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Animal and cellular models of familial dysautonomia

Frances Lefcort^{1,*}, Marc Mergy¹, Sarah B. Ohlen¹, Yumi Ueki¹, and Lynn George²

¹Department of Cell Biology and Neuroscience, Montana State University, Bozeman, MT 59717

²Department of Biological and Physical Sciences, Montana State University Billings, Billings, MT 59101

Abstract

Since Riley and Day first described the clinical phenotype of patients with familial dysautonomia (FD) over 60 years ago, the field has made considerable progress clinically, scientifically, and translationally in treating and understanding the etiology of FD. FD is classified as a hereditary sensory and autonomic neuropathy (HSAN Type III) and is both a developmental and a progressive neurodegenerative condition that results from an autosomal recessive mutation in the gene *IKBKAP*, also known as *ELP1*. FD primarily impacts the peripheral nervous system but also manifests in central nervous system disruption, especially in the retina and optic nerve. While the disease is rare, the rapid progress being made in elucidating the molecular and cellular mechanisms mediating the demise of neurons in FD should provide insight into degenerative pathways common to many neurological disorders. Interestingly, the protein encoded by *IKBKAP/ELP1*, IKAP or ELP1, is a key scaffolding subunit of the six-subunit Elongator complex, and variants in other Elongator genes are associated with amyotrophic lateral sclerosis (ALS), intellectual disability, and Rolandic Epilepsy. Here we review the recent model systems that are revealing the molecular and cellular pathophysiological mechanisms mediating FD. These powerful model systems can now be used to test targeted therapeutics for mitigating neuronal loss in FD and potentially other disorders.

Introduction

Familial dysautonomia (FD; OMIM 223900; also called Riley-Day Syndrome) is classified as one of the hereditary sensory and autonomic neuropathies (HSAN Type III) and afflicts Jews of eastern European, Ashkenazi, descent; 1:18 – 1:32 Ashkenazi Jews are carriers of the mutant haplotype [1, 2]. Its hallmark features are the consequence of a depletion of sensory and autonomic neurons, which manifests as reduced pain and temperature sensation, cardiovascular instability, gastrointestinal incoordination, respiratory dysfunction and frequent autonomic “crises” marked by vomiting, nausea, hypertension, tachycardia, and blotching – the consequences of unrestrained release of catecholamines in plasma [1, 3, 4]. With close supervision, now more than 50% of patients can reach age 30 [4] with the most common cause of death being sudden death during sleep. FD is both a developmental and a progressive neurodegenerative disease, and although considered an HSAN, its pathology is

*Corresponding author: Frances Lefcort, Ph.D, Department of Cell Biology and Neuroscience, Montana State University, Bozeman, MT 59717, Tel: 406.994.5656, lefcort@montana.edu, ORCID number: 0000-0002-2064-8678.

not restricted to the peripheral nervous system (PNS). In fact, one of the most debilitating hallmarks of the disease as patients age is blindness, as a consequence of progressive death of retinal ganglion cells [5–7]. In addition, the vast majority of patients develop scoliosis (in particular, kyphoscoliosis), and tend to have difficulty gaining and maintaining weight [1].

FD results from a point mutation in the *IKBKAP/ELP1* gene with over 99% of cases due to the same 6T>C substitution in the donor splice site in intron 20 [1, 8]. Exon 20 is variably deleted from the FD *IKBKAP* mRNA, according to tissue type, and is ultimately degraded by nonsense-mediated decay [8–10]. Although *IKBKAP/ELP1* is widely expressed throughout the body, the nervous system is least capable of correctly splicing the mutant pre-mRNA and hence is the most impaired system in FD [8].

IKBKAP/ELP1 encodes the protein IKAP, which is homologous to the protein that is encoded by the yeast *Elp1* gene. IKAP is the scaffolding subunit of the six subunit Elongator complex which is required for chemical modification of tRNAs [11, 12]; its absence in yeast and the nematode *Caenorhabditis elegans* (*C. elegans*), abrogates translation which induces ribosome pausing [13], protein aggregation and misfolding [14]. This essential function of the Elongator complex in the addition of 5-methoxycarbonylmethyl (mcm⁵) and 5-carbamoylmethyl (ncm⁵) moieties to wobble uridines within the tRNA anti-codon loop has been corroborated in mouse cells and importantly, has been shown to be deficient in human FD cells [15–17]. The Elongator complex has also been shown to be required in the nucleus for normal transcript elongation [18, 19] and to negatively regulate exocytosis in yeast and *C.elegans*, and is required for vesicle trafficking, cytoskeleton stability and dynamics [20–26], and neuronal survival [27–29]. Interestingly, IKAP is just one of six subunits of the Elongator complex and recently it has become clear that several of the other Elongator genes are also associated with neurological disorders: *ELP3* with amyotrophic lateral sclerosis, *ELP2* and *ELP4* with intellectual disability and *ELP4* with Rolandic epilepsy [30–36].

How one complex can exert such profound effects on multiple functions in various cell types has recently stimulated an exciting series of studies, that have contributed significantly to our understanding of the molecular, cellular and physiological mechanisms that underlie FD, and identify the critical role of the Elongator complex in the nervous system. IKAP/ELP1 has been investigated in numerous animal and cellular models. In light of the several excellent comprehensive review articles that have been published recently on FD, *IKBKAP* and the Elongator complex [1, 4, 37], the goal of this review is to summarize what the past 15 years of animal and cellular model studies of FD have taught us about the normal function of IKAP and how its reduction in FD disrupts cellular function to ultimately manifest in the clinical symptomatology of FD.

Non-mammalian models of Familial Dysautonomia

Loss of function studies on *Ikbkap* have been conducted in several species including yeast, *C. elegans*, chick, zebrafish, *Xenopus*, *Drosophila* and mice, and they complement and build on each other's findings. Studies in *C.elegans*, zebrafish, chick and *Drosophila* all show a requirement for IKAP and/or the Elongator complex in normal axonal morphology, with reductions in *Ikbkap/Elp1* causing abnormal branching [28, 38, 39], reduced vesicle

movement along microtubules, disrupted target innervation [38, 40], and compromised c-Jun N-terminal Kinase (JNK) and Nerve Growth Factor (NGF) signaling [38]. In addition, mutants lacking *Ikbkap/Elp1* and/or the Elongator complex had abnormal synaptic function [40], larval development [40–42] and learning impairment [41]. Gastrointestinal (GI) problems plague the majority of FD patients, and the investigation of GI function in the absence of *Ikbkap* has been done solely in zebrafish. Antisense RNA reduction of *Ikbkap/Elp1* led to a significant reduction in enteric neurons including complete agangliogenesis of the distal intestine in zebrafish embryos [39]. These findings are analogous to the human FD gastroenteropathy which most likely results from reduced numbers of enteric neurons [43]. While trunk neural crest migration in chick and mouse does not require *Ikbkap* [27–29], reduction of *Elp3* in *Xenopus* impairs neural crest migration [44]. Whether IKAP has functions independent from its fellow Elongator complex subunits remains an important question.

Mouse models of Familial Dysautonomia

Several attempts have been made to generate a useful mouse model for FD. Hims et al. first generated a humanized FD mouse using a bacterial artificial chromosome (BAC) to deliver the full human *IKBKAP* gene containing the human exon 20 splice site mutation (IVS20+T→C) into the mouse genome [45]. They demonstrated that the human FD *IKBKAP* gene is incorrectly spliced in the mouse in a tissue-specific manner that mirrors the splicing pattern observed in human FD patients, with neurons being the least capable of overcoming the splicing defect. This humanized FD mouse model did not manifest any FD symptoms and is phenotypically normal due to the fact that it expresses normal levels of endogenous mouse IKAP. A similar approach was taken by Bochner et al. [46] in which they generated a knock-in homozygous humanized mouse line by swapping the human exon 20 and its two flanking introns with the 3' intron having the same mutation that occurs in the FD *IKBKAP* gene for the endogenous mouse exon 20 and flanking intron sequences. Although this mouse exhibited some degree of tissue-specific skipping of the mutant human exon 20, this occurred primarily in the liver rather than in the nervous system, and consequently, this line did not express any FD phenotype. However, both of these models provide powerful systems for testing therapeutics that can modify splicing of the human *IKBKAP* gene.

Chen et al. subsequently utilized a knock-out (KO) approach to create a mouse model with FD characteristics [47]. Unfortunately, complete deletion of mouse *Ikbkap* results in early embryonic lethality. Analysis of *Ikbkap* null embryos revealed abnormal vasculature development, arrested embryonic development at the mid-gastrulation stage, and death between E10.5 – E12.5. Chromatin immunoprecipitation assays of KO embryos found that the loss of IKAP protein expression resulted in a loss of histone acetylation and transcriptional elongation. In an effort to rescue the embryonic lethality, the human *IKBKAP* gene was introduced into the mouse *Ikbkap* KO background; the resulting pups were viable and appeared to be phenotypically normal. However, introducing the FD version of human *IKBKAP* failed to rescue the embryonic lethality seen in the KO mouse. The authors speculate that there is a tissue-specific threshold of IKAP protein expression required for survival and it is not completely surprising that a full KO produces a non-viable mouse.

Since the major haplotype FD mutation results in the aberrant exclusion of exon 20 from the *IKBKAP* mRNA, Dietrich et al. created two lines of mice – one resulting in loss of *Ikbkap* expression, and one resulting in expression of *Ikbkap* mRNA that lacked exon 20 (*Ikbkap*^{-/-} mice) [48]. In both cases, however, embryo development is delayed and ultimately fails, including in the heart and the brain, leading to the conclusion that the embryos are likely dying from heart failure. In the brain, development of the diencephalon, midbrain, and hindbrain are grossly normal, suggesting that IKAP expression is not required for neural tube closure and formation of the neuroepithelium. However, development of the cerebral cortex was impaired, demonstrating a role for IKAP in forebrain development. Genetic analysis of the *Ikbkap*^{-/-} mice revealed a down-regulation of genes in the *Nkx2-5* homeobox transcription factor network, and a reduction in Transforming Growth Factor (TGF)β signaling components. Although the *Ikbkap*^{-/-} mice are not a useful model given the early embryonic lethality, this model did demonstrate that an *Ikbkap* mRNA lacking exon 20 retains no biological activity.

Dietrich et al. then generated mice harboring a loxP-flanked *Ikbkap* gene locus (*Ikbkap*^{flox/flox}) that results in a 90% reduction in IKAP protein expression, as well as a compound heterozygote mouse that combines the floxed and *Ikbkap*^{-/-} alleles (*Ikbkap*^{20/flox}) [49]. The compound heterozygote model has a 95% reduction in IKAP protein levels. However, in contrast to previous attempts, both the *Ikbkap*^{flox/flox} and *Ikbkap*^{20/flox} were viable, albeit they required special care in order to survive. Both lines of mice recapitulate a number of key features of FD, including small stature, ataxic gait, loss of fungiform papillae, loss of pain and temperature sensation, kyphosis or kyphoscoliosis, progressive eye and bladder pathology, and reduced lifespan. Furthermore, *Ikbkap*^{20/flox} embryos have smaller dorsal root ganglia (DRG), while *Ikbkap*^{flox/flox} embryos have normal sized DRG. This finding suggests that a small increase in IKAP protein expression – from 5% in *Ikbkap*^{20/flox} to 10% in *Ikbkap*^{flox/flox} – is sufficient to rescue the embryonic development of DRG neurons. In sympathetic ganglia, neurons are morphologically normal, but overall ganglia size and neuron number is reduced and are lost progressively as animals age. These data indicate that IKAP not only has a role in embryonic development, but is also needed for maintaining neurons throughout life. This demonstrates that postnatal reintroduction of *IKBKAP* expression may have clinical value for mitigating progressive FD symptoms.

In FD patients, inclusion of exon 20 varies with tissue type, with neurons least capable of generating normal transcripts. Consequently, IKAP levels are most compromised in the nervous system. The possibility that a small shift in the balance of splicing could raise neuronal IKAP levels above a threshold level where therapeutic benefit would be achieved prompted generation of the *TgFD9; Ikbkap*^{20/flox} mouse [50]. In this FD model, the complete human *IKBKAP* transgene with the major FD splice mutation (*TgFD9*) was introduced into the *Ikbkap*^{20/flox} mouse. This combination transgenic mouse produces mouse *Ikbkap* mRNA lacking exon 20 from the *Ikbkap*^{20/flox} allele, extremely low levels of mouse wild type (WT) *Ikbkap* mRNA from the flox allele, human *IKBKAP* mRNA lacking exon 20 from the *FD9* transgene, and tissue specific expression of correctly spliced human WT *IKBKAP* mRNA also from the *FD9* transgene, with brain and DRG expressing only minimal levels of the functional human transcript. As such, the *TgFD9; Ikbkap*^{20/flox}

mouse most closely models the tissue-specific mis-splicing defect seen in FD patients and also recapitulates many phenotypic features of the human disease and provides a useful model for testing compounds that could restore normal splicing.

An alternative approach for generating mouse models of FD are conditional knock-out (CKO) mice, in which the *Ikbkap* gene is selectively deleted from specific cell types at specific time points using *Cre-lox* mediated recombination. These models differ from FD in that (1) complete ablation of gene expression can be achieved in the target tissue, while in the human disease, cells express reduced levels of IKAP protein, and (2) given the selective, cell type-specific deletion of *Ikbkap*, the *in vivo* FD physiological environment is not replicated. However, generating CKO mouse models for major neurological diseases has proven extremely powerful for identifying molecular, cellular and pathophysiological mechanisms that mediate those disorders and they provide spatial and temporal control of gene deletion [51]. Furthermore, these models have successfully recapitulated key hallmarks of human FD including the selective death of pain and temperature afferent DRG neurons, sympathetic neurons and retinal ganglion cells, and as such, provide well defined systems for deducing the function of IKAP and for testing therapeutics that can mitigate the loss of specific cell populations.

Since the PNS is the system most severely impacted in FD, and as the PNS derives from the trunk region of the neural crest, it had been speculated that FD results from faulty neural crest migration [52, 53]. To test this hypothesis, two groups selectively deleted *Ikbkap* from the neural crest by constructing *Wnt1-Cre; Ikbkap^{LoxP/LoxP}* CKOs with *LoxP* sites flanking either Exon 4 [27] or Exon 20 [29]. In both systems, *Ikbkap* expression was specifically ablated in neural crest derivatives of the PNS, and CKO mice were born alive but died within 24 hours of birth. George et al. showed that IKAP is expressed throughout neurogenesis in both progenitor cells and neurons, and that IKAP-deficient trunk neural crest cells migrate and colonize their derivatives normally. This study also showed that a p53-mediated, activated Caspase-3 loss of neuronal progenitors and post-mitotic neurons results in significantly depleted numbers of tropomyosin-related kinase A (TrkA+) DRG neurons by E12.5. Interestingly, this work demonstrated that the first wave of neurogenesis in the DRG that generates the large diameter TrkC+ proprioceptive neurons is not dependent on *Ikbkap*, while the second wave in which the small diameter TrkA+ pain and temperature sensory neurons are born, does require *Ikbkap*. In contrast, Jackson et al. saw normal numbers of neurons, proliferating cells, and apoptotic cells in the DRG at E12.5, and instead, found diminished numbers of TrkA+ and TrkB+ neurons in newborn mice and attributed this loss to impairment in target tissue innervation. This same study also examined *DβH-iCre; Ikbkap^{LoxP/LoxP}* mice, in which *Ikbkap* expression is selectively ablated in lineage-specified sympathetic neuroblasts, and again found diminished target tissue innervation.

Naftelberg et al., 2016, used a *Tyrp2-Cre* driver to remove exon 20 from a humanized *IKBKAP* knock-in mouse. In *IKBKAPCKO^{Tyrp2}* offspring, Cre-mediated recombination becomes active at E12.5 in select tissues including the DRG. DRG cultures from these mice showed irregular neurite outgrowth and branching, and impaired Nerve growth factor (NGF) retrograde transport compared to DRG cultures from control littermates. This reduction may have been due to decreased acetylated alpha-tubulin levels, which they attribute to increased

levels of the histone deacetylase HDAC6. Addition of phosphatidylserine to cultured CKO *Tryp2* DRG explants improved neurite outgrowth and enhanced NGF transport in neurons that were capable of generating full-length IKAP. Reduction in retrograde transport of NGF was also reported in *Ikbkap* CKO sympathetic neurons [26]. IKAP has been reported to be expressed in a vesicular pattern in axons [26, 38] and to co-localize with proteins that mediate retrograde transport including tubulin, dynein and Rab7. Thus, given that both thermoreceptive and nociceptive sensory neurons and sympathetic neurons, three of the most severely affected cell types in FD, are heavily dependent on the retrograde transport of NGF for their survival during development, these intriguing findings warrant further study.

Using a pan-neuronal *Cre* to delete *Ikbkap*, Chaverra et al., 2017, generated a *Tuba1a-Cre; Ikbkap^{LoxP/LoxP}* CKO mouse in which *Ikbkap* is deleted in both the central nervous system (CNS) and PNS beginning in early development (approximately E12.5) [54]. Since CNS pathology has been documented in FD [55, 56] and because mutations in other Elongator subunits (*ELP2-4*) are associated with CNS disorders, the goal of this study was to investigate a potential CNS requirement for *Ikbkap*. Using an *Ikbkap* reporter, the authors showed that *Ikbkap* is expressed throughout the CNS both during development and in the adult. During embryogenesis, robust *Ikbkap* expression was detected in the ventricular zone of the spinal cord and in spinal motor neurons, as well as in the mitotic and post-mitotic zones of the developing cortex and in the choroid plexus. In the adult, *Ikbkap* was expressed throughout the brain, including the medulla, cortex, amygdala, hippocampus and hypothalamus. Using the *Tuba1a-Cre* CKO mouse, the authors show that deletion of *Ikbkap* in the CNS perturbs both the development and survival of cortical and spinal motor neurons. In addition, they show that *Ikbkap* is required for the maintenance of innervation of the neuromuscular junction, and for normal number and organization of primary cilia in embryonic cortical apical progenitors and motile cilia in adult ependymal cells. Loss of IKAP in the CNS ultimately manifests in a progressive neurodegenerative condition that includes kyphoscoliosis, an FD hallmark, as well as significant impairments in behavior including learning and memory. The former observation suggests that the deletion of *Ikbkap* solely in neurons is sufficient to trigger the pathophysiological processes underlying the kyphoscoliosis, most likely due to the progressive denervation of paraspinal muscles.

A major disability that marks older FD patients is a progressive loss of vision that ultimately culminates in blindness. Since *Ikbkap* is deleted from the majority of CNS neurons in *Tuba1a-Cre; Ikbkap^{LoxP/LoxP}* mice, Ueki et al. (2016) [57] investigated the development and survival of neurons in the retina in this strain. They demonstrated that while *Ikbkap* is not required for normal development of retinal ganglion cells (RGCs), its loss causes a slow, progressive RGC degeneration that is most severe in the temporal retina, recapitulating the human cell type and pattern of retina degeneration [5]. Further evidence for the validity of this model system includes the finding that the melanopsin RGCs were resistant to death as has also been found in FD patients [7]. The RGC degeneration was later followed by indirect photoreceptor loss and complete retinal disorganization. However, in this model, *Ikbkap* was deleted from the majority of CNS and some PNS neurons; consequently, whether all or some of these retinal phenotypes are directly due to the retinal-loss of IKAP is not clear. Work on

a retina-specific *Ikbkap* CKO is in progress to investigate the retinal cell types that autonomously depend on *Ikbkap*.

Importantly, retinal ganglion cell degeneration was more pronounced in the more metabolically-taxed temporal retina than in the less metabolically active nasal retina in both FD patients and in the mouse model described above. This pattern of degeneration is a classic hallmark of the major optic neuropathies, Leber's hereditary optic neuropathy and Dominant Optic Atrophy, which are both caused by mutations in mitochondrial genes [58]. FD patients also suffer frequently from rhabdomyolysis, which can result from mitochondrial-induced myopathy [59]. These findings provoked a detailed investigation of mitochondrial function in DRG neurons from *Wnt1-cre;Ikbkap^{LoxP/LoxP}* mice [60], which revealed that mitochondria in *Ikbkap* depleted DRG neurons were depolarized, fragmented and released elevated levels of reactive oxygen species (ROS) compared to their control littermate DRG neurons. Furthermore, this study also showed that the small hydroxylamine, BGP-15 which has been shown in numerous studies to be cytoprotective by exerting anti-oxidative damage effects, could not only significantly improve mitochondrial function in the CKO neurons, but could also prevent their death both *in vitro* and *in vivo* [60]. These findings and those from several other of the studies described here demonstrate not only the power of mouse models to provide the necessary cell types for elucidation of the pathophysiological mechanisms that cause the FD phenotype, but also their effectiveness as a readily accessible and defined system for testing potential therapeutics.

In summary, the diverse strategies for generating mouse models of FD have contributed significantly to our understanding of the spatial and temporal requirements for IKAP both during development and in the adult. From these and other studies we have learned that in the absence of IKAP, both CNS and PNS progenitors behave abnormally, that neurons undergo apoptosis both in the developing and adult PNS and CNS and suffer from intracellular stress, that targets are poorly innervated, and that this impaired innervation may be caused and/or exacerbated by diminished retrograde transport of key survival factors such as NGF. These developmental abnormalities could underlie the dysfunctional regulation of the adult sensory, autonomic, central and enteric nervous systems. These models may even provide an explanation for the FD kyphoscoliosis since it can be recapitulated with a neuronal-specific deletion of *Ikbkap* in the nervous system, identifying a potential target tissue for therapeutics. Finally, these systems provide heuristic models for generating and multiplexing large data sets from "omic" studies that can form the basis for therapeutic screens. In fact, several of these model systems are already proving invaluable for testing potential therapeutics both *in vitro* and *in vivo*, that can mitigate the progressive visual impairment and hopefully rescue additional neuronal cell populations.

Stem cell models of Familial Dysautonomia

An alternative approach to genetically-modified mice for investigating FD, is to generate stem cell models. Due to their pluripotent nature, stem cells can be differentiated into neuronal cells, thereby providing a powerful system for modeling human diseases *in vitro*. FD human embryonic stem cell (hESC) or induced pluripotent stem cell (iPSC)-derived peripheral nervous system progenitor cells (neural crest) and neurons have been generated

and can serve as a potential tool to investigate the molecular mechanisms mediating the demise of neurons in FD [24, 61–64]. In addition, the feasibility of large-scale small molecule screening using FD-iPSCs [62] has facilitated the discovery of potential therapeutic compounds that can increase splicing of the mutated *IKBKAP* mRNA in FD-patient iPSC-derived neural crest cells, and increase expression of genes that regulate the differentiation of sympathetic neurons such as *ASCL1* and *SCG10*.

A major unresolved clinical conundrum in FD, is the variation in the disease phenotypic severity amongst patients, in spite of the fact that they share the identical genetic point mutation. Given that patient cells are mosaic in their ability to successfully splice the mutant *IKBKAP* transcript, it has been postulated that disease severity might be the consequence of, and correlate with, varying levels of IKAP protein production. As described above, one mouse model study did find that slight increases in IKAP protein expression could ameliorate the disease phenotype [49]. In fact, a recent study reported that patient iPSC-derived neurons may provide a powerful model system for investigating the mechanisms mediating variation in disease severity [64]. This study showed that neural crest precursor cells and peripheral sensory neurons derived from iPSCs from more severely affected FD patients compared to more mildly affected FD patients, did not differ significantly in their ratio of mutant *IKBKAP* mRNA to wild type *IKBKAP* mRNA, however they did differ in their level of IKAP protein, with severely affected FD patient-derived neural crest precursor cells expressing lower IKAP levels than those of mildly-affected patients. In addition, differentiation into sensory and sympathetic neurons was reduced in the severely-affected patient iPSCs compared to the mildly-affected patient iPSCs, as was neuronal survival. Whole exome sequencing of three severe vs. three mildly affected patient cell lines revealed secondary mutations in the severely-affected patient cell lines not present in the mildly-affected lines, suggesting the presence of genetic modifiers that regulate the severity of a patient's FD phenotype.

Other types of stem cells such as human olfactory ecto-mesenchymal stem cells (hOR-MSCs) from FD patients, have also been used to generate a useful FD model. hOR-MSCs can be obtained through patient biopsy, are pluripotent, and express stem cell, neuronal, and glial markers [10]. Genome-wide analysis (both mRNA and microRNA screening) using FD hOR-MSCs was performed to investigate dysregulated genes in FD and to test potential therapeutics [10, 65, 66]. Recent work with these FD and control hOR-MSCs indicated over-activity of the 26S proteasome in patient cells compared to controls, which lowered the level of IKAP protein, and that this reduction could be reversed by proteasome inhibitors [67]. Since some proteasome inhibitors have been clinically approved for cancer treatment, this study warrants further investigation. Finally, a method to directly convert fibroblasts into neurons has been developed [68], providing another mechanism for induction of neuronal (iN) cells that can model neurological diseases. Using defined transcription factors, Wainger *et al.* reprogrammed FD patient fibroblasts into functional nociceptor neurons with native subtype diversity [69]. This direct conversion method could be useful for analyzing the consequences of the *IKBKAP* mutation in a specific neuronal type *in vitro*. Obvious challenges of stem cell models include modeling complicated *in vivo* cellular networks, which is necessary to assess safety and dose of a potential therapeutic compound as well as to determine their ability to cross the blood-brain-barrier. Nonetheless, stem cell models of

FD provide an unlimited number of FD patient-derived neurons that are necessary for high-throughput studies.

Conclusion

While all of these models provide a framework and a tool kit for determining the function and requirement for *IKBKAP* in the developing and adult nervous system, the pathophysiological mechanisms that ultimately cause some of the major debilitating clinical autonomic symptoms of FD have not yet been investigated in animal models. Slice preparations from various levels of the neuraxis can be generated and recorded from electrophysiologically to investigate the integrity of key neural circuits that mediate clinical sensory and autonomic hallmarks of FD. One outstanding question is the physiological mechanisms mediating the erratic, elevated levels of dopamine in FD patient plasma that trigger the vomiting crises during “autonomic storms” [70]. Studies in yeast have shown that IKAP negatively regulates exocytosis [20], and evidence has been provided for IKAP localization to synaptic vesicles [24] raising the testable question of whether aberrant regulation of synaptic transmission may explain some of the autonomic dysfunction in FD. Another question that can be addressed with these animal and cellular models are the underlying metabolic problems experienced by FD patients and recapitulated in mice: patients have a significantly decreased body mass index (BMI), and multiple studies on FD mouse models report weight reductions of 60–70% in mutant mice with an absence of white adipose tissue. Is this due to impaired mitochondrial function and/or dysfunctions in the GI tract and associated gut dysbiosis, and/or hypothalamus-pituitary-adrenal axis? These questions call out for physiological investigations of the functionality of the autonomic nervous system in mouse models in order to identify key steps for potential therapeutic intervention. Outside of the autonomic nervous system, the sensory nervous system needs to be more thoroughly investigated. For example, FD patients suffer a progressive degenerative gait ataxia most likely due to the absence of functional muscle spindles [71, 72]. Histological analysis of muscle spindles in one of the mouse FD models [49] showed that they were present in adult gastrocnemius muscle. However, to what extent they were normally innervated by proprioceptive afferents has not yet been determined. Interestingly, two mouse studies reported that in contrast to the pain and temperature sensory afferents, proprioceptors develop and survive normally in the absence of IKAP, but it’s not yet been determined whether they degenerate in adulthood. Since α -motor neurons innervating the neuromuscular junction degenerate in an FD mouse model [54], it’s possible that γ -motor neurons might also degenerate and thereby indirectly affect the integrity of proprioceptors and muscle spindle function.

Generation of these powerful animal and cellular systems can be used to solve several outstanding genetic, molecular and cellular questions regarding FD. For example, what precipitates the cell type-specific splicing patterns: lymphoblasts and multiple non-neuronal cells can very effectively splice the mutant FD pre-mRNA, conferring protection on those cells, while most neurons are much less capable of accommodating the splice site mutation and consequently suffer from reduced levels of IKAP protein. Investigation of neuron-specific splicing factors could clarify this question [67]. Another important problem that can be pursued with the existing model systems is a search for genetic modifiers that may

mediate the variation in FD phenotype severity. In addition, fundamental questions remain concerning the cellular function(s) of IKAP/ELP1 and to what extent the cellular pathology of FD is a direct and/or indirect consequence of loss of Elongator complex-mediated modification of tRNAs required for translation. Is the mitochondrial impairment the direct and/or indirect upstream trigger for the P53, Caspase-3 mediated neuronal apoptosis in FD or are other intracellular stress pathways activated by translation impairment that lead to elevated P53 and neuronal death? Finally, since the discovery that IKAP is a key subunit of the Elongator complex, important work remains to elucidate why mutations in the other Elongator subunits (*ELP2*, *ELP3* and *ELP4*) all cause neurological disorders, but in distinct cell types and circuits. In summary, from the model systems described here, four exciting and promising therapeutic avenues have emerged for mitigating neuronal death in FD: increasing inclusion of exon 20 in mutant *IKBKAP* pre-mRNA to ultimately increase IKAP cellular levels, blocking IKAP degradation by proteasome inhibition, preventing the apoptotic death of neurons by reducing intracellular stress, and reintroducing *IKBKAP* by gene therapy. Although the past 15 years have transformed our genetic, molecular, cellular and neurobiological understanding of FD, if the past is prologue, then the next 15 years should be even more illuminating.

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Table 1

Mouse models of Familial Dysautonomia

<i>Ikbkap</i> status	Phenotype	References
Human FD-<i>IKBKAP</i> via BAC	No FD symptoms; tissue-specific splicing defect mirrors human FD	Hims et al., 2007
Full KO mouse	Embryonic lethality	Chen et al., 2009
20 <i>Ikbkap</i> allele	Embryonic lethality	Dietrich et al., 2011
Floxed <i>Ikbkap</i>	90% reduction in IKAP protein; small stature, ataxic gait, kyphoscoliosis, loss of pain and temperature sensation, loss of fungiform papillae, progressive eye and bladder problems	Dietrich et al., 2012
Floxed/ 20 <i>Ikbkap</i> compound heterozygote	95% reduction in IKAP protein; small stature, ataxic gait, kyphoscoliosis, loss of pain and temperature sensation, loss of fungiform papillae, progressive eye and bladder problems; loss of DRG neurons	Dietrich et al., 2012
<i>Wnt1-Cre;Ikbkap^{LoxP/LoxP}</i> PNS-specific ablation	Loss of 2nd wave neuronal progenitors and post-mitotic neurons during development; pups die within 24 hrs of birth	George et al., 2013
<i>TgFD9; Ikbkap^{20/flox}</i> low levels of WT <i>Ikbkap</i>, tissue specific expression of correctly spliced human WT <i>IKBKAP</i>	Reduced growth rate, reduced number of fungiform papillae, spinal abnormalities, sensory and sympathetic impairments, and recreates the same tissue-specific mis-splicing defect seen in FD patients	Morini et al., 2016
<i>Wnt1-Cre;Ikbkap^{LoxP/LoxP}</i> PNS-specific ablation	Innervation abnormalities; pups die shortly after birth	Jackson, et al., 2014
<i>DβH-iCre; Ikbkap^{LoxP/LoxP}</i> Selective ablation in lineage-specified sympathetic neuroblasts	Innervation abnormalities	Jackson, et al., 2014
Humanized <i>IKBKAP</i> CKO^{Tryp2} DRG-specific ablation beginning at E12.5	Irregular neurite outgrowth and branching, impaired NGF transport, decreased acetylated alpha-tubulin	Naftelberg et al., 2016
<i>Tuba1a-Cre; Ikbkap^{LoxP/LoxP}</i> Pan-neuronal deletion of <i>Ikbkap</i> in both CNS and PNS beginning at E12.5	Abnormal muscle innervation, cortical and motor neuron number, and number and organization of primary cilia; progressive neurodegenerative condition that includes kyphoscoliosis, and impairments in behavior including learning and memory; progressive retinal degeneration	Chaverra et al., 2017 Ueki et al., 2016