利用果蝇模型研究人类II型腓骨肌萎缩症的进展

余凌奇 谢甲钰 原垚 凌圣安 陈文锋*
(福州大学生物科学与工程学院, 福州 350108)

摘要 腓骨肌萎缩症(Charcot-Marie-Tooth, CMT)通常是由神经元中某些蛋白质缺陷引起的一种常见家族遗传性外周神经系统疾病, 患者表现为远端感觉和运动神经元的缺陷, 行动能力不足, 严重者可丧失行动能力。根据临床和电生理特征, CMT主要分为原发性脱髓鞘病变CMT1、原发性轴突病变CMT2以及继发性脱髓鞘和轴突病变的DI-CMT。越来越多的研究利用果蝇模型来模拟人类疾病和人类健康相关过程的各个方面。果蝇没有被髓鞘包围的轴突, 因此不适合建立脱髓鞘型的CMT模型, 而比较适合轴突病变CMT2的研究。该文主要针对CMT2进行分析, 总结了人类CMT2涉及的相关致病基因, 以及如何利用果蝇模型进行CMT2模型构建和病理分析。这对于CMT2疾病的生物学和医学研究具有重要的意义。

关键词 腓骨肌萎缩症; CMT2; 果蝇; 疾病模型

Advances in the Research of Human Charcot-Marie-Tooth II Disease Using the *Drosophila*

YU Lingqi, XIE Jiayu, YUAN Yao, LING Sheng'an, CHEN Wenfeng*
(*College of Biological Science and Engineering, Fuzhou University, Fuzhou 350108, China*)

Abstract CMT (Charcot-Marie-Tooth) is a common family-genetic peripheral nervous system disease usually caused by mutations in certain protein defects in neurons, which are characterized by defects in distal sensory and motor neurons. CMT patients show insufficient mobility and severe cases can lose mobility. According to clinical and electrophysiological characteristics, CMT is mainly divided into primary demyelinating lesions CMT1, primary axonal lesion CMT2, secondary demyelinating and axonal lesions DI-CMT. More and more researches are using the *Drosophila* models to simulate various aspects of human disease and human health related processes. *Drosophila* has no axons surrounded by myelin, so it is not suitable for establishing a demyelinating CMT model, and is more suitable for CMT2 researches. Here, this review mainly analyzes CMT2, summarizing the related pathogenic genes involved in human CMT2, and describes how to use the *Drosophila* for CMT2 model construction and pathological analysis. This will be of great significance for the biological and medical researches of CMT2 diseases.

Keywords Charcot-Marie-Tooth; CMT2; *Drosophila*; disease model

CMT(Charcot-Marie-Tooth)[又称遗传性运动和感觉神经病(hereditary motor sensory neuropathy, HMSN)]是一种常见的家族性周围神经病之一, 发病率约为1/2 500[1-2], 本疾病多为儿童或青少年期发病, 临床主要特征是四肢远端进行性的迟缓性肌无力、远端肌肉萎缩, 伴肢体远端感觉障碍。病情发展可

收稿日期: 2022-06-28 接受日期: 2022-09-07
福建省自然科学基金(批准号: 2020J02027)和国家自然科学基金(批准号: 31970461)资助的课题
*通讯作者。Tel: 18659194662, E-mail: chenwenfeng@fzu.edu.cn
Received: June 28, 2022 Accepted: September 7, 2022
This work was supported by the Natural Science Foundation of Fujian Province (Grant No.2020J02027) and the National Natural Science Foundation of China (Grant No.31970461)
*Corresponding author. Tel: +86-18659194662, E-mail: chenwenfeng@fzu.edu.cn

致跨越步态, 出现“鹤型腿”等畸形。部分患者可有骨骼畸形, 如“高弓足”[3], 也可能存在其他现象。传统CMT分类(CMT1、CMT2和DI-CMT)主要基于神经传导速度NCV(nerve conduction velocity)检测确定病变类型, 比如脱髓鞘型CMT1定义为NCV<35 m/s, 轴索型CMT2定义为NCV>45 m/s, 中间型DI-CMT定义为NCV=35~45 m/s。患者症状和体征比较对称, 多数患者有家族史, 具有明显的遗传异质性。CMT1型又称肥大型或脱髓鞘型(hypertrophic type), 由于果蝇没有被髓鞘或施万细胞包围的轴突, 因此建立脱髓鞘CMT模型并不合适[3]。CMT2型又称轴索型(neurnal type), 此类型适合在果蝇中建立模型。DI-CMT属于二者兼具的中间形式[4]。目前已有超过100个CMT相关基因被确定[3], CMT疾病多数呈常染色体显性遗传, 也有少数为常染色体隐性或伴X遗传即CMTX。这里将对该疾病尤其是CMT2型腓骨肌萎缩症进行探讨, 并概括相关致病基因。此外, 介绍如何构建CMT2疾病的果蝇模型, 并对其生理病理指标做出评价, 从而更深层次地了解该疾病。

1 CMT2型(轴突型, 晚发病)介绍

CMT2占遗传学诊断的CMT患者的20%, 常表现为常染色体显性遗传模式, 神经动作电位波幅降低, 神经传导速度正常(提示轴索病)。CMT2的特征在于由长度依赖性的运动和感觉神经变性引起的慢性进行性无力和远端肢体肌肉萎缩[5]。CMT2包括多种类型, 涉及多个基因。比如*MFN2*基因的突变与轴突类型的CMT2A2有关[6]。CMT2B由基因*RAB7*、*LMNA*、*MED25*发生病变所致[7]。CMT2C由基因*TRPV4*介导。CMT2D是由*GARS*基因突变造成的[8]。CMT2F是由*HSPB1*基因突变引起的, 可导致自噬功能障碍[9]。CMT2K是由*GDAP1*突变引起[10], CMT2L是通过位于*HSPB8*的染色体突变所致的[11]。此外, *AARS*、*MARS*、*HARS*的异常都与CMT2型有关。尽管轴索性周围神经病与脱髓鞘性周围神经病有广泛的临床重叠, 但一般而言, 轴索性神经病患者的残疾程度较低, 感觉丧失也比脱髓鞘性神经病患者少。总的来说, CMT2型病因分为两点: 一是基因突变直接导致神经发育产生变化, 二是关键步骤酶的作用受影响。在表1中我们概括了至今发现的CMT2主要致病基因及这些基因已知的生物学功能。

2 果蝇CMT2模型建立方法

CMT2果蝇疾病模型大致可分为三种, 前两种主要利用Gal4/UAS二元表达系统[81], 使用不同的Gal4进行组织特异性的目的片段驱动表达, 包括表达CMT2致病基因和表达基因干扰片段敲降果蝇内源同源基因, 第三种是修改果蝇内源基因模拟特定的CMT2突变。

2.1 表达CMT2致病基因

该方法通过组织特异性的增强子序列驱动的Gal4转基因品系与UAS转基因品系进行杂交, 组织特异性驱动UAS连接的目的片段表达, 比如表达一段编码蛋白的序列(图1A)。可以直接把发现的人源致病基因构建成UAS转基因果蝇, 然后在特定组织中进行表达观察病理表型。还可以通过人源和果蝇的蛋白质序列同源性比对, 找出保守位点, 然后把果蝇对应基因的保守位点修改成人源发现的特定点突变并构建成UAS转基因果蝇, 在特定组织中进行表达观察病理表型。

2.2 敲降果蝇内源同源基因

另外, 还可以通过RNA干扰(RNAi)敲降果蝇内源基因制备CMT2疾病模型。通过使用Gal4/UAS系统表达短发夹RNA(shRNA)或长双链RNA(dsRNA), 可以在特定组织或细胞中有效地实现基因敲降(图1A)。目前, 有多个公共果蝇品系中心可以获得大部分果蝇内源基因的UAS-RNAi转基因品系(表2)。

2.3 突变果蝇内源基因成致病突变

临床上发现的遗传性CMT疾病大多数是某个基因特定碱基点突变导致氨基酸发生变化导致的, 可以改造果蝇对应的内源基因模拟人类基因的突变类型, 获得所需CMT果蝇模型。首先, 从氨基酸水平上比对人类与果蝇基因, 找到CMT相关基因突变位置对应果蝇基因的保守性(图1B)。其次, 利用CRISPR/Cas9介导的基因编辑技术修改果蝇内源基因, 本课题组常用CRISPR/Cas9基因编辑介导的同源重组对果蝇内源基因进行修改(图1C)[82-83]。比如, 针对已经发现的*ATP1A1*基因存在的部分CMT2致病突变(例如ATP1A1$^{p.I572T}$、ATP1A1$^{p.A597T}$、ATP1A1$^{p.P600T}$、ATP1A1$^{p.D601F}$)[2], 本课题组在果蝇同源基因*ATPα*中利用基因编辑方法构建了多个基于*ATPα*的CMT2模型(例如ATPα$^{p.I571T}$、ATPα$^{p.A576T}$、ATPα$^{p.P579T}$、ATPα$^{p.D580F}$)。

表1 CMT2型不同分型涉及的基因及这些基因的生物学功能

Table 1 Genes involved in different types of CMT2 and the biological functions of these genes

不同分型或疾病相关基因 OMIM号* Classification and phenotype OMIM number	遗传方式 Inheritance	基因的生物学功能 Biological functions of genes	果蝇同源基因 *Drosophila* homologues	临床相关文献 References
CMT2A1:*KIF1Bβ* (kinesin family member 1B) #118210	AD	A motor protein that transports mitochondria and synaptic vesicle precursors	CG8566 (*unc-104*)	[12-13]
CMT2A2:*MFN2* (mitofusin 2) #609260	AD, AR	A mitochondrial membrane protein that participates in mitochondrial fusion	CG3869 (*Marf*)	[12,14-18]
CMT2B:*RAB7* (member RAS oncogene family) #600882	AD	Endocytic and autophagic vesicle trafficking, maturation, and fusion	CG5915 (*Rab7*)	[19]
CMT2B1:*LMNA* (lamin A/C) #605588	AR	Structural protein components of the nuclear lamina	CG6944 (*Lam*) CG10119 (*LamC*)	[20]
CMT2B2:*PNKP* (polynucleotide kinase 3'-phosphatase) # 605589	AR	Polynucleotide kinase 3'-phosphatase	CG9601	[21]
CMT2C:*TRPV4* (transient receptor potential cation channel subfamily V member 4) # 606071	AD	Cation channels mediate calcium flux	CG5842 (*nan*), CG4536 (*iav*)	[22-29]
CMT2D:*GARS1* (glycyl-tRNA synthetase 1) #601472	AD	Glycyl-tRNA synthetase	CG6778 (*GlyRS*)	[30-32]
CMT2DD:*ATP1A1* (ATPase Na^+/K^+ transporting subunit alpha 1) #618036	AD	Establishes and maintains electrochemical gradients of Na^+ and K^+ across the plasma membrane	CG5670(*Atpα*)	[2,33]
CMT2E:*NEFL* (neurofilament light chain) #607684	AD, AR	Major components of the neuronal cytoskeleton	—	[34-38]
CMT2F:*HSPB1* (heat shock protein family B (small) member 1) #606595	AD, AR	Acts as a molecular chaperone to facilitate proper folding of other proteins	CG4167 (*Hsp67Ba*)	[39-40]
CMT2GG:*GBF1* (golgi brefeldin A resistant guanine nucleotide exchange factor 1) #606483	AD	A guanine nucleotide exchange factor that regulates the recruitment of proteins to membranes by mediating GDP to GTP exchange	CG8487 (*garz*)	[41]
CMT2I/J:*MPZ* (myelin protein zero) #607677	AD	Major structural protein of peripheral myelin	—	[42-44]
CMT2K:*GDAP1* (ganglioside induced differentiation associated protein 1) #607831	AD, AR	Signal transduction pathways during neuronal development	CG4623 (*Gdap1*)	[45-50]
CMT2L:*HSPB8* (heat shock protein family B (small) member 8) #608673	AD	A superfamily of small stress-inducible proteins	—	[40,51]
CMT2M:*DNM2* (dynamin 2) #606482	AD	Involved in clathrin-dependent and -independent endocytosis and intracellular membrane trafficking	CG18102 (*shi*)	[52-55]
CMT2N:*AARS1* (alanyl-tRNA synthetase 1) #613287	AD	Alanyl-tRNA synthetase	—	[56-59]
CMT2O:*DYNC1H1* (dynein cytoplasmic 1 heavy chain 1) # 614228	AD	A large (over 530 kDa) critical subunit of the cytoplasmic dynein complex	CG7507 (*Dhc64C*)	[60]
CMT2P:*LRSAM1* (leucine rich repeat and sterile alpha motif containing 1) #614436	AD, AR	Regulates cell adhesion molecules with ubiquitin ligase activity	—	[61-63]
CMT2Q:*DHTKD1* (dehydrogenase E1 and transketolase domain containing 1) #615025	AD	Involved in the degradation pathway of various amino acids, including lysine	CG1544	[64]

续表1

不同分型或疾病相关基因 OMIM号* Classification and phenotype OMIM number	遗传方式 Inheritance	基因的生物学功能 Biological functions of genes	果蝇同源基因 *Drosophila* homologues	临床相关文献 References
CMT2R:*TRIM2* (tripartite motif containing 2) # 615490	AR	E3 ubiquitin ligase	CG15105 (*tn*), CG10719 (*brat*)	[65-66]
CMT2S:*IGHMBP2* (immunoglobulin mu DNA binding protein 2) #616155	AR	Helicase superfamily member	CG30094	[67-68]
CMT2T:*MME* (membrane metalloendopeptidase) # 617017	AD,AR	Membrane metalloendopeptidase	CG5905 (*Nep1*), CG9761 (*Nep2*)	[69]
CMT2U:*MARS1* (methionyl-tRNA synthetase 1) # 616280	AD	Methionyl-tRNA synthetase	CG15100 (*MetRS*)	[70-71]
CMT2V:*NAGLU* (*N*-acetyl-alpha-glucosaminidase) #616491	AD	Hydrolysis of terminal N-acetyl-D-glucosamine residues in N-acetyl-α-D-glucosamine to degrade heparan sulfate	CG13397	[72]
CMT2W:*HARS1* (histidyl-tRNA synthetase 1) # 616625	AD	Histidyl-tRNA synthetase	CG6335 (*HisRS*)	[73]
CMT2X:*SPG11* (SPG11 vesicle trafficking associated, spatacsin) #616668	AR	Proteins with roles in neuronal axon growth, function, and intracellular cargo transport	CG13531	[74]
CMT2Y:*VCP* (valosin containing protein) #616687	AD	Organelle biogenesis, ubiquitin-dependent protein degradation	CG2331 (*TER94*)	[75-76]
CMT2Z:*MORC2* (MORC family CW-type zinc finger 2) #616688	AD	Chromatin remodeling, DNA repair, and transcriptional regulation	—	[77-80]

*OMIM(Online Mendelian Inheritance in Man): https://www.omim.org/about。AD: 常染色体显性遗传; AR: 常染色体隐性遗传。

*OMIM (Online Mendelian Inheritance in Man): https://www.omim.org/about. AD: autosomal dominant; AR: autosomal recessive.

3 果蝇CMT2模型病理评价指标

根据已经报道的CMT果蝇模型的研究, 目前主要从运动能力、眼部特征、寿命、肌肉神经分析等方面探讨疾病对果蝇的影响。

3.1 眼部特征

GMR-Gal4、*ey*-Gal4果蝇可在眼部特异性驱动UAS下游元件表达。借助这些Gal4在果蝇眼睛中表达致病蛋白或干扰特定基因, 观察果蝇眼睛表型可以评估病理模型(图2A和图2B)。比如研究涉及氨酰tRNA合成酶相关基因的CMT2模型时常用该方法。还可以用特定抗体免疫染色对病理模型果蝇幼虫视盘中的感光细胞轴突投射进行观察(图2C)。

3.2 爬管能力

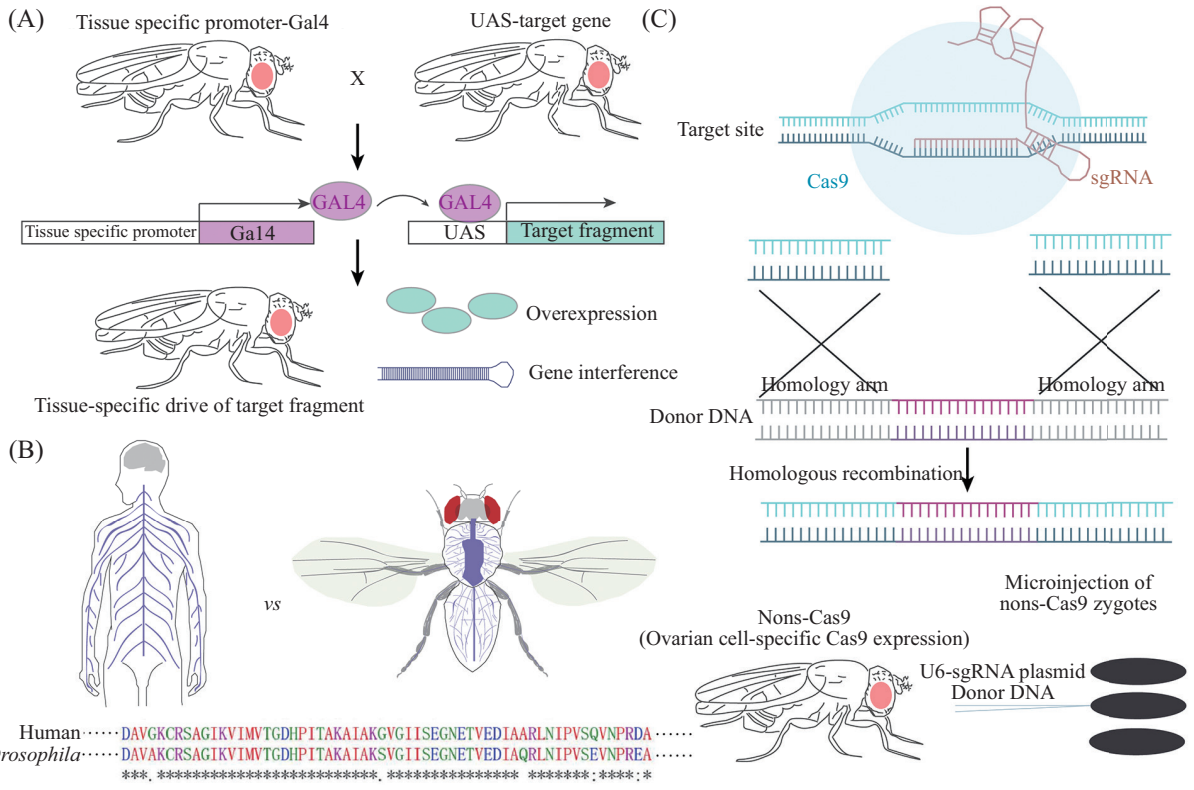
Elav-GAL4、*OK371*-Gal4、*tubulin*-Gal4果蝇可分别以泛神经系统、运动神经元、泛表达的方式驱动UAS下游元件表达。借助特定Gal4在果蝇特定组织中表达致病蛋白或干扰特定基因, 观察其爬管能力, 可以评估病理模型(图2D)。病理果蝇模型常表现出明显的爬管缓慢, 爬管过程中掉落的情况, 证明运动能力受损, 符合CMT2患者远端的肌肉控制不良和远端肌无力等症状。

3.3 寿命

有些病理模型果蝇表现出寿命缩短的表型, 可以用寿命作为病理评价指标之一(图2E)。常用*elav*-Gal4、*tubulin*-Gal4等驱动表达致病蛋白或干扰特定基因, 分析模型果蝇的寿命。

3.4 神经细胞分析

对果蝇神经细胞进行分析, 可以从细胞水平上对病理模型进行评价。比较常用的系统包括幼虫神经肌肉接头(neuromuscular junction, NMJ)、幼虫多树突感觉神经元、成虫巨型纤维等。NMJ是一个已建立的用于研究突触发育和可塑性的模型系统。果蝇幼虫每个半节有30块肌肉, 共有31个运动神经元附着在这些肌肉上。利用*D42*-Gal4驱动UAS-nSyb-



A: 果蝇Gal4/UAS二元表达系统; B: 果蝇和人类同源基因比对; C: CRISPR/Cas9介导的果蝇内源基因修改。

A: *Drosophila* Gal4/UAS binary expression system; B: *Drosophila* and human homologous gene alignment; C: CRISPR/Cas9-mediated endogenous gene modification in *Drosophila*.

图1 CMT2果蝇模型构建方法

Fig.1 Construction of *Drosophila* CMT2 disease models

表2 常用的公共果蝇品系中心

Table 2 Commonly used public *Drosophila* stock centers

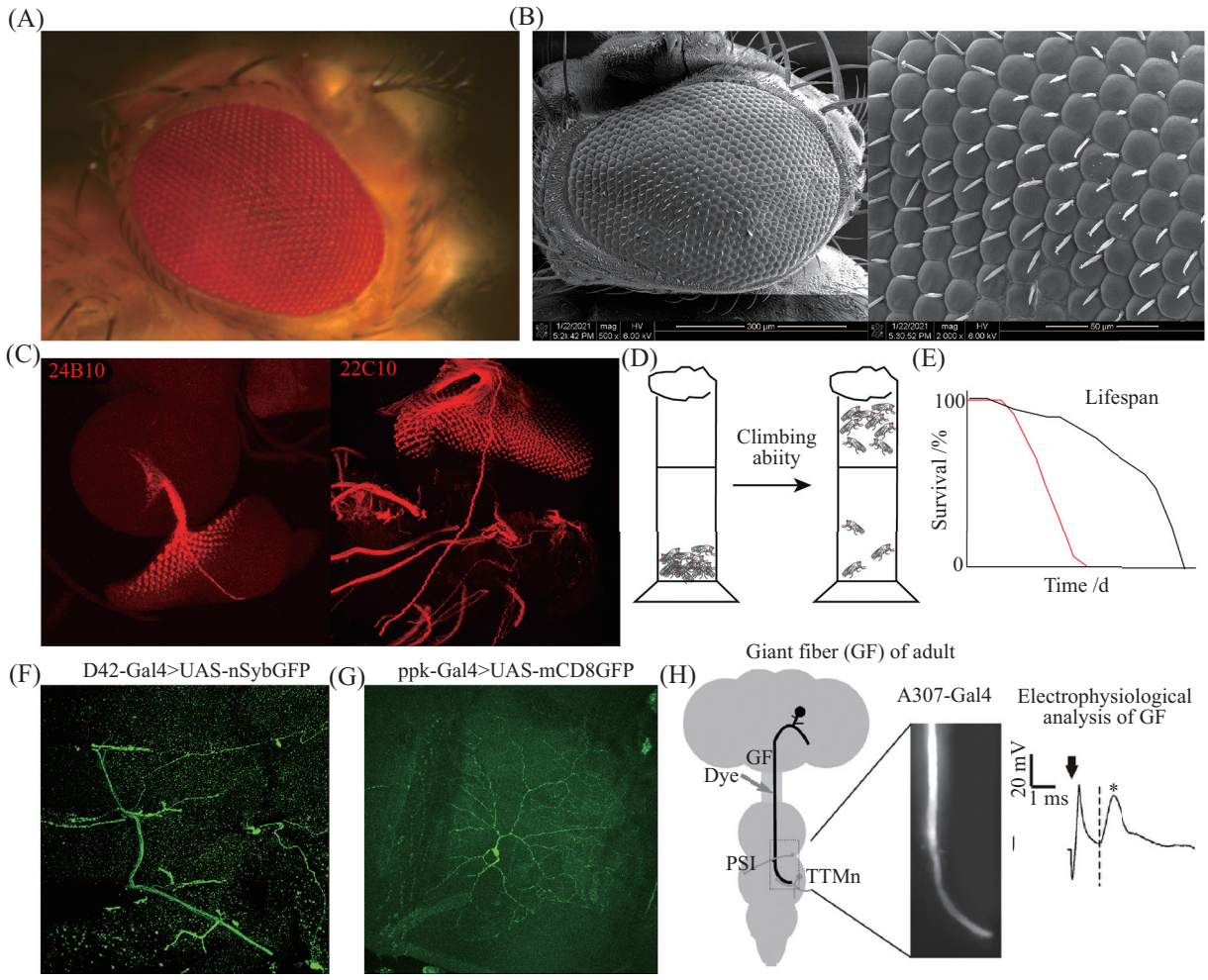
公共果蝇中心 Public *Drosophila* stock centers	网址 Websites	主要品系 Main stocks
Vienna *Drosophila* resource center	https://stockcenter.vdrc.at/control/main	RNAi
TsingHua fly center	https://thfc.zzbd.org/	RNAi
Bloomington *Drosophila* stock center	https://bdsc.indiana.edu/	Multiple *Drosophila* stocks center
Kyoto stock center	https://bdsc.indiana.edu/	Multiple *Drosophila* stocks center

GFP进行荧光标记或利用HRP、CSP、SYT、DLG等抗体进行免疫组织化学染色标记可清晰地对NMJ进行成像(图2F)。比如, 有些病理果蝇模型中可以观察到NMJ运动神经元形态学缺陷, 神经肌肉的突触数量变少, 最长突触分支的长度缩短[84-85]。幼虫体壁中用*ppk*-Gal4驱动UAS-mCD8GFP进行荧光标记可观察到多树突感觉神经元(图2G)。该表型也可用于评估CMT2病理模型, 比如表达GARS的突变体引起了树突状覆盖率显著降低的现象[85]。另外, 有些研究利用成虫巨型纤维进行观察, 成虫巨型纤维是一个特征明确的神经元回路, 可以进行常规的观察或进行电生理记录(图2H)。比如在研究GARS相关的CMT2模型中就应用了该表型的观测[86-87]。模型果蝇巨型纤维电生理功能障碍包括突触强度和可靠性降低, 其特征是单次刺激后输出信号的响应潜伏期较长和/或振幅较小。

4 果蝇已有的CMT2模型

4.1 与线粒体相关的果蝇CMT2模型

线粒体是高度动态的细胞器, 负责细胞活力。



A: 果蝇复眼体式显微镜拍照; B: 果蝇复眼扫描电镜拍照; C: 三龄果蝇幼虫眼盘24B10和22C10抗体染色; D: 果蝇成虫爬管能力检测; E: 果蝇成虫寿命检测; F: 三龄果蝇幼虫肌肉神经检测分析突触长度和数量; G: 三龄果蝇幼虫表皮感觉神经检测分析树枝状覆盖的百分比; H: 果蝇成虫巨形纤维检测(根据参考文献[86]修改)。

A: *Drosophila* compound eye stereomicroscope photography; B: *Drosophila* compound eye scanning electron microscope photography; C: third-instar *Drosophila* larval eye discs stained with 24B10 and 22C10 antibodies; D: detection of the climbing ability of adult *Drosophila*; E: *Drosophila* adult lifespan assay; F: detection of neuromuscular junction in third instar *Drosophila* larvae to quantify the length and number of synapses; G: detection of epidermal sensory nerves in third instar *Drosophila* larvae to quantify the percentage of dendritic coverage; H: *Drosophila* adult giant fiber detection (modified from reference [86]).

图2 果蝇CMT2模型常见病理评价指标

Fig.2 Common pathological evaluation indicators of *Drosophila* CMT2 models

由于线粒体的供能作用, 线粒体受损或减少会对能量供应产生巨大影响, 进而直接影响神经系统工作。线粒体还具有调节细胞凋亡、钙信号和活性氧(reactive oxygen species, ROS)的产生等功能。线粒体不断重复融合和分裂, 以维持其稳态和功能。线粒体异常如何导致CMT2疾病还不完全清楚, 但是几个涉及线粒体功能并与CMT2有关的分子已在果蝇中进行了相关的研究。

线粒体融合由多种GTP酶控制, 包括作用于线粒体外膜的MFN1和MFN2 [88]。MFN2功能异常导致常染色体显性的CMT2A2。ESCHENBACHER等[89]较早在果蝇中研究人类*Mfn2*特定突变, 发现MFN2上特定点突变(M393I和R400Q)具有潜在的破坏性, 这些点突变的MFN2蛋白在果蝇眼中的表达导致眼部面积减少。DEBATTISTI等[90]发现果蝇线粒体装配调节因子(*Marf*)(人*Mfn2*的果蝇同源物)的敲除会导致多个表型: 运动功能障碍(攀爬能力降低), 表达野生型的人MFN2而不是具有R94Q突变(与CMT2A相关的最常见突变之一)的MFN2可以挽救线粒体向轴突远端的转运受损、线粒体分裂和成簇。这

些表型可以重现患者的关键症状。FISSI等[91]在果蝇神经元中表达果蝇MARF模拟多个导致CMT2的MFN2突变(R94Q、R364W、T105M和 L76P), 研究不同MFN2突变对线粒体活性和神经元功能的影响。发现所有的MARF转基因果蝇都引发与神经肌肉接头处线粒体耗竭、氧化代谢减少和线粒体DNA(mtDNA)突变增加相关的运动缺陷, 但它们对线粒体的形态和组织有不同影响[91]。在MARF的GTPase结构域(对应于人MFN2中的R94Q和T105M)内携带突变的果蝇显示未融合和聚集的线粒体[91]。在MARF的螺旋束1结构域(对应于人MFN2中的R364W和L76P)内携带突变的果蝇表现出增强的线粒体融合和巨大线粒体[91]。根据这些结果, 不仅受损的线粒体融合, 线粒体的过度融合也可能是MFN2突变引起CMT的基础, *MFN2*基因突变位点的多样性以及每个MFN2突变引起的MFN2蛋白的各种功能改变可能是导致CMT2A2患者个体差异的原因。

GDAP1存在于线粒体外膜上, 结构类似谷胱甘肽*S*-转移酶(glutathione *S*-transferase, GST)但无GST活性, 功能异常可诱发CMT2K。GDAP1参与线粒体形态和功能的调节, 但仍有许多未知之处。LOPEZ等[92]发现, 敲低果蝇中人类*GDAP1*的同系物*dGDAP1*会导致果蝇眼睛和肌肉退化, 而人类*GDAP1*的表达可以挽救这些表型[92]。敲低和过表达*dGDAP1*均导致果蝇攀爬能力降低, 敲低*dGDAP1*也导致线粒体聚集和肌肉中产生大而长的线粒体。此外, *dGDAP1*的过表达和敲除都会导致胰岛素途径的失活并伴随着碳水化合物的积累和脂质的β-氧化增加[93]。这些基于*dGDAP1*的结果表明, 线粒体功能障碍导致的能量代谢受损可能与神经退行性变化密切相关。

细胞色素c氧化酶组装因子7(cytochrome C oxidase assembly factor 7, COA7)控制负责氧化磷酸化的线粒体呼吸链复合物(mitochondrial respiratory chain, MRC)的组装。虽然在OMIM上, *COA7*与脊髓小脑性共济失调伴轴突性神经病变3(spinocerebellar ataxia with axonal neuropathy type 3, SCAN3)相关联, 但是*COA7*的隐性突变是从1 396例日本CMT或其他遗传性周围神经病患者中发现的。果蝇含有与人类同源的*dCOA7*基因, HIGUCHI等[94]发现, 敲低*dCOA7*的模型显示出粗糙的眼睛表型、寿命缩短、运动能力受损和运动神经元突触分支缩短。

4.2 与氨基酰基-tRNA合成酶相关的果蝇CMT2模型

氨酰-tRNA合成酶是一类参与将氨基酸结合到其对应的tRNA上的酶, 是体内蛋白质翻译合成的重要成分, 多个氨酰-tRNA合成酶功能异常与CMT2疾病密切相关[95]。四种不同的氨酰-tRNA合成酶基因的显性突变会产生CMT2:*GARS*(CMT2D)、*AARS*(CMT2N)、*MARS*(CMT2U)和*HARS*(CMT2W)。两种氨酰-tRNA合成酶基因的显性突变产生DI-CMT:酪氨酰-tRNA合成酶(*YARS*)和赖氨酰-tRNA合成酶(*KARS*)。多个与氨酰-tRNA合成酶相关的果蝇模型已被建立[96]。由氨酰-tRNA合成酶编码基因突变引起的CMT2果蝇模型再现了CMT2患者的临床表型, 如运动功能受损、轴突变性、肌肉去神经支配和突触功能障碍[85-87], 然而在这些模型中, 运动症状的发展并不依赖于氨酰-tRNA合成酶的酶活性。CHIHARA等[97]发现, 果蝇神经元中*GARS*的缺失影响轴突和树突末端树枝状化的精细化和稳定性, 他们还构建研究了*GARS*的两个致病突变(E71G和L219P)在果蝇神经投射中的功能。ERMANOSKA等[87]在果蝇中表达*GARS*的两个致病突变(G240R、P234KY)产生CMT2表型。GRICE等[98]在果蝇中表达*GARS*致病突变(P234KY), 观察其对运动、寿命、肌肉神经等的影响。NIEHUES等[85]报道, 表达*GARS*突变(E71G、G240R、G526R)的果蝇运动能力受损, 表现出外周神经元的整体蛋白质合成减少, 并且蛋白质合成减少不能通过野生型果蝇*GARS*的过表达来挽救。ZHAO等[99]利用果蝇模型研究发现NAD^+-依赖性去乙酰化酶*Sirt2*的敲降能挽救*GARS*引起的CMT2病变并延长寿命。三种*YARS*突变体(G41R、E196K、153-156delVKQV、K265N)在果蝇中过表达都产生了类似CMT2的表型[85-87]。这些*YARS*突变体表达后, 相关的转录调控网络受到干扰, 使用药理学和遗传方法从细胞核中排除突变的YARS可抑制果蝇模型中CMT的标志性表型[100]。关于氨基酰基-tRNA合成酶与CMT相关的最新进展可参考两篇最近的综述文章[101-102]。关于氨基酰基-tRNA合成酶相关的果蝇CMT模型的更详细信息可参考MORANT等[96]撰写的文章。

4.3 与膜运输缺陷相关的果蝇CMT2模型

Rab7是一种参与晚期内涵体成熟的小GTP酶, 其功能异常是CMT2B的病因。JANSSENS等[103]开

发了第一个CMT2B的动物模型, 通过在果蝇中表达*Rab7*的一个突变(L129F)模拟CMT2B。行为分析表明, 该模型模拟了人类疾病的几个特征。在感觉神经元中表达突变的*Rab7*后, 幼虫出现温度和疼痛感知降低[103]。此外, 当突变蛋白在运动神经元中表达时, 幼虫表现出爬行缺陷。对果蝇幼虫感觉神经元中Rab7阳性囊泡轴突运输的分析表明, 表达*Rab7*突变囊泡的停顿少于其野生型对应物[103]。这些发现表明, 囊泡运输的改变可能是CMT2B的重要病理机制。

5 总结与展望

迄今为止, 已有超过100个CMT疾病相关基因被发现。正是由于这些基因的重复缺失或是突变, 导致了分型多样的CMT疾病, 先前被认为占比较低的CMT类型也逐渐被科研人员重视。虽然还没有发现能够完全治疗该疾病的方法, 但是对CMT疾病的研究, 尤其是相关基因和分子机制的研究对治疗该疾病具有重要的现实意义。已鉴定出近30多种与CMT2相关的人类基因, 但是并非所有基因都能在果蝇中找到同源基因(表1)。对存在同源基因的CMT2, 利用果蝇研究相关致病机理, 可以为相关CMT治疗提供思路。对于无果蝇同源基因或同源性比较差的基因, 在小鼠或斑马鱼上进行研究也是一种选择。

果蝇在CMT疾病的研究中扮演了重要的角色, 尤其是在轴突病变类型的研究中表现突出, 在果蝇上通过基因操作得到了与CMT疾病非常相似的表型。可以对寿命长短、运动机能、远端神经缺陷以及粗糙的眼睛表型等进行研究。还可以在诸多方面如: 内体分拣、细胞信号转导、线粒体维护、内质网和高尔基体功能、mRNA的加工、蛋白酶体和蛋白质的降解、离子通道的调节、轴突的转运和突触的传递等进行探讨[104]。果蝇用于CMT2疾病的研究还有很多方面可以进行, 通过这些研究, 我们能够更好地揭示CMT2疾病并为CMT2疾病治疗提供新的解决方案。

参考文献 (References)

[1] FRIDMAN V, BUNDY B, REILLY M M, et al. CMT subtypes and disease burden in patients enrolled in the Inherited Neuropathies Consortium natural history study: a cross-sectional analysis [J]. J Neurol Neurosurg Psychiatry, 2015, 86(8): 873-8.

[2] LASSUTHOVA P, REBELO A P, RAVENSCROFT G, et al. Mutations in ATP1A1 cause dominant Charcot-Marie-Tooth type 2 [J]. Am J Hum Genet, 2018, 102(3): 505-14.

[3] YAMAGUCHI M, TAKASHIMA H. *Drosophila* Charcot-Marie-Tooth disease models [J]. Adv Exp Med Biol, 2018, 1076: 97-117.

[4] XU Y, ZHANG J Y, YANG B Y, et al. Advances in genetic studies of Charcot-Marie-Tooth disease type 4 (CMT4) [J]. Yi Chuan, 2015, 37(6): 501-9.

[5] AHARONI S, BARWICK K E, STRAUSSBERG R, et al. Novel homozygous missense mutation in GAN associated with Charcot-Marie-Tooth disease type 2 in a large consanguineous family from Israel [J]. BMC Med Genet, 2016, 17(1): 82.

[6] BANCHS I, CASASNOVAS C, MONTERO J, et al. Two Spanish families with Charcot-Marie-Tooth type 2A: clinical, electrophysiological and molecular findings [J]. Neuromuscul Disord, 2008, 18(12): 974-8.

[7] NAGAMATSU M, JENKINS R B, SCHAID D J, et al. Hereditary motor and sensory neuropathy type 2C is genetically distinct from types 2B and 2D [J]. Arch Neurol, 2000, 57(5): 669-72.

[8] MOTLEY W W, TALBOT K, FISCHBECK K H. GARS axonopathy: not every neuron's cup of tRNA [J]. Trends Neurosci, 2010, 33(2): 59-66.

[9] HAIDAR M, ASSELBERGH B. Neuropathy-causing mutations in HSPB1 impair autophagy by disturbing the formation of SQSTM1/p62 bodies [J]. Autophagy, 2019, 15(6): 1051-68.

[10] QIN L, YANG C, LU T, et al. Analysis of GDAP1 gene mutation in a pedigree with autosomal dominant Charcot-Marie-Tooth disease [J]. Nan Fang Yi Ke Da Xue Xue Bao, 2019, 39(1): 63-8.

[11] LI F, XIAO H, HU Z, et al. Exploring the multifaceted roles of heat shock protein B8 (HSPB8) in diseases [J]. Eur J Cell Biol, 2018, 97(3): 216-29.

[12] SAITO M, HAYASHI Y, SUZUKI T, et al. Linkage mapping of the gene for Charcot-Marie-Tooth disease type 2 to chromosome 1p (CMT2A) and the clinical features of CMT2A [J]. Neurology, 1997, 49(6): 1630-5.

[13] XU F, TAKAHASHI H, TANAKA Y, et al. KIF1Bbeta mutations detected in hereditary neuropathy impair IGF1R transport and axon growth [J]. J Cell Biol, 2018, 217(10): 3480-96.

[14] ZUCHNER S, MERSIYANOVA I V, MUGLIA M, et al. Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A [J]. Nat Genet, 2004, 36(5): 449-51.

[15] MUGLIA M, ZAPPIA M, TIMMERMAN V, et al. Clinical and genetic study of a large Charcot-Marie-Tooth type 2A family from southern Italy [J]. Neurology, 2001, 56(1): 100-3.

[16] VUCIC S, KENNERSON M, ZHU D, et al. CMT with pyramidal features. Charcot-Marie-Tooth [J]. Neurology, 2003, 60(4): 696-9.

[17] ZHU D, KENNERSON M L, WALIZADA G, et al. Charcot-Marie-Tooth with pyramidal signs is genetically heterogeneous: families with and without MFN2 mutations [J]. Neurology, 2005, 65(3): 496-7.

[18] LAWSON V H, GRAHAM B V, FLANIGAN K M. Clinical and electrophysiologic features of CMT2A with mutations in the mitofusin 2 gene [J]. Neurology, 2005, 65(2): 197-204.

[19] HOULDEN H, KING R H, MUDDLE J R, et al. A novel RAB7 mutation associated with ulcero-mutilating neuropathy [J]. Ann

Neurol, 2004, 56(4): 586-90.

[20] ZHANG R X, GUO P, REN Z J, et al. Mutation analysis of LITAF, RAB7, LMNA and MTMR2 genes in Chinese Charcot-Marie-Tooth disease [J]. Hereditas, 2010, 32(8): 817-23.

[21] LEAL A, BOGANTES-LEDEZMA S, EKICI A B, et al. The polynucleotide kinase 3'-phosphatase gene (PNKP) is involved in Charcot-Marie-Tooth disease (CMT2B2) previously related to MED25 [J]. Neurogenetics, 2018, 19(4): 215-25.

[22] DYCK P J, LITCHY W J, MINNERATH S, et al. Hereditary motor and sensory neuropathy with diaphragm and vocal cord paresis [J]. Ann Neurol, 1994, 35(5): 608-15.

[23] KLEIN C J, CUNNINGHAM J M, ATKINSON E J, et al. The gene for HMSN2C maps to 12q23-24: a region of neuromuscular disorders [J]. Neurology, 2003, 60(7): 1151-6.

[24] CHEN D H, SUL Y, WEISS M, et al. CMT2C with vocal cord paresis associated with short stature and mutations in the TRPV4 gene [J]. Neurology, 2010, 75(22): 1968-75.

[25] DONAGHY M, KENNETT R. Varying occurrence of vocal cord paralysis in a family with autosomal dominant hereditary motor and sensory neuropathy [J]. J Neurol, 1999, 246(7): 552-5.

[26] MCENTAGART M E, REID S L, IRRTHUM A, et al. Confirmation of a hereditary motor and sensory neuropathy IIC locus at chromosome 12q23-q24 [J]. Ann Neurol, 2005, 57(2): 293-7.

[27] LANDOURE G, ZDEBIK A A, MARTINEZ T L, et al. Mutations in TRPV4 cause Charcot-Marie-Tooth disease type 2C [J]. Nat Genet, 2010, 42(2): 170-4.

[28] AHARONI S, HARLALKA G, OFFIAH A, et al. Striking phenotypic variability in familial TRPV4-axonal neuropathy spectrum disorder [J]. Am J Med Genet A, 2011, 155A(12): 3153-6.

[29] LANDOURE G, SULLIVAN J M, JOHNSON J O, et al. Exome sequencing identifies a novel TRPV4 mutation in a CMT2C family [J]. Neurology, 2012, 79(2): 192-4.

[30] IONASESCU V, SEARBY C, SHEFFIELD V C, et al. Autosomal dominant Charcot-Marie-Tooth axonal neuropathy mapped on chromosome 7p (CMT2D) [J]. Hum Mol Genet, 1996, 5(9): 1373-5.

[31] SAMBUUGHIN N, SIVAKUMAR K, SELENGE B, et al. Autosomal dominant distal spinal muscular atrophy type V (dSMA-V) and Charcot-Marie-Tooth disease type 2D (CMT2D) segregate within a single large kindred and map to a refined region on chromosome 7p15 [J]. J Neurol Sci, 1998, 161(1): 23-8.

[32] YALCOUYE A, DIALLO S H, COULIBALY T, et al. A novel mutation in the GARS gene in a Malian family with Charcot-Marie-Tooth disease [J]. Mol Genet Genomic Med, 2019, 7(7): e00782.

[33] HE J, GUO L, LIN S, et al. ATP1A1 mutations cause intermediate Charcot-Marie-Tooth disease [J]. Hum Mutat, 2019, 40(12): 2334-43.

[34] MERSIYANOVA I V, PEREPELOV A V, POLYAKOV A V, et al. A new variant of Charcot-Marie-Tooth disease type 2 is probably the result of a mutation in the neurofilament-light gene [J]. Am J Hum Genet, 2000, 67(1): 37-46.

[35] GEORGIOU D M, ZIDAR J, KOROSEC M, et al. A novel NF-L mutation Pro22Ser is associated with CMT2 in a large Slovenian family [J]. Neurogenetics, 2002, 4(2): 93-6.

[36] FABRIZI G M, CAVALLARO T, ANGIARI C, et al. Giant axon and neurofilament accumulation in Charcot-Marie-Tooth disease type 2E [J]. Neurology, 2004, 62(8): 1429-31.

[37] MILTENBERGER-MILTENYI G, JANECKE A R, WANSCHITZ J V, et al. Clinical and electrophysiological features in Charcot-Marie-Tooth disease with mutations in the NEFL gene [J]. Arch Neurol, 2007, 64(7): 966-70.

[38] AGRAWAL P B, JOSHI M, MARINAKIS N S, et al. Expanding the phenotype associated with the NEFL mutation: neuromuscular disease in a family with overlapping myopathic and neurogenic findings [J]. JAMA Neurol, 2014, 71(11): 1413-20.

[39] KALMAR B, INNES A, WANISCH K, et al. Mitochondrial deficits and abnormal mitochondrial retrograde axonal transport play a role in the pathogenesis of mutant Hsp27-induced Charcot Marie Tooth Disease [J]. Hum Mol Genet, 2017, 26(17): 3313-26.

[40] TANG B S, ZHAO G H, LUO W, et al. Small heat-shock protein 22 mutated in autosomal dominant Charcot-Marie-Tooth disease type 2L [J]. Hum Genet, 2005, 116(3): 222-4.

[41] MENDOZA-FERREIRA N, KARAKAYA M, CENGIZ N, et al. *De Novo* and inherited variants in GBF1 are associated with axonal neuropathy caused by golgi fragmentation [J]. Am J Hum Genet, 2020, 107(4): 763-77.

[42] MARROSU M G, VACCARGIU S, MARROSU G, et al. Charcot-Marie-Tooth disease type 2 associated with mutation of the myelin protein zero gene [J]. Neurology, 1998, 50(5): 1397-401.

[43] SENDEREK J, HERMANNS B, LEHMANN U, et al. Charcot-Marie-Tooth neuropathy type 2 and P0 point mutations: two novel amino acid substitutions (Asp61Gly; Tyr119Cys) and a possible "hotspot" on Thr124Met [J]. Brain Pathol, 2000, 10(2): 235-48.

[44] AUER-GRUMBACH M, STRASSER-FUCHS S, ROBL T, et al. Late onset Charcot-Marie-Tooth 2 syndrome caused by two novel mutations in the MPZ gene [J]. Neurology, 2003, 61(10): 1435-7.

[45] BIROUK N, AZZEDINE H, DUBOURG O, et al. Phenotypical features of a Moroccan family with autosomal recessive Charcot-Marie-Tooth disease associated with the S194X mutation in the GDAP1 gene [J]. Arch Neurol, 2003, 60(4): 598-604.

[46] XIN B, PUFFENBERGER E, NYE L, et al. A novel mutation in the GDAP1 gene is associated with autosomal recessive Charcot-Marie-Tooth disease in an Amish family [J]. Clin Genet, 2008, 74(3): 274-8.

[47] KABZINSKA D, STRUGALSKA-CYNOWSKA H, KOSTERA-PRUSZCZYK A, et al. L239F founder mutation in GDAP1 is associated with a mild Charcot-Marie-Tooth type 4C4 (CMT4C4) phenotype [J]. Neurogenetics, 2010, 11(3): 357-66.

[48] CHUNG K W, KIM S M, SUNWOO I N, et al. A novel GDAP1 Q218E mutation in autosomal dominant Charcot-Marie-Tooth disease [J]. J Hum Genet, 2008, 53(4): 360-4.

[49] CRIMELLA C, TONELLI A, AIROLDI G, et al. The GST domain of GDAP1 is a frequent target of mutations in the dominant form of axonal Charcot Marie Tooth type 2K [J]. J Med Genet, 2010, 47(10): 712-6.

[50] ZIMON M, BAETS J, FABRIZI G M, et al. Dominant GDAP1 mutations cause predominantly mild CMT phenotypes [J]. Neurology, 2011, 77(6): 540-8.

[51] TANG B S, LUO W, XIA K, et al. A new locus for autosomal dominant Charcot-Marie-Tooth disease type 2 (CMT2L) maps to chromosome 12q24 [J]. Hum Genet, 2004, 114(6): 527-33.

[52] KENNERSON M L, ZHU D, GARDNER R J, et al. Dominant intermediate Charcot-Marie-Tooth neuropathy maps to chromosome 19p12-p13.2 [J]. Am J Hum Genet, 2001, 69(4): 883-8.
[53] CLAEYS K G, ZUCHNER S, KENNERSON M, et al. Phenotypic spectrum of dynamin 2 mutations in Charcot-Marie-Tooth neuropathy [J]. Brain, 2009, 132(Pt 7): 1741-52.
[54] FABRIZI G M, FERRARINI M, CAVALLARO T, et al. Two novel mutations in dynamin-2 cause axonal Charcot-Marie-Tooth disease [J]. Neurology, 2007, 69(3): 291-5.
[55] GALLARDO E, CLAEYS K G, NELIS E, et al. Magnetic resonance imaging findings of leg musculature in Charcot-Marie-Tooth disease type 2 due to dynamin 2 mutation [J]. J Neurol, 2008, 255(7): 986-92.
[56] LATOUR P, THAUVIN-ROBINET C, BAUDELET-MERY C, et al. A major determinant for binding and aminoacylation of tRNA(Ala) in cytoplasmic Alanyl-tRNA synthetase is mutated in dominant axonal Charcot-Marie-Tooth disease [J]. Am J Hum Genet, 2010, 86(1): 77-82.
[57] LIN K P, SOONG B W, YANG C C, et al. The mutational spectrum in a cohort of Charcot-Marie-Tooth disease type 2 among the Han Chinese in Taiwan [J]. PLoS One, 2011, 6(12): e29393.
[58] MCLAUGHLIN H M, SAKAGUCHI R, GIBLIN W, et al. A recurrent loss-of-function alanyl-tRNA synthetase (AARS) mutation in patients with Charcot-Marie-Tooth disease type 2N (CMT2N) [J]. Hum Mutat, 2012, 33(1): 244-53.
[59] WETERMAN M A J, KUO M, KENTER S B, et al. Hypermorphic and hypomorphic AARS alleles in patients with CMT2N expand clinical and molecular heterogeneities [J]. Hum Mol Genet, 2018, 27(23): 4036-50.
[60] WEEDON M N, HASTINGS R, CASWELL R, et al. Exome sequencing identifies a DYNC1H1 mutation in a large pedigree with dominant axonal Charcot-Marie-Tooth disease [J]. Am J Hum Genet, 2011, 89(2): 308-12.
[61] GUERNSEY D L, JIANG H, BEDARD K, et al. Mutation in the gene encoding ubiquitin ligase LRSAM1 in patients with Charcot-Marie-Tooth disease [J]. PLoS Genet, 2010, 6(8): e1001081.
[62] WETERMAN M A, SORRENTINO V, KASHER P R, et al. A frameshift mutation in LRSAM1 is responsible for a dominant hereditary polyneuropathy [J]. Hum Mol Genet, 2012, 21(2): 358-70.
[63] NICOLAOU P, CIANCHETTI C, MINAIDOU A, et al. A novel LRSAM1 mutation is associated with autosomal dominant axonal Charcot-Marie-Tooth disease [J]. Eur J Hum Genet, 2013, 21(2): 190-4.
[64] XU W Y, GU M M, SUN L H, et al. A nonsense mutation in DHTKD1 causes Charcot-Marie-Tooth disease type 2 in a large Chinese pedigree [J]. Am J Hum Genet, 2012, 91(6): 1088-94.
[65] YLIKALLIO E, POYHONEN R, ZIMON M, et al. Deficiency of the E3 ubiquitin ligase TRIM2 in early-onset axonal neuropathy [J]. Hum Mol Genet, 2013, 22(15): 2975-83.
[66] PEHLIVAN D, COBAN AKDEMIR Z, KARACA E, et al. Exome sequencing reveals homozygous TRIM2 mutation in a patient with early onset CMT and bilateral vocal cord paralysis [J]. Hum Genet, 2015, 134(6): 671-3.
[67] COTTENIE E, KOCHANSKI A, JORDANOVA A, et al. Truncating and missense mutations in IGHMBP2 cause Charcot-Marie Tooth disease type 2 [J]. Am J Hum Genet, 2014, 95(5): 590-601.
[68] SCHOTTMANN G, JUNGBLUTH H, SCHARA U, et al. Recessive truncating IGHMBP2 mutations presenting as axonal sensorimotor neuropathy [J]. Neurology, 2015, 84(5): 523-31.
[69] HIGUCHI Y, HASHIGUCHI A, YUAN J, et al. Mutations in MME cause an autosomal-recessive Charcot-Marie-Tooth disease type 2 [J]. Ann Neurol, 2016, 79(4): 659-72.
[70] GONZALEZ M, MCLAUGHLIN H, HOULDEN H, et al. Exome sequencing identifies a significant variant in methionyl-tRNA synthetase (MARS) in a family with late-onset CMT2 [J]. J Neurol Neurosurg Psychiatry, 2013, 84(11): 1247-9.
[71] HYUN Y S, PARK H J, HEO S H, et al. Rare variants in methionyl- and tyrosyl-tRNA synthetase genes in late-onset autosomal dominant Charcot-Marie-Tooth neuropathy [J]. Clin Genet, 2014, 86(6): 592-4.
[72] TETREAULT M, GONZALEZ M, DICAIRE M J, et al. Adult-onset painful axonal polyneuropathy caused by a dominant NAGLU mutation [J]. Brain, 2015, 138(Pt 6): 1477-83.
[73] SAFKA BROZKOVA D, DECONINCK T, GRIFFIN L B, et al. Loss of function mutations in HARS cause a spectrum of inherited peripheral neuropathies [J]. Brain, 2015, 138(Pt 8): 2161-72.
[74] MONTECCHIANI C, PEDACE L, LO GIUDICE T, et al. ALS5/SPG11/KIAA1840 mutations cause autosomal recessive axonal Charcot-Marie-Tooth disease [J]. Brain, 2016, 139(Pt 1): 73-85.
[75] GONZALEZ M A, FEELY S M, SPEZIANI F, et al. A novel mutation in VCP causes Charcot-Marie-Tooth Type 2 disease [J]. Brain, 2014, 137(Pt 11): 2897-902.
[76] JERATH N U, CROCKETT C D, MOORE S A, et al. Rare manifestation of a c.290 C>T, p.Gly97Glu VCP mutation [J]. Case Rep Genet, 2015, 2015: 239167.
[77] SEVILLA T, LUPO V, MARTINEZ-RUBIO D, et al. Mutations in the MORC2 gene cause axonal Charcot-Marie-Tooth disease [J]. Brain, 2016, 139(Pt 1): 62-72.
[78] ALBULYM O M, KENNERSON M L, HARMS M B, et al. MORC2 mutations cause axonal Charcot-Marie-Tooth disease with pyramidal signs [J]. Ann Neurol, 2016, 79(3): 419-27.
[79] ANDO M, OKAMOTO Y, YOSHIMURA A, et al. Clinical and mutational spectrum of Charcot-Marie-Tooth disease type 2Z caused by MORC2 variants in Japan [J]. Eur J Neurol, 2017, 24(10): 1274-82.
[80] HYUN Y S, HONG Y B, CHOI B O, et al. Clinico-genetics in Korean Charcot-Marie-Tooth disease type 2Z with MORC2 mutations [J]. Brain, 2016, 139(Pt 7): e40.
[81] LAI S L, LEE T. Genetic mosaic with dual binary transcriptional systems in *Drosophila* [J]. Nat Neurosci, 2006, 9(5): 703-9.
[82] CHEN W, WERDANN M, ZHANG Y. The auxin-inducible degradation system enables conditional PERIOD protein depletion in the nervous system of *Drosophila melanogaster* [J]. FEBS J, 2018, 285(23): 4378-93.
[83] CHEN W, XUE Y, SCARFE L, et al. Loss of prune in circadian cells decreases the amplitude of the circadian locomotor rhythm in *Drosophila* [J]. Front Cell Neurosci, 2019, 13: 76.
[84] KYOTANI A, AZUMA Y, YAMAMOTO I, et al. Knockdown of the *Drosophila* FIG4 induces deficient locomotive behavior, shortening of motor neuron, axonal targeting aberration, reduction of life span and defects in eye development [J]. Exp Neurol, 2016, 277: 86-95.

[85] NIEHUES S, BUSSMANN J, STEFFES G, et al. Impaired protein translation in *Drosophila* models for Charcot-Marie-Tooth neuropathy caused by mutant tRNA synthetases [J]. Nat Commun, 2015, 6: 7520.

[86] STORKEBAUM E, LEITAO-GONCALVES R, GODENSCHWEGE T, et al. Dominant mutations in the tyrosyl-tRNA synthetase gene recapitulate in *Drosophila* features of human Charcot-Marie-Tooth neuropathy [J]. Proc Natl Acad Sci USA, 2009, 106(28): 11782-7.

[87] ERMANOSKA B, MOTLEY W W, LEITAO-GONCALVES R, et al. CMT-associated mutations in glycyl- and tyrosyl-tRNA synthetases exhibit similar pattern of toxicity and share common genetic modifiers in *Drosophila* [J]. Neurobiol Dis, 2014, 68: 180-9.

[88] LARREA D, PERA M, GONELLI A, et al. MFN2 mutations in Charcot-Marie-Tooth disease alter mitochondria-associated ER membrane function but do not impair bioenergetics [J]. Hum Mol Genet, 2019, 28(11): 1782-800.

[89] ESCHENBACHER W H, SONG M, CHEN Y, et al. Two rare human mitofusin 2 mutations alter mitochondrial dynamics and induce retinal and cardiac pathology in *Drosophila* [J]. PLoS One, 2012, 7(9): e44296.

[90] DEBATTISTI V, PENDIN D, ZIVIANI E, et al. Reduction of endoplasmic reticulum stress attenuates the defects caused by *Drosophila* mitofusin depletion [J]. J Cell Biol, 2014, 204(3): 303-12.

[91] EL FISSI N, ROJO M, AOUANE A, et al. Mitofusin gain and loss of function drive pathogenesis in *Drosophila* models of CMT2A neuropathy [J]. EMBO Rep, 2018, 19(8): e45241.

[92] LOPEZ DEL AMO V, SECO-CERVERA M, GARCIA-GIMENEZ J L, et al. Mitochondrial defects and neuromuscular degeneration caused by altered expression of *Drosophila* Gdap1: implications for the Charcot-Marie-Tooth neuropathy [J]. Hum Mol Genet, 2015, 24(1): 21-36.

[93] LOPEZ DEL AMO V, PALOMINO-SCHATZLEIN M, SECO-CERVERA M, et al. A *Drosophila* model of GDAP1 function reveals the involvement of insulin signalling in the mitochondria-dependent neuromuscular degeneration [J]. Biochim Biophys Acta Mol Basis Dis, 2017, 1863(3): 801-9.

[94] HIGUCHI Y, OKUNUSHI R, HARA T, et al. Mutations in COA7 cause spinocerebellar ataxia with axonal neuropathy [J]. Brain, 2018, 141(6): 1622-36.

[95] FUCHS S A, SCHENE I F, KOK G, et al. Aminoacyl-tRNA synthetase deficiencies in search of common themes [J]. Genet Med, 2019, 21(2): 319-30.

[96] MORANT L, ERFURTH M L, JORDANOVA A. *Drosophila* models for Charcot-Marie-Tooth nneuropathy related to aminoacyl-tRNA synthetases [J]. Genes, 2021, 12(10): 1519.

[97] CHIHARA T, LUGINBUHL D, LUO L. Cytoplasmic and mitochondrial protein translation in axonal and dendritic terminal arborization [J]. Nat Neurosci, 2007, 10(7): 828-37.

[98] GRICE S J, SLEIGH J N, MOTLEY W W, et al. Dominant, toxic gain-of-function mutations in gars lead to non-cell autonomous neuropathology [J]. Hum Mol Genet, 2015, 24(15): 4397-406.

[99] ZHAO Y, XIE L, SHEN C, et al. SIRT2-knockdown rescues GARS-induced Charcot-Marie-Tooth neuropathy [J]. Aging Cell, 2021, 20(6): e13391.

[100] BERVOETS S, WEI N, ERFURTH M L, et al. Transcriptional dysregulation by a nucleus-localized aminoacyl-tRNA synthetase associated with Charcot-Marie-Tooth neuropathy [J]. Nat Commun, 2019, 10(1): 5045.

[101] WEI N, ZHANG Q, YANG X L. Neurodegenerative Charcot-Marie-Tooth disease as a case study to decipher novel functions of aminoacyl-tRNA synthetases [J]. J Biol Chem, 2019, 294(14): 5321-39.

[102] ZHANG H, ZHOU Z W, SUN L. Aminoacyl-tRNA synthetases in Charcot-Marie-Tooth disease: a gain or a loss [J]? J Neurochem, 2021, 157(3): 351-69.

[103] JANSSENS K, GOETHALS S, ATKINSON D, et al. Human Rab7 mutation mimics features of Charcot-Marie-Tooth neuropathy type 2B in *Drosophila* [J]. Neurobiol Dis, 2014, 65: 211-9.

[104] KITANI-MORII F, NOTO Y I. Recent advances in *Drosophila* models of Charcot-Marie-Tooth disease [J]. Int J Mol Sci, 2020, 21(19): 7419.