Drugs of the Future 2010, 35(6): 489-494

CCC: 0377-8282/2010
DOI: 10.1358/dof.2010.35.6.1488338

FAMILIAL DYSAUTONOMIA

Y.-T. Chen and S.A. Slaugenhaupt

Center for Human Genetic Research, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

CONTENTS

SUMMARY

Familial dysautonomia (FD), also known as Riley–Day syndrome or recessive hereditary sensory and autonomic neuropathy type III (HSAN III), is caused by a single-base noncoding mutation in intron 20 (IVS20+6T > C) of the IKBKAP/ELP1 gene. This mutation results in variable skipping of exon 20 in IKBKAP/ELP1 transcripts, which leads to tissue-specific reduction of ELP1 (IKBKAP) protein, particularly in the nervous system. FD is a devastating disorder with a high mortality rate due primarily to autonomic dysfunction. The identification of the gene and the disease-causing mutation has promised the development of potential treatments that directly target mRNA splicing to increase normal mRNA and protein. FD is a developmental disease with diagnostic symptoms present at birth, whereas patients show progressive neurodegeneration throughout life. Drugs that can increase ELP1 protein may slow this degeneration and improve the quality of life in aging patients. Since these compounds target the mRNA splicing mechanism and not a specific gene, it is likely that they will prove useful in other disorders with similar splice-site mutations. Given that 20-30% of human mutations are predicted to alter mRNA splicing, direct modification of splicing efficiency poses an important target for the design of therapeutics in the future.

INTRODUCTION

History

Familial dysautonomia (FD), also known as Riley–Day syndrome or hereditary sensory and autonomic neuropathy type III (HSAN III, OMIM #223900), was first described in 1949 by Conrad M. Riley, Richard L. Day, David McL. Greeley and William S. Langford, physicians in New York City (1). The authors pointed out that while FD is similar to several well-defined disorders such as congenital deficiency of lacrimation, autonomic diencephalic epilepsy, postencephalitic parkinsonism, essential hypertension and autonomic imbalance, its unique constellation of features allowed classification of FD, later named Riley–Day syndrome, as a distinct clinical entity. In the original report, the primary features of FD were deficiency of lacrimation, characteristics of anxiety, such as excessive sweating and red blotching of the skin, arterial hypertension, hyporeflexia, motor incoordination and cyclic vomiting. Moreover, it was noted that all patients described were of Ashkenazi Jewish heritage. Since the first description of FD, numerous clinical studies have contributed substantially to the diagnosis and supportive medical treatment of this disorder, dramatically improving patient survival over the past 30 years (2-6).

Correspondence: Susan A. Slaugenhaupt, Ph.D, Center for Human Genetic Research, Massachusetts General Hospital, CPZN-5254, 185 Cambridge Street, Boston, MA 02114, USA. E-mail: Slaugenhaupt@chgr.mgh.harvard.edu.

Epidemiology

FD is one of the most common and best known of the hereditary sensory and autonomic neuropathies (7). To date, FD has only been described in the Ashkenazi Jewish population. In 2003, a single non-Jewish mutation was described in a patient of mixed Ashkenazi ancestry (8). Following identification of *IKBKAP/ELP1* as the FD gene, the carrier frequency was estimated to range from 1 in 17 in Ashkenazi Jews of Polish descent to 1 in 27 in the general Ashkenazi Jewish population (5, 9, 10).

Genetics

FD was first suggested to be an autosomal recessive disorder in 1970 (11). In 1993, the FD gene was mapped to chromosome 9q31 using genetic linkage analysis performed in 26 families with multiple affected children. Haplotype analysis showed that there was a single major founder mutation present in the majority of FD patients (12). In 1999, using detailed haplotype analysis, the location of the disease gene was narrowed down to a 471-kilobase candidate region on chromosome 9 (13). The major intronic FD mutation was identified in 2000 by direct comparison of genomic DNA sequences between FD patients and controls (14-17). The major FD mutation is an intronic noncoding point mutation located at the sixth base pair of intron 20 in the last position of the consensus splice donor site (IVS20+6T > C) of the *IKBKAP/ELP1* gene. Subsequently, two missense mutations, R696P and P914L, were identified in patients who were heterozygous for the major FD haplotype. Importantly, all FD patients identified to date carry at least one copy of the splice mutation, and more than 99.5% of FD patients are homozygous for the intronic noncod-

ing mutation (8, 16). Surprisingly, examination of lymphoblast and fibroblast cell lines derived from FD patients homozygous for the IVS20+6T > C mutation revealed that wild-type (WT) mRNA and protein were produced. This finding was unexpected given that FD is a recessive disease and thus the expectation was that the mutation would cause complete loss of function of the ELP1 (IKBKAP) protein. The presence of normal *IKBKAP/ELP1* mRNA and ELP1 protein in patient cells suggests that the intronic point mutation weakens but does not completely disrupt the inclusion of exon 20 during mRNA splicing. It is likely that the T to C mutation in the exon 20 splice donor site reduces the efficiency of the splicing machinery in a tissue-specific manner and results in variable expression of ELP1 protein during early development and throughout life (16).

It is known that the WT *IKBKAP/ELP1* mRNA in the FD patient-derived cells is translated into full-length, functional ELP1 protein. The exclusion of exon 20 in the mutant (MU) *IKBKAP/ELP1* transcript results in a frameshift and introduces a premature translation termination codon in exon 21, which would be expected to trigger the nonsense-mediated mRNA decay pathway (NMD) and lead to degradation of the mutant transcript (16, 18). This was later supported by the observations of increased MU *IKBKAP/ELP1* transcript in FD patient-derived cells that were treated with cycloheximide, an inhibitor of NMD, as well as by the lack of truncated protein in FD patient tissues (16, 19, 20). The data generated to date strongly suggest that FD is caused by a tissue-specific reduction of ELP1 protein below the critical threshold necessary to support proper development of the sensory and autonomic nervous system, and not by the activity of a truncated mutant protein. Indeed, if a pathogenic mutant protein was produced, one might expect a dominant mode of inheritance and symptoms in FD carriers, and to date none have been described.

PATHOLOGICAL MECHANISMS

Although we now know that FD results from reduction of ELP1 protein levels, the precise pathological mechanism is largely unknown. Given that FD is a sensory and autonomic neuropathy, it is likely that reduction of ELP1 protein leads to deficits in neuronal development and/or migration, coupled with progressive age-related degeneration in both the peripheral and central nervous system (CNS) (21-25).

In FD patients, the number of myelinated and unmyelinated axons in the sural nerve is severely decreased, as are the intermediolateral neurons in the spinal cord. The total size and neuronal count of both the sensory and sympathetic ganglia are also reduced, and the sphenopalatine ganglion (parasympathetic) is a tenth of normal size. In addition, no dense-core vesicle nerve terminals are observed on peripheral blood vessels. The number of dorsal root ganglia is further reduced with age, suggesting that progressive neuronal degeneration contributes to the worsening of neurological function, such as loss of pain and vibration sensation in FD patients with age (21-23, 26, 27). Consistent with the pathological findings, microarray analyses performed using the post mortem cerebrum from two FD patients revealed that the expression of several genes that have been implicated in oligodendrocyte differentiation and myelination was downregulated in the FD brain (28). In FD patients, several clinical features, such as emotional liability and ataxic gait, indicate functional compromise of the CNS. Consistent with these clinical observations, imaging analyses using magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) revealed structural abnormalities in the CNS, including a reduction in the volume of white matter and the integrities of optic radiation and cerebellar peduncle, respectively (29).

The identification of *IKBKAP/ELP1* as the FD gene has ushered in a new era for the study of the underlying mechanism of FD. In FD patients homozygous for the IVS20+6T > C mutation, the relative amount of WT and MU *IKBKAP/ELP1* transcripts varies between tissues, with the central and peripheral nervous tissues showing the lowest levels of WT *IKBKAP/ELP1* mRNA (Fig. 1B) and ELP1 protein (16, 17, 30). These findings, together with the neurological and pathological observations of FD patients, suggest that excessive reduction of ELP1 protein, specifically in the developing nervous system, underlies the FD phenotype. Moreover, the fact that carriers with a single IVS20+6T > C mutation have no neurological abnormalities, suggests that there is a threshold of ELP1 protein that is required for normal development and maintenance of the nervous system.

It is now appreciated that the ELP1 is expressed ubiquitously in most tissues. ELP1 was originally identified as an IκB kinase complex-associated protein (31). However, later studies demonstrated that

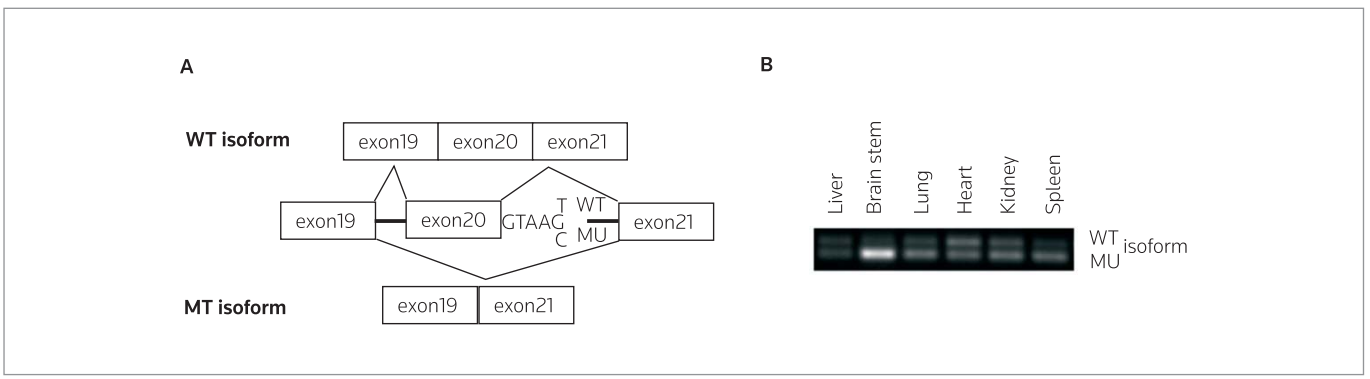

Figure 1. A. Schematic diagram of the IVS20+6T > C mutation in the *IKBKAP/ELP1* gene showing the two RNA isoforms transcribed from this allele. WT, wild-type; MU, mutant. **B.** Tissue-specific splicing pattern seen in familial dysautonomia (FD). Different levels of WT and MU transcript are produced from the IVS20+6T > C containing allele in different tissues.

ELP1 is not involved in the NF-κB signaling cascade (32). Instead, ELP1 is homologous to the Elp1 protein, one of the subunits in the holo-elongator complex encoded by the *ELP1* gene in *Saccharomyces cerevisiae*. It is now known that the holo-elongation complex associates with hyperphosphorylated RNA polymerase II during transcriptional elongation in yeast (32-35). The human Elongator complex was subsequently shown to be a six-subunit complex containing IKBKAP/hELP1, hELP2/StIP1, hELP3, hELP4, hELP5 and hELP6. Intriguingly, despite an obvious role for Elongator in the nucleus, ELP1 can also be isolated from cytoplasmic fractions that lack detectable hELP3 (36). This finding raises the possibility that Elongator might have multiple functions in addition to its role in transcriptional elongation, perhaps functioning independently in different compartments (37). This assumption was supported by various studies that have demonstrated the putative functions of ELP1 in tRNA modification, exocytosis and activation of JNK signaling (38-40). Recently, it was demonstrated that a decrease of ELP1 alters the integrity of the Elongator complex, and subsequently interferes with histone H3 acetylation and alters the expression of genes that are important for cell motility (19). A role for ELP1 in cell adhesion and migration has also been suggested using RNA interference-based depletion of ELP1 (41). Most recently, the Elongator complex has been shown to regulate the maturation of projection neurons through defective α-tubulin acetylation (42, 43). The development of an *Ikbkap/Elp1* mouse knockout model has provided new insights into the role of ELP1 during early embryonic development (44). Complete loss of ELP1 function leads to early embryonic lethality, demonstrating an essential role for the protein during embryogenesis. The fact that complete ELP1 loss is incompatible with life explains the unique nature of the FD mutation observed in patients in that some level of ELP1 protein is required for embryonic development.

Examination of *Ikbkap/Elp1* knockout embryos demonstrated that ELP1 is required for neurogenesis and vasculogenesis, as evidenced by disoriented dorsal primitive neural alignment and failure to establish the embryonic vascular system. Expression of several genes that are important for neural and vascular development in the *Ikbkap/Elp1* knockout animals was shown to be reduced or absent due to defects in transcriptional elongation (44). While these studies have begun to shed light on the normal cellular function of *IKBKAP/ELP1* and the Elongator complex, there is still much to be learned about how disruption of these processes leads to the autonomic and sensory dysfunction seen in FD.

PHENOTYPE

FD is a neuropathy that results from dysfunction in the sensory and autonomic nervous systems. The common clinical features that are considered to be diagnostic criteria for FD include absence of lacrimation (1), no axon flare formation when performing the intradermal histamine test (45), repressed deep tendon reflex (46, 47) and no fungiform papillae on the tongue (48-50). Interestingly, Ashkenazi Jewish ancestry is one of the major diagnostic criteria, which may partially explain the fact that, to date, FD has not been diagnosed outside the Ashkenazi Jewish population. FD patients also show impairment of pain and temperature sensation that worsens with increasing age (27, 51). Also, as reported in 1949, diminished corneal reflexes, postural hypotension, excessive sweating and blotching, and episodic vomiting are present in the majority of FD patients (1). Patients with FD also suffer from various degrees of aspiration and insensitivity to hypoxia (52), ataxic gait (53), oropharyngeal incoordination (54), esophageal dysmotility and gastroesophageal reflux (55), and spinal curvature (56).

CURRENT MEDICAL TREATMENTS

Since FD was first described in 1949, numerous clinical studies have contributed substantially to our understanding and treatment of this devastating disease. Although there is currently no cure for FD, remarkable progress has been made in developing supportive treatments, primarily by Dr. Felicia Axelrod, Director of the Dysautonomia Treatment and Evaluation Center at New York University Medical Center. In 1970, 50% of FD children died before their 5th birthday (11). Children born with FD today have a 50% probability of surviving beyond 40 years of age (57). Current clinical reports indicate that autonomic dysfunction, such as arrhythmia, pulmonary or renal failure, are the major causes of death (58). Listed below are the major clinical symptoms of FD and the typical supportive treatments:

Absent or reduced lacrimation. Topical lubricants and artificial tear solutions, or moisture chamber spectacle attachments and goggles, are used to retain eye moisture and protect the eye from the outside environment (59). These methods have been proven to significantly reduce corneal abrasions that are common in FD patients due to disturbances in the central autonomic system (47).

Oropharyngeal incoordination. Most FD infants have oropharyngeal incoordination, which causes difficulties in sucking and swallowing. Consequently, infants usually suffer from malnutrition or aspiration that results from misdirected liquids. Gastrostomy is frequently performed to assist with feeding and insure that sufficient calories are obtained (6).

Gastroesophageal reflux. Gastroesophageal reflux, also called acid reflux or acid regurgitation, is common in FD patients. In 1991, a clinical study estimated that over 95% of FD patients suffered from gastroesophageal reflux (54). Fundoplications and gastrostomy are frequently performed if the patients develop complications of reflux, such as pneumonia, apnea and poor weight gain (54, 60).

Episodic vomiting. Episodic vomiting typically accompanies crisis in FD patients (1), and the use of anticonvulsant drugs such as clonazepam, lamictal or diazepam has been shown to reduce the duration and frequency of the crisis (59).

Recurrent pneumonia. In FD patients, aspiration due to misdirected swallowing or gastroesophageal reflux often leads to recurrent pneumonia. However, the frequency of lung infections can be significantly reduced with aggressive management of gastrointestinal dysfunction. Measures are used to decrease the risk of aspiration, and chest physiotherapy and postural drainage are often employed (59).

Postural hypotension. Postural hypotension is one of the characteristic features of FD. Exercise to reinforce lower body muscle tone, combined with increased fluid intake, is advised for all FD patients. Fludrocortisone and midodrine are also prescribed to manage hypotension (59).

Spinal curvature. The progression of scoliosis often occurs in FD patients, and severe spinal curvature might further affect the respi-

ratory system (61, 62). Surgical intervention and brace therapy are often required to slow down the progression of scoliosis (59).

DEVELOPMENT OF NEW DRUGS FOR THE TREATMENT OF FD

The noncoding point mutation IVS20+6T > C in the *IKBKAP/ELP1* gene interferes with splicing efficiency of *IKBKAP/ELP1* pre-mRNA and consequently results in tissue-specific reduction of ELP1 protein. Studies performed since the mutation was discovered strongly suggest that a reduction of ELP1 leads to inefficient transcription of other genes and impairs the development of the nervous system. Thus, the search for drugs that can improve splicing accuracy and increase ELP1 protein levels in FD patients has been a major focus for developing new, targeted treatments for FD. Based on this concept, screens for drugs that can alter splicing have been performed, and the plant cytokinin kinetin (Fig. 2) has been shown to dramatically enhance splicing efficiency of FD cell lines (20). In addition to kinetin, two other compounds, tocotrienol and epigallocatechin gallate (EGCG), have been reported to increase the WT *IKBKAP/ELP1* transcripts (63-65).

Kinetin (N^6-furfuryladenine) is one of the plant cytokinins that has been shown to stimulate tRNA synthesis, cell cycle progression and the catalytic activity of cyclin-dependent kinase in plant cells (66-68). In addition to its described functions in plants, kinetin was shown to delay the onset and decrease the extent of aging characteristics in cultured human fibroblasts (69). Kinetin was also reported to protect against oxidative damage to both DNA and protein (70, 71). Currently, kinetin is widely used in skincare products, such as Kinerase (Valient Pharmaceuticals) and Kinetin Skincare (Almay), which are advertised to improve skin roughness, mottling and fine wrinkling (72).

The precise mechanism by which kinetin improves splicing efficiency has yet to be determined. Experiments using a series of minigenes with targeted alterations in *IKBKAP/ELP1* exon 20 have shown that the CAA motif at the distal end of exon 20 of the *IKBKAP/ELP1* gene is essential for kinetin activity (73). Importantly, kinetin treatment has been shown to significantly improve exon 20 inclusion and increase the amount of ELP1 protein amount in various patient cell lines, as well as in iPSC-derived neural crest cells from FD patients (20, 73, 74). Additionally, using mice that carry human IVS20+6T > C transgenes, we also observed improved exon 20 inclusion in all tissues examined, including brain, following oral administration of kinetin (unpublished observations). In 2009, a short-term oral administration study was performed using a group of healthy, non-symptomatic FD carriers bearing one copy of the IVS20+6T > C mutation in the *IKBKAP/ELP1* gene. The results from this study demonstrate that, after kinetin treatment, WT *IKBKAP/ELP1* mRNA increased in leukocytes from nonsymptomatic carriers, and there was a linear association between the plasma kinetin concentrations and WT *IKBKAP/ELP1* mRNA level (75). These exciting results show that kinetin can improve *IKBKAP/ELP1* splicing in humans. Coupled with the latest results in transgenic mice showing similar improvement in *IKBKAP/ELP1* splicing in all tissues including brain, there is strong evidence that kinetin will have a therapeutic impact in FD, and potentially in other splicing disorders as well. Given that 20-30% of all disease-causing mutations are predicted to alter mRNA splicing, a number that will surely increase as the regulatory effects of single nucleotide polymorphisms (SNPs) associated with complex, common diseases are revealed, the demonstration of successful modification of splicing in FD illustrates that treatments which target precise molecular splicing defects can be successfully developed.

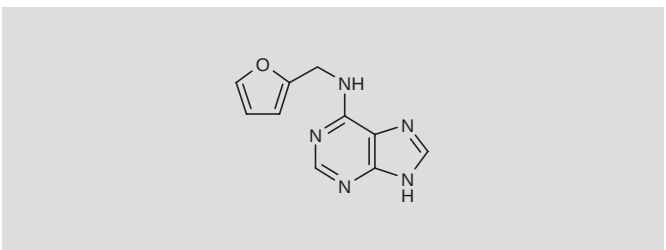

Figure 2. Structure of kinetin.

DISCLOSURES

The authors state no conflicts of interest.

REFERENCES

1. Riley, C.M., Day, R.L., Greeley, M.D., Langford, S.W. *Central autonomic dysfunction with defective lacrimation; report of five cases*. Pediatrics 1949, 3(4): 468-78.
2. Smith, A.A. *Familial dysautonomia.* Br Med J 1968, 2(5607): 763.
3. Pearson, J. *Familial dysautonomia (a brief review).* J Auton Nerv Syst 1979, 1(2): 119-26.
4. Slaugenhaupt, S.A. *Genetics of familial dysautonomia. Tissue-specific expression of a splicing mutation in the IKBKAP gene.* Clin Auton Res 2002, 12(Suppl. 1): I15-9.
5. Slaugenhaupt, S.A., Gusella, J.F. *Familial dysautonomia*. Curr Opin Genet Dev 2002, 12(3): 307-11.
6. Axelrod, F.B. *Familial dysautonomia.* Muscle Nerve 2004, 29(3): 352-63.
7. Verpoorten, N., De Jonghe, P., Timmerman, V. *Disease mechanisms in hereditary sensory and autonomic neuropathies.* Neurobiol Dis 2006, 21(2): 247-55.
8. Leyne, M., Mull, J., Gill, S.P. et al. *Identification of the first non-Jewish mutation in familial Dysautonomia.* Am J Med Genet A 2003, 118(4): 305-8.
9. Dong, J., Edelmann, L., Bajwa, A.M., Kornreich, R., Desnick, R.J. *Familial dysautonomia: detection of the IKBKAP IVS20(+6T —> C) and R696P mutations and frequencies among Ashkenazi Jews.* Am J Med Genet 2002, 110(3): 253-7.
10. Lehavi, O., Aizenstein, O., Bercovich, D., Pavzner, D., Shomrat, R., Orr-Urtreger, A., Yaron, Y. *Screening for familial dysautonomia in Israel: Evidence for higher carrier rate among Polish Ashkenazi Jews*. Genet Test 2003, 7(2): 139-42.
11. Brunt, P.W., McKusick, V.A. *Familial dysautonomia. A report of genetic and clinical studies, with a review of the literature.* Medicine (Baltimore) 1970, 49(5): 343-74.
12. Blumenfeld, A., Slaugenhaupt, S.A., Axelrod, F.B. et al. *Localization of the gene for familial dysautonomia on chromosome 9 and definition of DNA markers for genetic diagnosis.* Nat Genet 1993, 4(2): 160-4.

13. Blumenfeld, A., Slaugenhaupt, S.A., Liebert, C.B. et al. *Precise genetic mapping and haplotype analysis of the familial dysautonomia gene on human chromosome 9q31.* Am J Hum Genet 1999, 64(4): 1110-8.
14. Chadwick, B.P., Gill, S., Leyne, M. et al. *Cloning, genomic organization and expression of a putative human transmembrane protein related to the Caenorhabditis elegans M01F1.4 gene.* Gene 1999, 240(1): 67-73.
15. Chadwick, B.P., Mull, J., Helbling, L.A. et al. *Cloning, mapping, and expression of two novel actin genes, actin-like-7A (ACTL7A) and actin-like-7B (ACTL7B), from the familial dysautonomia candidate region on 9q31.* Genomics 1999, 58(3): 302-9.
16. Slaugenhaupt, S.A., Blumenfeld, A., Gill, S.P. et al. *Tissue-specific expression of a splicing mutation in the IKBKAP gene causes familial dysautonomia.* Am J Hum Genet 2001, 68(3): 598-605.
17. Anderson, S.L., Coli, R., Daly, I.W. et al. *Familial dysautonomia is caused by mutations of the IKAP gene.* Am J Hum Genet 2001, 68(3): 753-8.
18. Byers, P.H. *Killing the messenger: New insights into nonsense-mediated mRNA decay.* J Clin Invest 2002, 109(1): 3-6.
19. Close, P., Hawkes, N., Cornez, I. et al. *Transcription impairment and cell migration defects in elongator-depleted cells: Implication for familial dysautonomia.* Mol Cell 2006, 22(4): 521-31.
20. Slaugenhaupt, S.A., Mull, J., Leyne, M. et al. *Rescue of a human mRNA splicing defect by the plant cytokinin kinetin.* Hum Mol Genet 2004, 13(4): 429-36.
21. Pearson, J., Pytel, B. *Quantitative studies of ciliary and sphenopalatine ganglia in familial dysautonomia.* J Neurol Sci 1978, 39(1): 123-30.
22. Pearson, J., Pytel, B.A. *Quantitative studies of sympathetic ganglia and spinal cord intermedio-lateral gray columns in familial dysautonomia.* J Neurol Sci 1978, 39(1): 47-59.
23. Pearson, J., Pytel, B.A., Grover-Johnson, N., Axelrod, F., Dancis, J. *Quantitative studies of dorsal root ganglia and neuropathologic observations on spinal cords in familial dysautonomia.* J Neurol Sci 1978, 35(1): 77-92.
24. Pearson, J., Brandeis, L., Goldstein, M. *Tyrosine hydroxylase immunoreactivity in familial dysautonomia.* Science 1979, 206(4414): 71-2.
25. Axelrod, F.B., Iyer, K., Fish, I., Pearson, J., Sein, M.E., Spielholz, N. *Progressive sensory loss in familial dysautonomia.* Pediatrics 1981, 67(4): 517-22.
26. Pearson, J., Dancis, J., Axelrod, F., Grover, N. *The sural nerve in familial dysautonomia.* J Neuropathol Exp Neurol 1975, 34(5): 413-24.
27. Axelrod, F.B., Pearson, J. *Congenital sensory neuropathies. Diagnostic distinction from familial dysautonomia.* Am J Dis Child 1984, 138(10): 947-54.
28. Cheishvili, D., Maayan, C., Smith, Y., Ast, G., Razin, A. *IKAP/hELP1 deficiency in the cerebrum of familial dysautonomia patients results in down regulation of genes involved in oligodendrocyte differentiation and in myelination.* Hum Mol Genet 2007, 16(17): 2097-104.
29. Axelrod, F.B., Hilz, M.J., Berlin, D. et al. *Neuroimaging supports central pathology in familial dysautonomia.* J Neurol 2010, 257(2): 198-206.
30. Cuajungco, M.P., Leyne, M., Mull, J. et al. *Tissue-specific reduction in splicing efficiency of IKBKAP due to the major mutation associated with familial dysautonomia.* Am J Hum Genet 2003, 72(3): 749-58.
31. Cohen, L., Henzel, W.J., Baeuerle, P.A. *IKAP is a scaffold protein of the IkappaB kinase complex.* Nature 1998, 395(6699): 292-6.
32. Krappmann, D., Hatada, E.N., Tegethoff, S. et al. *The I kappa B kinase (IKK) complex is tripartite and contains IKK gamma but not IKAP as a regular component*. J Biol Chem 2000, 275(38): 29779-87.
33. Krogan, N.J., Greenblatt, J.F. *Characterization of a six-subunit holo-elongator complex required for the regulated expression of a group of genes in Saccharomyces cerevisiae.* Mol Cell Biol 2001, 21(23): 8203-12.
34. Otero, G., Fellows, J., Li, Y. et al. *Elongator, a multisubunit component of a novel RNA polymerase II holoenzyme for transcriptional elongation.* Mol Cell 1999, 3(1): 109-18.
35. Winkler, G.S., Petrakis, T.G., Ethelberg, S., Tokunaga, M., Erdjument-Bromage, H., Tempst, P., Svejstrup, J.Q. *RNA polymerase II elongator holoenzyme is composed of two discrete subcomplexes.* J Biol Chem 2001, 276(35): 32743-9.
36. Hawkes, N.A., Otero, G., Winkler, G.S. et al. *Purification and characterization of the human elongator complex.* J Biol Chem 2002, 277(4): 3047-52.
37. Svejstrup, J.Q. *Elongator complex: How many roles does it play?* Curr Opin Cell Biol 2007, 19(3): 331-6.
38. Jablonowski, D., Zink, S., Mehlgarten, C., Daum, G., Schaffrath, R. *tRNAGlu wobble uridine methylation by Trm9 identifies Elongator's key role for zymocin-induced cell death in yeast.* Mol Microbiol 2006, 59(2): 677-88.
39. Rahl, P.B., Chen, C.Z., Collins, R.N. *Elp1p, the yeast homolog of the FD disease syndrome protein, negatively regulates exocytosis independently of transcriptional elongation.* Mol Cell 2005, 17(6): 841-53.
40. Holmberg, C., Katz, S., Lerdrup, M., Herdegen, T., Jaattela, M., Aronheim, A., Kallunki, T. *A novel specific role for I kappa B kinase complex-associated protein in cytosolic stress signaling.* J Biol Chem 2002, 277(35): 31918-28.
41. Johansen, L.D., Naumanen, T., Knudsen, A. et al. *IKAP localizes to membrane ruffles with filamin A and regulates actin cytoskeleton organization and cell migration.* J Cell Sci 2008, 121(Pt. 6): 854-64.
42. Gardiner, J., Barton, D., Marc, J., Overall, R. *Potential role of tubulin acetylation and microtubule-based protein trafficking in familial dysautonomia.* Traffic 2007, 8(9): 1145-9.
43. Creppe, C., Malinouskaya, L., Volvert, M. L. et al. *Elongator controls the migration and differentiation of cortical neurons through acetylation of alpha-tubulin.* Cell 2009, 136(3): 551-64.
44. Chen, Y.T., Hims, M.M., Shetty, R.S., Mull, J., Liu, L., Leyne, M., Slaugenhaupt, S.A. *Loss of mouse Ikbkap, a subunit of elongator, leads to transcriptional deficits and embryonic lethality that can be rescued by human IKBKAP.* Mol Cell Biol 2009, 29(3): 736-44.
45. Smith, A.A., Dancis, J. *Response to intradermal histamine in familial dysautonomia - A diagnostic test.* J Pediatr 1963, 63: 889-94.
46. Mahloudji, M., Brunt, P.W., McKusick, V.A. *Clinical neurological aspects of familial dysautonomia.* J Neurol Sci 1970, 11(4): 383-95.
47. Axelrod, F.B. *A world without pain or tears.* Clin Auton Res 2006, 16(2): 90-7.
48. Smith, A.A., Dancis, J. *Taste discrimination in familial dysautonomia.* Pediatrics 1964, 33: 441-3.
49. Smith, A., Farbman, A., Dancis, J. *Absence of taste-bud papillae in familial dysautonomia.* Science 1965, 147: 1040-1.
50. Smith, A.A., Farbman, A., Dancis, J. *Tongue in familial dysautonomia, a diagnostic sign.* Am J Dis Child 1965, 110: 152-4.
51. Hilz, M.J., Azelrod, F.B., Schweibold, G., Kolodny, E.H. *Sympathetic skin response following thermal, electrical, acoustic, and inspiratory gasp stimulation in familial dysautonomia patients and healthy persons.* Clin Auton Res 1999, 9(4): 165-77.
52. Bernardi, L., Hilz, M., Stemper, B., Passino, C., Welsch, G., Axelrod, F.B. *Respiratory and cerebrovascular responses to hypoxia and hypercapnia in familial dysautonomia*. Am J Respir Crit Care Med 2003, 167(2): 141-9.
53. Ganz, S.B., Levine, D.B., Axelrod, F.B., Kahanovitz, N. *Physical therapy management of familial dysautonomia.* Phys Ther 1983, 63(7): 1121-4.
54. Axelrod, F.B., Gouge, T.H., Ginsburg, H.B., Bangaru, B.S., Hazzi, C. *Fundoplication and gastrostomy in familial dysautonomia.* J Pediatr 1991, 118(3): 388-94.

55. Krausz, Y., Maayan, C., Faber, J., Marciano, R., Mogle, P., Wynchank, S. *Scintigraphic evaluation of esophageal transit and gastric emptying in familial dysautonomia.* Eur J Radiol 1994, 18(1): 52-6.
56. Hensinger, R.N., MacEwen, G.D. *Spinal deformity associated with heritable neurological conditions: spinal muscular atrophy, Friedreich's ataxia, familial dysautonomia, and Charcot-Marie-Tooth disease.* J Bone Joint Surg Am 1976, 58(1): 13-24.
57. Axelrod, F.B., Goldberg, J.D., Ye, X.Y., Maayan, C. *Survival in familial dysautonomia: Impact of early intervention.* J Pediatr 2002, 141(4): 518-23.
58. Hilz, M.J., Kolodny, E.H., Neuner, I., Stemper, B., Axelrod, F.B. *Highly abnormal thermotests in familial dysautonomia suggest increased cardiac autonomic risk.* J Neurol Neurosurg Psychiatry 1998, 65(3): 338-43.
59. Axelrod, F.B. *Familial dysautonomia: a review of the current pharmacological treatments.* Expert Opin Pharmacother 2005, 6(4): 561-7.
60. Axelrod, F.B., Schneider, K.M., Ament, M.E., Kutin, N.D., Fonkalsrud, E.W. *Gastroesophageal fundoplication and gastrostomy in familial dysautonomia.* Ann Surg 1982, 195(3): 253-8.
61. Yoslow, W., Becker, M.H., Bartels, J., Thompson, W.A. *Orthopaedic defects in familial dysautonomia. A review of sixty-five cases.* J Bone Joint Surg Am 1971, 53(8): 1541-50.
62. Hayek, S., Laplaza, F.J., Axelrod, F.B., Burke, S.W. *Spinal deformity in familial dysautonomia. Prevalence, and results of bracing.* J Bone Joint Surg Am 2000, 82-A(11): 1558-62.
63. Anderson, S.L., Qiu, J., Rubin, B.Y. *EGCG corrects aberrant splicing of IKAP mRNA in cells from patients with familial dysautonomia.* Biochem Biophys Res Commun 2003, 310(2): 627-33.
64. Anderson, S.L., Qiu, J., Rubin, B.Y. *Tocotrienols induce IKBKAP expression: A possible therapy for familial dysautonomia.* Biochem Biophys Res Commun 2003, 306(1): 303-9.
65. Anderson, S.L., Rubin, B.Y. *Tocotrienols reverse IKAP and monoamine oxidase deficiencies in familial dysautonomia.* Biochem Biophys Res Commun 2005, 336(1): 150-6.
66. Miller, C.O., Skoog, F., Okumura, F.S., Von-Saltza, M.H., Strong, F. *Structure and synthesis of kinetin.* J Am Chem Soc 1955, 78: 2662-3.
67. Miller, C.O., Skoog, F., Okumura, F.S., Von-Saltza, M.H., Strong, F. *Isolation, structure and synthesis of kinetin, a substance promoting cell division.* J Am Chem Soc 1956, 78: 1375-80.
68. Miller, C.O., Skoog, F., Von-Saltza, M.H., Strong, F. *Kinetin, a cell division factor from deoxyribonucleic acid.* J Am Chem Soc 1955, 77: 1392.
69. Rattan, S.I., Clark, B.F. *Kinetin delays the onset of ageing characteristics in human fibroblasts.* Biochem Biophys Res Commun 1994, 201(2): 665-72.
70. Olsen, A., Siboska, G.E., Clark, B.F., Rattan, S.I. *N(6)-Furfuryladenine, kinetin, protects against Fenton reaction-mediated oxidative damage to DNA.* Biochem Biophys Res Commun 1999, 265(2): 499-502.
71. Verbeke, P., Siboska, G. E., Clark, B.F., Rattan, S.I. *Kinetin inhibits protein oxidation and glycoxidation in vitro.* Biochem Biophys Res Commun 2000, 276(3): 1265-70.
72. McCullough, J.K. *Clinical study of safety and efficacy of using topical kinetin 0.1% (Kinerase) to treat photodamaged skin*. Cosmet Derma 2002, 15: 29.
73. Hims, M.M., Ibrahim, E.C., Leyne, M. et al. *Therapeutic potential and mechanism of kinetin as a treatment for the human splicing disease familial dysautonomia.* J Mol Med 2007, 85(2): 149-61.
74. Lee, G., Papapetrou, E.P., Kim, H. et al. *Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs.* Nature 2009, 461(7262): 402-6.
75. Gold-von Simson, G., Goldberg, J.D., Rolnitzky, L.M. et al. *Kinetin in familial dysautonomia carriers: Implications for a new therapeutic strategy targeting mRNA splicing.* Pediatr Res 2009, 65(3): 341-6.