR) production and the PAR-dependent signaling cascade that is essential for NF- κ B activation and anti-apoptotic gene expression. As a consequence, Sam68 deleted cells are hypersensitive to genotoxicity caused by DNA damaging agents. Moreover, upregulated Sam68 levels coincide with elevated PAR production and NF- κ B-mediated anti-apoptotic gene transcription in both human and mouse colon tumors. Knockdown of Sam68 substantially sensitizes human colon cancer cells to genotoxic stress-induced apoptosis and genetic deletion of Sam68 dramatically dampens colon tumor burden and survival in mice. Hence our data reveal an unexpected function for Sam68 in DNA damage-triggered early signaling and the genotoxic stress-initiated “nuclear to cytoplasmic” NF- κ B transcription activation and uncovers a critical role of Sam68 in the growth and survival of colon cancer.

Keywords

Genotoxic Stress, Colon Cancer, NF- κ B

不同供体来源克隆胚胎重编程过程中的转录组学分析

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摘要

自然条件下已分化细胞不会自发发生转变为其他类型细胞。通过核移植等实验手段可以将终末分化的体细胞重编程为类胚胎干细胞的多能性干细胞状态, 从而实现细胞类型的转化也即重编程。然而体细胞核重编程效率低下, 严重制约该技术发展及其应用。虽然大量研究表明体细胞核重编程过程中存在多重的转录缺陷导致核重编程效率低下, 但具体机制不清。因而构建精细的转录组图谱特别是长非编码RNA图谱以全面阐明体细胞核重编程的分子机制显得迫在眉睫。针对这些问题, 本研究利用体细胞核移植技术, 构建了与小鼠体内胚胎遗传背景相同的两种克隆胚胎。利用单细胞转录组测序技术结合生物信息学分析绘制了两种不同供体的克隆胚胎及正常体内胚胎植入前发育全期的mRNA特别是带有polyA尾的lncRNA转录组图谱。结果发现体内胚mRNA和lncRNA均呈现出与发育时期相关的表达模式, 而克隆胚的表达模式与之相差甚远, 大量本该重编程的基因未被正确编程。经GO功能富集分析和KEGG通路分析发现, 植入前胚胎发育早期克隆胚(合子期至4-细胞期)在RNA加工、修饰和翻译起始等过程存在严重缺陷, 造成克隆胚早期发育过程中出现表观缺陷, 无法有效开启基因组使染色质重构。此外, 我们还发现大量各期特异性且与表观遗传修饰相关的新mRNA和lncRNA转录本, 进一步暗示表观重编程不彻底是导致基因再激活和重编程效率低的主要原因。本研究结果将加深对重编程理解, 较为全面的揭示非编码RNA在不同类型供体细胞核重编程过程中的作用和功能, 也为核重编程技术在再生医学和农业等行业的推广和应用奠定基础。

Exposure of Mouse Early Embryos to Ethanol Cause Abnormal Development of Placenta

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Abstract

Harmful effects of ethanol on pregnant mothers and their offspring have still being paid attention to, and it was known that oxidative stress damage play crucial role to impact fetus growth and development. Although massive data indicated abnormal development of mammalian embryos by gestational ethanol exposure in diverse animal model, and our study with embryo culture in vitro, the roles of placenta in the process of ethanol interfering embryos development remains unclear. To better understand effects of ethanol on placenta, in this study, proteomic analysis was utilized to investigated the profile change of placentas (E13.5) resulted from mouse preimplantation embryos cultured in medium with 1.0% ethanol till blastocyst stage, then transferred into uterus. Experiment of embryos culture indicated that both embryo development and placenta formation (E13.5) were influenced by ethanol. Results from mass spectrometry showed that there were 4334 proteins to be found in placenta, in which 412 proteins significantly differently expressed in ethanol exposure placenta including 225 up-regulated and 287 down-regulated proteins. These alerted proteins contain related proteins to hypoxia response, lipid metabolic, molecular transport and localization, nervous development and protein modification. GSEA analysis showed that several novel cellular processes, such as shh pathway, glycosylation, neoplastic transformation and basement membrane presented significant alteration in ethanol exposure group. But, differently from early studies, no obviously change occurred on facets of nutrition transportation and anti-oxidative stress mentioned in early studies. All above suggest that using of ethanol exposure of early embryos is helpful to explore the cause of abnormal development and function defects of placenta, which distinguished from effect of routine gestational ethanol exposure on placenta. Meanwhile, these findings also point out that to prevent injury effect of ethanol on early embryos is more critical in avoiding damaged impact of alcohol to offspring.

Keywords

Ethanol, Placenta, Proteomic Analysis

Toxic Effects of NanoAg on Mouse Embryo Development Are Dose- and Time- Dependent

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Abstract

Nanoparticle Ag (NanoAg) has being attracted more and more attention from researchers in diversity of fields for their amazing quality and application. Safety and toxic effects of NanoAg on human health have being investigated in many tissue and cell from the facets of physiology, cellular biology and molecular mechanisms because it can pass through almost all of tissue wall and impact greatly normal physiology action. However, it remains unknown that the direct effects of NanoAg on human embryonic development for only few studies investigated development of mouse embryos by injection of NanoAg into tail vein or gastric perfusion, besides others using zebrafish as model. To clarify the direct effects of NanoAg on mammal embryos and related pathway, this study analyzed the developmental pattern of mouse early embryos cultured in medium containing NanoAg with different particle sizes and concentrations. Meanwhile, Physical and chemical characters of NanoAg in medium were analyzed to contract the link between embryonic development toxicity and NanoAg quality. ROS level and membrane potential of cell and mitochondria in embryos under different environment conditions were detected respectively by DCFH, DiBAC4(3) and Mito-Tracker Red assay. Results showed that toxic effect of NanoAg on embryos development presented particle size and does dependent, and a critical timing was existed to effect of different concentration of NanoAg in medium on embryos. Data suggested that both of cell apoptosis triggered by changing of membrane potential at lower dose of NanoAg and cell death induced by high ROS level at higher dose of NanoAg play the function in ethanol toxicity to early embryos. All these findings defined that NanoAg owned direct toxic effect on mouse embryo development no matter at lower or higher dose, which remind us to note its dangerous to reproduction in its daily production and application.

Keywords

NanoAg, Embryo Development, ROS, Apoptosis