

IKBKAP/ELP1 gene mutations: mechanisms of familial dysautonomia and gene-targeting therapies

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Abstract: The successful completion of the Human Genome Project led to the discovery of the molecular basis of thousands of genetic disorders. The identification of the mutations that cause familial dysautonomia (FD), an autosomal recessive disorder that impacts sensory and autonomic neurons, was aided by the release of the human DNA sequence. The identification and characterization of the genetic cause of FD have changed the natural history of this disease. Genetic testing programs, which were established shortly after the disease-causing mutations were identified, have almost completely eliminated the birth of children with this disorder. Characterization of the principal disease-causing mutation has led to the development of therapeutic modalities that ameliorate its effect, while the development of mouse models that recapitulate the impact of the mutation has allowed for the in-depth characterization of its impact on neuronal development and survival. The intense research focus on this disorder, while clearly benefiting the FD patient population, also serves as a model for the positive impact focused research efforts can have on the future of other genetic diseases. Here, we present the research advances and scientific breakthroughs that have changed and will continue to change the natural history of this centuries-old genetic disease.

Keywords: HSAN, splicing, MAO, tocotrienol, EGCG, Ashkenazi

Introduction

Familial dysautonomia (FD, OMIM #223900), also known as Riley–Day syndrome, is an autosomal recessive congenital neuropathy that affects the development and survival of sensory and autonomic neurons. The identification and characterization of the FD-causing mutations in the *ELP1* (formerly known as *IKBKAP*) gene have enabled the rapid elucidation of the cellular, molecular and physiological action of this gene product and the role it plays in the pathophysiology of FD. The observed incomplete penetrance of the most common FD-causing mutation, which allows for the production of some functional gene product, has heralded a new era in the development of novel therapeutics that target the increased production of the functional gene product. The clinical use of several of these newly identified therapeutics has already demonstrated their ability to ameliorate the life-threatening symptoms of this disorder and will likely increase the long-term survival of this patient population. The rapid translation of the mutation discovery into the identification of novel targeted therapies represents a model for the development of new therapies for other disease-causing mutations.

The FD phenotype

The compromised development and survival of sensory, sympathetic and some parasympathetic neurons are hallmark symptoms of FD^{1,2} and it is based on these

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deficits that FD is characterized as a hereditary sensory and autonomic neuropathy (HSAN). Neuronal deficits have also recently been detected in the central nervous system of these patients.^{3,4} Examination of the neuronal development and deficits in individuals with FD have revealed: 1) diminished numbers of myelinated and non-myelinated axons in the sural nerve^{5,6}; 2) diminished neuronal presence in dorsal root ganglia in young patients and the progressive loss of neurons with age⁷; 3) depletion of axons in the spinal cord⁸; 4) a reduced volume of the superior cervical ganglia with a concomitant decrease in the number of neurons⁸; 5) diminished presence of nerve fibers in the epidermis, subepidermal neural plexus and the deep epidermis⁹; 6) decreased cardiac sympathetic innervation¹⁰; 7) reduced sympathetic innervation of peripheral blood vessels¹¹; 8) delayed and diminished brain stem reflexes,⁴ and 9) progressive optic nerve degeneration.¹² Due to the various neuronal deficits, individuals with FD typically exhibit a reduced sensitivity to pain and temperature, difficulty swallowing, decreased or absent deep tendon reflexes, an ataxic gait, cardiovascular instability, defective lacrimation and gastrointestinal dysfunction.¹³ Additional manifestations of FD include: the reduced presence of fungiform papillae on the tongue, orthostatic hypotension, spinal deformities (scoliosis/kyphoscoliosis/kyphosis), dysautonomic crises and frequent aspiration pneumonias.^{13,14} During dysautonomic crisis, FD patients experience uncontrollable bouts of nausea, retching, vomiting, sialorrhea, erythematous skin rashes and hyperhidrosis, which are accompanied by hypertension and tachycardia.^{13,15} These crises can occur following emotional or physiologic stresses that trigger the release of catecholamines^{13,15,16} and in response to tyramine ingestion.^{17,18} Aspiration pneumonias are common in these patients and they typically occur as the result of either misdirected swallowing or the inhaling of stomach contents during dysautonomic crises.¹⁹ Aspiration pneumonias are a leading cause of death for this patient population.¹⁴

The FD genotype

FD is a member of a genetically and clinically heterogeneous group of HSANs and is classified as HSAN type III. FD is almost exclusively confined to individuals of Ashkenazi Jewish (AJ) descent and is the result of the inheritance of mutations in the *ELP1* gene.^{20,21} Greater than 99% of individuals with FD are homozygous for a T → C transition at position 6 of the 5' (donor) splice site of intron 20 of the *ELP1* gene (termed IVS20+6T>C). This mutation, which is termed the "major" mutation because of its prevalence, compromises the base pairing of the U1 small nuclear ribonucleic protein

to the intron 20 donor splice site,²² resulting in the skipping of the exon 20-encoded sequence. The mis-splicing of this transcript introduces a frameshift in the transcript and the translation of this mRNA generates a truncated 79 kDa protein.²⁰ The IVS20+6T>C mutation is incompletely penetrant, allowing for the production of some full-length transcript. A second rare (termed "minor") disease-causing mutation present in the AJ population is a G → C transversion in exon 19 (c.2087G>C) of the reported IkB kinase complex-associated protein (IKAP) cDNA that results in an arginine to proline substitution of amino acid 696 (p.Arg696Pro).^{20,21} This amino acid change disrupts a consensus serine/threonine kinase phosphorylation site (RIVT→pIVT), resulting in reduced phosphorylation at this site.²⁰ The heterozygous inheritance of the IVS20+6T>C and p.Arg696Pro mutations results in the typical FD phenotype. To date, no individuals have been identified who are homozygous for the p.Arg696Pro mutation. A third FD-causing mutation, and the first non-Jewish FD-causing mutation, was identified in a patient who inherited an IVS20+6T>C bearing allele from his AJ father and a proline to leucine missense mutation in exon 26 (p.Pro914Leu) from his non-Jewish mother.²³ This patient exhibits the typical symptoms of this disease. A second non-Jewish disease-causing mutation has been identified in 2 children who inherited a single base insertion in exon 11 of the *ELP1* gene from their non-Jewish father and the IVS20+6T>C bearing allele from their AJ mother. The single base insertion in exon 11 generates a frameshift and the encoded protein lacks the amino acids encoded by exon 12 through 37. The children who inherited both of these mutations exhibited severe neurological deficits and died before the age of 2 months (Anderson et al, manuscript in preparation).

Based on the birth incidence of FD in the AJ population, the carrier frequency of the defective gene was predicted to be ~1 in 30.²⁴ Genotype screening for the FD-causing mutations performed on 3,246 non-selected healthy individuals of AJ descent demonstrated a carrier frequency of 1 in 30 for the IVS20+6T>C mutation and 1 in 1,623 for the p.Arg696Pro mutation.²⁵ Mutation screening performed on a small number of individuals of AJ descent suggested a higher carrier frequency among individuals of Polish ancestry.²⁶ The most robust data on the carrier frequency of these 2 mutations comes from the Dor Yeshorim, Committee for Prevention of Jewish Genetic Diseases, screening program²⁷ that has, to date, performed genetic testing for this disease on nearly 300,000 individuals of AJ descent. This program reports a carrier frequency of 1 in 27.5 for the IVS20+6T>C mutation and 1 in 1,897 for the p.Arg696Pro mutation (Rabbi J Ekstein,

Dor Yeshorim Executive Director, email communication, September, 2017). While the FD mutation clearly originated in the AJ population,²⁸ genetic testing of ~18,000 Jewish individuals who identified themselves as being of pure Sephardic lineage revealed a carrier frequency of 1 in 3,031 for the IVS20+6T>C mutation. No carriers of the p.Arg696Pro mutation were detected in this group of individuals (Rabbi J Ekstein, email communication, September, 2017). The presence of the IVS20+6T>C mutation in individuals who identify themselves as being of pure Sephardic lineages likely reflects the incomplete genealogical knowledge of the participants and highlights the need to perform FD carrier testing on individuals who report themselves to be of pure Sephardic descent.

The ongoing FD genetic screening programs and the recommendation of the American College of Obstetricians and Gynecologists that carrier screening for FD be offered to couples of AJ descent²⁹ has resulted in a dramatic decrease in the number of children born with this disorder.³⁰

The pleiotropic activities of the *ELP1* gene product

The *IKBKAP*-encoded IKAP, originally named because of its purported role in the assembly of the IκB complex,³¹ was later determined to play no role in this pathway.³² IKAP was subsequently determined to be the Elongator protein 1 (*ELP1*) subunit of the highly conserved Elongator complex.³³ The Elongator complex is detected in both the nucleus and cytoplasm. In the nucleus, the Elongator complex interacts with RNA polymerase II, playing a critical role in RNA polymerase II transcriptional activity and histone acetylation.^{33,34} In the cytoplasm, *ELP1*, either as a component of the Elongator complex or acting independently, is involved in diverse cellular functions, including cell migration and adhesion,^{35,36} intracellular trafficking,³⁷ tRNA modification,^{38,39} cytoplasmic kinase signaling,⁴⁰ and p53 activation.⁴¹ Whether these diverse biological activities represent distinct functions or are the result of a single biological activity that leads to various different downstream effects, remains to be determined.⁴²

Monoamine oxidase deficiency in FD patients and the role it plays in the pathophysiology of the disease

In the early 1960s, Smith and Dancis demonstrated that individuals with FD exhibit an exaggerated blood pressure response to systemic infusion of low doses of norepinephrine (NE) and proposed that this increased sensitivity plays a role

in the symptomatology of these patients.⁴³ The dysregulated response of FD patients to catecholamines was further established in studies demonstrating: 1) an exaggerated hypotensive response to infusion with methacholine⁴⁴; 2) excessive miosis following instillation of methacholine drops into the conjunctival sac⁴⁵; and 3) prolonged vasoconstriction in peripheral blood vessels following NE application.⁴⁶ The elevated presence of NE and dopamine (DA) detected in the plasma of FD patients during hypertensive crises^{13,15} supports the proposed role of increased catecholamines in the precipitation of hypertensive crises.¹⁹

The observed elevated levels of catecholamines in the plasma of FD patients during hypertensive crises and the overlapping symptoms manifested in FD patients during a hypertensive crisis¹³ with those observed in individuals treated with monoamine oxidase inhibitors (MAOIs) who have ingested biogenic amines,^{47,48} prompted a study of the levels of the MAOs, key isoenzymes responsible for the degradation of biogenic and dietary monoamines, in FD-derived tissues and cells.¹⁷ A reduced presence of the *MAO-A* encoding transcript and the *MAO-A* protein was observed in liver and kidney tissue samples from fetuses homozygous for the IVS20+6T>C mutation and the *MAO-A* encoding transcript was found to be reduced in peripheral blood cells of FD-affected individuals. No deficit in *MAO-B* gene expression was detected in the FD-derived tissues/cells.¹⁷ The reduced presence of the *MAO-A* gene product in individuals with FD, which would result in a prolonged circulating presence of various catecholamines, likely explains the exaggerated hypertensive response to infusion of low doses of NE,⁴³ the altered pattern of catecholamine metabolites observed in the plasma of individuals with FD,⁴⁹ the prolonged vasoconstriction in peripheral blood vessels following NE application,⁴⁶ the onset of dysautonomic crisis following emotional events that trigger the release of NE and DA,¹³ the elevated circulating levels of NE and DA observed in the plasma of FD patients during hypertensive/dysautonomic crises^{13,15} and the onset of hypertensive/dysautonomic crises following the ingestion of foods containing tyramine,^{17,18} a biogenic amine that also triggers hypertensive crises in individuals taking MAOIs.

The demonstrated *MAO-A* deficiency in FD patients has informed physicians of the need to avoid the use of serotonergic medications for these patients and has enabled anesthesiologists to select anesthetics and analgesics that do not precipitate the labile blood pressures and hypertensive crises that commonly occurred during and following anesthesia in these patients.⁵⁰⁻⁵³

Treating FD: symptom-targeting therapies

Pharmacological approaches currently used to treat FD patients have primarily focused on treating the symptoms manifested in these individuals. Hypertension and hypertensive/dysautonomic crises are typically treated with a combination of clonidine, a centrally acting α_2 adrenergic agonist, and diazepam, a benzodiazepine.⁵⁴ Hypotension is treated with fludrocortisone, a synthetic mineralocorticoid, which increases the blood volume and elevates blood pressure by increasing renal sodium and water reabsorption,⁵⁵ and/or midodrine, a peripherally acting α_1 -adrenergic agonist.⁵⁶ Gastroesophageal reflux is treated with either histamine H₂-receptor antagonists and/or proton pump inhibitors¹⁹ and insufficient tear production is treated with the administration of artificial tears.⁵⁷ Many of the medications currently used to treat these patients are sedating and their ongoing use can severely compromise the quality of life of these patients. In addition, it has recently been reported that medications used to control FD-related symptoms may be responsible for the high incidence of sudden unexpected death during sleep observed in this patient population.⁵⁸ An extensive overview of the medications currently used to treat the symptomology of FD has recently been published.¹⁹

Treating FD: the search for gene-targeting therapies

The absence of FD symptoms in carriers of the IVS20+6T>C mutation that produces a reduced amount of the *ELP1* gene product, due to the presence of only 1 non-mutated *ELP1* allele, clearly indicates that there is a threshold amount of the IKAP protein that is sufficient to enable normal neuronal development and survival. The observed ability of the IVS20+6T>C-bearing *ELP1* allele to produce some functional gene product in FD patients prompted the screening of chemical libraries for compounds capable of facilitating the production of the functional *ELP1* gene product that might be used to ameliorate or reverse the neuronal dysfunction observed in FD patients.

When considering the clinical use of compounds that alter gene expression or the RNA splicing process, it is important to consider the potential off-target toxic effects these compounds may have.^{59,60} In an effort to avoid or minimize potential off-target-mediated toxicities, Anderson et al screened a library of commonly ingested compounds for their impact on the expression of the correctly spliced *ELP1* transcript and IKAP protein in FD-derived cells. In 2003, Anderson et al reported that tocotrienols, a form of vitamin E found in brown rice, and epigallocatechin gallate

(EGCG), a polyphenol present in green tea, increase expression of the correctly spliced *ELP1* transcript in FD-derived cells by upregulating transcription of the *ELP1* gene⁶¹ and by altering the splicing of the *ELP1* transcript.⁶²

In 2004, kinetin (6-furfurylaminopurine), a plant cytokinin, was shown to facilitate the production of the exon 20-containing *ELP1* transcript by altering the splicing process.⁶³

A full listing of the chemical compounds and other substances identified to date capable of increasing the production of the exon 20-containing (wild-type) *ELP1* transcript in cells homozygous for the FD-causing IVS20+6T>C mutation is presented in Table 1.

Treating FD: the in vivo response to gene-targeting therapies

The first in vitro-identified gene-targeting agent clinically evaluated in FD patients was the tocotrienols. As tocotrienols are commonly ingested as a component of rice bran and no adverse side effects have been observed in humans at doses as high as 200 mg/day,^{71,72} the risk of toxicity was considered minimal, and FD patients were enrolled in a study in which they ingested a daily dose of 100 mg of tocotrienol for a period of up to 6 months. The tocotrienol treatment resulted in: 1) an increase in the production of the exon 20-containing *ELP1* transcript and the MAO-A transcript in peripheral blood cells;¹⁷ 2) a reduction in the frequency of hypertensive crises in excess of 82% of the participants enrolled in the study and a complete elimination of these crises in 40% of the study participants;⁷³ 3) an improved cardiac response to exercise⁷³; and 4) increased lacrimation.⁷³⁻⁷⁵

The results of a second clinical trial, which assessed the impact of tocotrienol ingestion on a cohort of 28 FD patients, were recently published.⁷⁶ The participants in this open label study ingested either 50 or 100 mg of tocotrienols for an initial 3-month period and then either 100 or 200 mg of tocotrienols for a second 3-month period. This study demonstrated that tocotrienol mediated: 1) reduction in the number of pneumonias; 2) reduced sweating, blotching and dizziness; and 3) increased stability in walking.⁷⁶ After 3 months of the tocotrienol therapy, a significant increase in the expression of the exon 20-containing *ELP1* transcript was detected in peripheral blood cells of these patients. For reasons that are unclear, no increase in the exon 20-containing *ELP1* transcript in the peripheral blood cells was detected at the 6-month time point.

The tocotrienol-mediated reduction in the frequency of pneumonias⁷⁶ and hypertensive crises,⁷³ which are the leading

Table 1 Substances that increase levels of the exon 20-containing *ELPI* transcript

Substance(s)	Impact on the <i>ELPI</i> transcript	Mechanism of action
Tocotrienols ⁵¹	Increases transcription rate	Not determined
EGCG ⁶²	Exon 20 inclusion	Downregulates hnRNP A2/B1
Kinetin ⁶³	Exon 20 inclusion	Not determined
Phosphatidylserine ^{64,65}	Increases transcription rate	Activates MAPK/ERK signaling pathway
Genistein/daidzein ⁶⁶	Exon 20 inclusion	Not determined
Genistein + EGCG ⁶⁶	Exon 20 inclusion	Not determined
Cardiac glycosides ⁶⁷	Exon 20 inclusion	Downregulate SRSF3
RECTAS ⁶⁸	Exon 20 inclusion	Not determined
Proteasome inhibitors ⁶⁹	Exon 20 inclusion	Not determined
RBM24 ⁷⁰	Exon 20 inclusion	Promotes UI snRNP recognition of mutated 5' splice site

Abbreviations: EGCG, epigallocatechin gallate; ERK, extracellular-signal-regulated kinase; hnRNP, heterogeneous nuclear ribonucleoproteins; MAPK, mitogen activated protein kinase; RBM24, RNA binding motif protein 24; RECTAS, RECTifier of aberrant splicing; snRNP, small nuclear ribonucleoproteins; SRSF3, serine/arginine-rich splicing factor 3.

causes of death in FD patients,^{14,77} strongly support their continued administration. The tocotrienol-mediated increased tear production^{73–75} has helped FD patients avoid the recurrent corneal abrasions/ulcers that typically result from the lack of tear production.⁵⁷ The observed tocotrienol-mediated increased stability in walking,⁷⁶ reduced sweating and blotching⁷⁶ and increased lacrimation^{73–75} suggest improvement in sympathetic and parasympathetic neuronal function. These findings demonstrate the in vivo impact of the tocotrienols and suggest that the long-term ingestion of the tocotrienols may preserve neuronal function and extend the life span of FD patients.

Additional clinical studies assessing the impact of tocotrienol administration on the FD patient population are needed and the recent report demonstrating the absence of any adverse side effects in humans at doses as high as 1,000 mg/day⁷⁸ suggests that the therapeutic impact of higher doses of the tocotrienols can be safely evaluated.

In light of the significant autonomic instability that occurs in FD patients during the perioperative period,^{52,53} Cook-Sather et al monitored the perioperative course in an FD patient receiving daily administration of 600 mg of tocotrienols and 700 mg of EGCG.⁵¹ The daily administration of these nutraceuticals was not interrupted in preparation of the surgery and the child received the normal dosing on the morning of the procedure. Other than a brief drop in arterial blood pressure that was observed during the induction of anesthesia with sevoflurane, no significant alterations in heart rate or arterial blood pressure were observed during the procedure. Following the surgical procedure, the patient's blood pressure remained stable, the patient did not experience any nausea or vomiting, and there was no evidence of any autonomic dysfunction. The authors noted that this perioperative course was very different than that experienced

by this child during a previous surgery that was performed prior to the discovery of the functional IKAP-enhancing activity of the tocotrienols and EGCG. Following the previous procedure, the child experienced post-operative apnea, hypotension and severe refractory vomiting. Based on their findings, the authors strongly recommend the continued use of tocotrienol and green tea supplementation for FD patients undergoing surgery.⁵¹

Kinetin's ability to alter the splicing process and facilitate exon 20 inclusion in the *ELPI* transcript produced in FD-derived cells prompted an examination of the impact of kinetin ingestion on carriers of the IVS20+6T>C mutation. The kinetin ingestion, for an 8-day period, resulted in increased functional *ELPI* mRNA expression in the peripheral blood cells of these test subjects. The most commonly reported side effect reported by the participants in the study was nausea.⁷⁹ Based on these findings, kinetin was administered at a dose of 23.5 mg/kg for 28 days to 8 FD patients homozygous for the IVS20+6T>C allele. The average percentage of correctly spliced *ELPI* mRNA present in the blood cells of these patients before kinetin ingestion was 54%. After 8 and 28 days of kinetin ingestion, the percentage increased to 57% and 71%, respectively. Blood tests performed on these patients revealed significant hepatotoxicity in 3 of the 8 patients in this cohort and 1 patient experienced a decrease in platelet and white blood cell counts. The kinetin administration did not improve the blood pressure, heart rate or neurological function in these patients.⁸⁰

The development of FD mouse models

Recent scientific studies have challenged the validity of using mouse models to recapitulate a human disease phenotype and predict the responsiveness of humans to drug candidates.^{81,82}

The use of mouse models to study compounds that alter the splicing process in human cells or transcriptionally upregulate genes of interest are further fraught with challenges, as the splicing process in humans and mice is very different^{83,84} and transcriptional regulation and promoter sequences have evolutionarily diverged in these species.^{85,86} Despite these potential pitfalls, investigators have endeavored to create a suitable FD mouse model in which to characterize the in vivo effects of chemical compounds demonstrated to increase the production of the exon 20-containing *ELP1* transcript in cell lines^{61,63} and induced pluripotent stem cell-derived lineages⁸⁷ generated from individuals homozygous for the FD-causing IVS20+6T>C mutation. Attempts to generate an FD mouse model bearing the major FD-causing mutation in the conserved intron 20 donor splice site of the mouse *ELP1* homolog, *Ikkkap* (current nomenclature as per the Mouse Genomic Nomenclature Committee), which would most closely model the human FD-causing mutation, failed.^{88,89} Using an alternative approach, Bochner and coworkers generated a knock-in humanized mouse model in which the human exon 20, with its flanking introns bearing the IVS20+6T>C mutation, replaced the corresponding mouse genomic sequence.⁸⁹ This effort failed to generate a suitable FD mouse model, as the homozygous inheritance of this humanized allele generated animals that were fully viable and did not display any FD symptoms.⁸⁹ The failure of both of these approaches to generate an FD mouse model clearly indicates that the regulation of splicing and the impact of the IVS20+6T>C is very different in man and mouse. It is likely that the lack of identity in the exon and intron sequences of the human *ELP1* and mouse *Ikkkap* genes and the differential expression of critical components of the splicing machinery in humans and mouse tissues⁹⁰ play a role in this differential response. The inability to generate a mouse model homozygous for the major FD mutation that recapitulates the FD phenotype undermines the ability to perform pre-clinical testing in mice using chemical compounds found to enhance the production of the wild-type *ELP1* transcript in FD-derived human cells.

To study the role IKAP plays in the development of the nervous system and in the pathophysiology of FD, investigators attempted to generate mice that were homozygous for either a disrupted murine *Ikkkap* gene (termed *Ikkkap*^{-/-})^{91,92} or were homozygous for an exon 20-lacking murine *Ikkkap* gene (termed *Ikkkap*^{Δ20/Δ20}).⁹² The homozygous inheritance of these altered *Ikkkap* genes resulted in embryonic lethality. Morphological examination of these embryos revealed developmental delay, vascular and cardiovascular defects

and neuronal malformations. Interestingly, the introduction of the normal human *ELP1* transgene into the *Ikkkap*^{-/-} mice generated animals that were phenotypically normal while the introduction of a human *ELP1* transgene bearing the FD-causing mutation into these animals failed to rescue the embryonic lethality.⁹¹ These findings suggest that the level of expression of the correctly spliced transcript from the transgene bearing the FD-causing mutation was insufficient to enable the survival of these animals.

Two mouse models producing a reduced amount of the IKAP protein were successfully developed through the generation of: 1) animals homozygous for an altered murine *Ikkkap* allele bearing an insertion of loxP sites flanking exon 20 sequence of the *Ikkkap* gene (termed *Ikkkap*^{flax/flax}), which exhibited a 90% reduction in IKAP protein expression, and 2) animals heterozygous for the loxP site-containing *Ikkkap* murine gene and an exon 20-lacking murine *Ikkkap* allele (termed *Ikkkap*^{Δ20/flax}), which exhibited a 95% reduction in functional IKAP expression.⁸⁸ Histological studies of the superior cervical ganglia, the dorsal root ganglia and epidermal innervation in these mice reveal that they recapitulate the neuronal deficits seen in individuals with FD. These mouse models also exhibit the unsteady gait, poor motor coordination, postural instability, gastrointestinal dysfunction, reduced number of fungiform papillae on the tongue, skeletal abnormalities and the reduced lifespan observed in FD patients. These animals also display the reduced expression of genes involved in oligodendrocyte maturation and myelin formation that is observed in the brains of FD patients.⁹³ The FD-like phenotype and the neuronal deficits in the *Ikkkap*^{flax/flax} mouse model, which produce ~10% of the wild-type levels of the IKAP protein, were significantly milder than those observed in the *Ikkkap*^{Δ20/flax} mouse model, which produce ~5% of the wild-type levels of the IKAP protein. Furthermore, the *Ikkkap*^{flax/flax} mice had a longer lifespan than the *Ikkkap*^{Δ20/flax} mice. These findings support the hypothesis that therapeutic modalities that generate even slight increases in wild-type ELP1 (IKAP) levels in FD patients may greatly ameliorate the severity of this disease.

The absence of the disease-causing IVS20+6T>C mutation in these animals, which precluded their use as a model to study the clinical and molecular impact of the in vitro identified splice-altering and *ELP1* transcription-upregulating compounds, prompted the introduction of the complete human *ELP1* transgene bearing the IVS20+6T>C mutation into the *Ikkkap*^{Δ20/flax} mice. The resulting animals exhibit many of the features of the FD phenotype and the splicing of the human *ELP1* transcript in these animals displays

the same tissue-specific splicing observed in FD patients. Kinetin ingestion by these animals facilitates the inclusion of the exon 20 sequence in the *ELP1* transcript produced by the human IVS20+6T>C bearing allele.⁹⁴ The impact of the kinetin treatment on the FD phenotype of these animals has not yet been reported.

Conclusion

The discovery of the splice-altering nature of the mutation in the *ELP1* (*IKBKAP*) gene that causes FD has rapidly led to an increased understanding of: 1) the pathophysiology of this disorder; 2) the role this gene plays in neuronal development and survival; and 3) the identification and utilization of novel therapeutics that facilitate the production of the correctly spliced gene product. As approximately one-third of disease-causing alleles are predicted to impact the splicing process,⁹⁵ the successes achieved in identifying potential therapies for individuals with FD should serve as a model to address the pathogenicity of other splice-altering mutations. While genetic screening efforts are successfully reducing the numbers of children born with FD,³⁰ the recent scientific progress made will likely result in the improved health and the prolonged survival of the current FD population.

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Disclosure

The authors report no conflicts of interest in this work.

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