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The Molecular Basis of Familial Dysautonomia: Overview, New Discoveries and Implications for Directed Therapies

Berish Y. Rubin · Sylvia L. Anderson

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Abstract Familial dysautonomia (FD) is a sensory and autonomic neuropathy that affects the development and survival of sensory, sympathetic, and some parasympathetic neurons. It is autosomally inherited and occurs almost exclusively among individuals of Ashkenazi Jewish descent. The pathological and clinical manisfestations of FD have been extensively studied and therapeutic modalities have, until recently, focused primarily on addressing the symptoms experienced by those with this fatal disorder. The primary FD-causing mutation is an intronic nucleotide substitution that alters the splicing of the *IKBKAP*-derived transcript. Recent efforts have resulted in the development of new therapeutic modalities that facilitate the increased production of the correctly spliced transcript and mitigate the symptoms of those with FD. Furthermore, the recent demonstration of the reduced presence of monoamine oxidase A in cells and tissues of individuals with FD has provided new insight into the cause of hypertensive crises experienced by these patients.

Keywords Autonomic nervous system · Familial dysautonomia · IKAP · *IKBKAP* · Monoamine oxidase · RNA splicing

Introduction

Described in 1949 (Riley et al. 1949), familial dysautonomia (FD), also known as Riley-Day syndrome, is an autosomal recessive disorder affecting the development and survival of sensory, sympathetic, and some parasympathetic neurons. FD is a member of a group of genetically distinct disorders known as Hereditary Sensory and Autonomic Neuropathies (HSAN) that impact the development, survival, function, and migration of sensory and autonomic nerves (Klein and Dyck 2005). FD is characterized as HSANIII.

Infants with FD often present with feeding difficulties that result from poor oral coordination and difficulty swallowing. Initial clinical diagnosis of FD is supported by criteria, which include: (1) parents of Ashkenazi Jewish (AJ) descent (Brunt and McKusick 1970); (2) absence of axonal flare after intradermal histamine injection (Smith and Dancis 1963); (3) absence of fungiform papillae on the tongue (Smith et al. 1965); (4) diminished deep tendon response (Riley 1974); and (5) defective lacrimation (Riley et al. 1949). Since the identification of the genetic cause of FD (Anderson et al. 2001; Slaugenhaupt et al. 2001), the diagnosis is now confirmed by the presence of the causative mutation.

Recent advances have provided new insight into the underlying genetic and biochemical deficits present in this patient population, and are enabling the development of new therapeutic approaches that address these deficits.

Genetics

FD is found almost exclusively among individuals of AJ descent (Brunt and McKusick 1970) and, in this population, is transmitted as an autosomal recessive disorder. In 1993, Blumenfeld and coworkers localized the gene for FD to chromosome 9q31–q33 (Blumenfeld et al. 1993); further mapping studies published in 1999 provided a more precise

B. Y. Rubin (✉) · S. L. Anderson
Department of Biological Sciences, Laboratory for Familial Dysautonomia Research, Fordham University, 441 East Fordham Road, Bronx, NY 10458, USA
e-mail: rubin@fordham.edu

chromosomal mapping of the disease-causing locus (Blumenfeld et al. 1999). In 2001, the causative mutations responsible for this genetic disorder were determined to be located in the *IKBKAP* gene, which encodes a protein termed IKAP (IkB kinase complex associated protein). Two mutations have been identified in individuals of AJ descent (Anderson et al. 2001; Slaugenhaupt et al. 2001). The more common mutation, termed IVS20 + 6T→C, is present in greater than 98% of the disease-causing alleles, and is a T→C transition in the sixth base of the donor splice site of intron 20. This mutation causes for the inefficient use of the intron 20 donor splice site, which results in the simultaneous removal of the intron 19, exon 20, and intron 20 sequences from the mature IKAP transcript. The skipping of the exon 20 sequence generates a frameshift, which introduces a stop codon in the reading frame of exon 21 (Fig. 1). The protein generated from this transcript lacks the amino acids encoded by exons 20–37 of *IKBKAP*.

The less common mutation present in those of AJ descent is a G→C transversion in exon 19 that results in an arginine to proline substitution (R696P) that disrupts a consensus serine/threonine kinase phosphorylation site and results in defective phosphorylation of IKAP (Anderson et al. 2001). This rare mutation has never been detected in a homozygous state.

The carrier frequency of the IVS20 + 6T→C mutation in the AJ population is approximately 1 in 30 and the carrier frequency of R696P is approximately 1 in 400 (Anderson et al. 2001, Slaugenhaupt et al. 2001). This carrier frequency is close to that predicted by the reported incidence rate of FD of 1 in 3,703 live births in Israel (Maayan et al. 1987). A recent study of subpopulations of individuals of AJ descent reveals that in individuals of Polish descent, the carrier frequency of the IVS20 + 6 T→C mutation is 1 in 18 (Lehavi et al. 2003).

The recent genetic characterization of a child with FD who inherited the IVS20 + 6T→C mutation from his father and an unknown mutation from his non-AJ mother, led to the discovery of a novel mutation, which is a C→T transition in exon 26 of *IKBKAP*, which results in a proline to leucine substitution (P914L) (Leyne et al. 2003).

The *IKBKAP* Gene and its Gene Product, IKAP

The *IKBKAP* gene consists of 37 exons and encodes a protein 1,332 amino acids in length. Northern blot analysis reveals that this gene generates two transcripts that are 5.9 and 4.8 kb in length (Slaugenhaupt et al. 2001). Both transcripts encode the identical protein and differ in the length of their 3′ untranslated region.

IKAP, the product of the *IKBKAP* gene, was originally, incorrectly reported to be a scaffold protein involved in the assembly of the IkB kinase complex (Cohen et al. 1998). It has subsequently been determined to be a component of the human Elongator complex (Hawkes et al. 2002). Biological activities ascribed to the Elongator complex include a role in transcription (Otero et al. 1999), histone acetylation (Winkler et al. 2002), cellular exocytosis (Rahl et al. 2005), tRNA modification (Esberg et al. 2006), and control of expression of genes involved in cell motility (Close et al. 2006). IKAP has also been reported to be a c-Jun N-terminal kinase (JNK)-associated protein capable of JNK stress kinase activation (Holmberg et al. 2002). The biological activities of IKAP and the Elongator complex, as well as the mechanism, by which the reduced presence of IKAP mediates the neurological dysfunction observed in

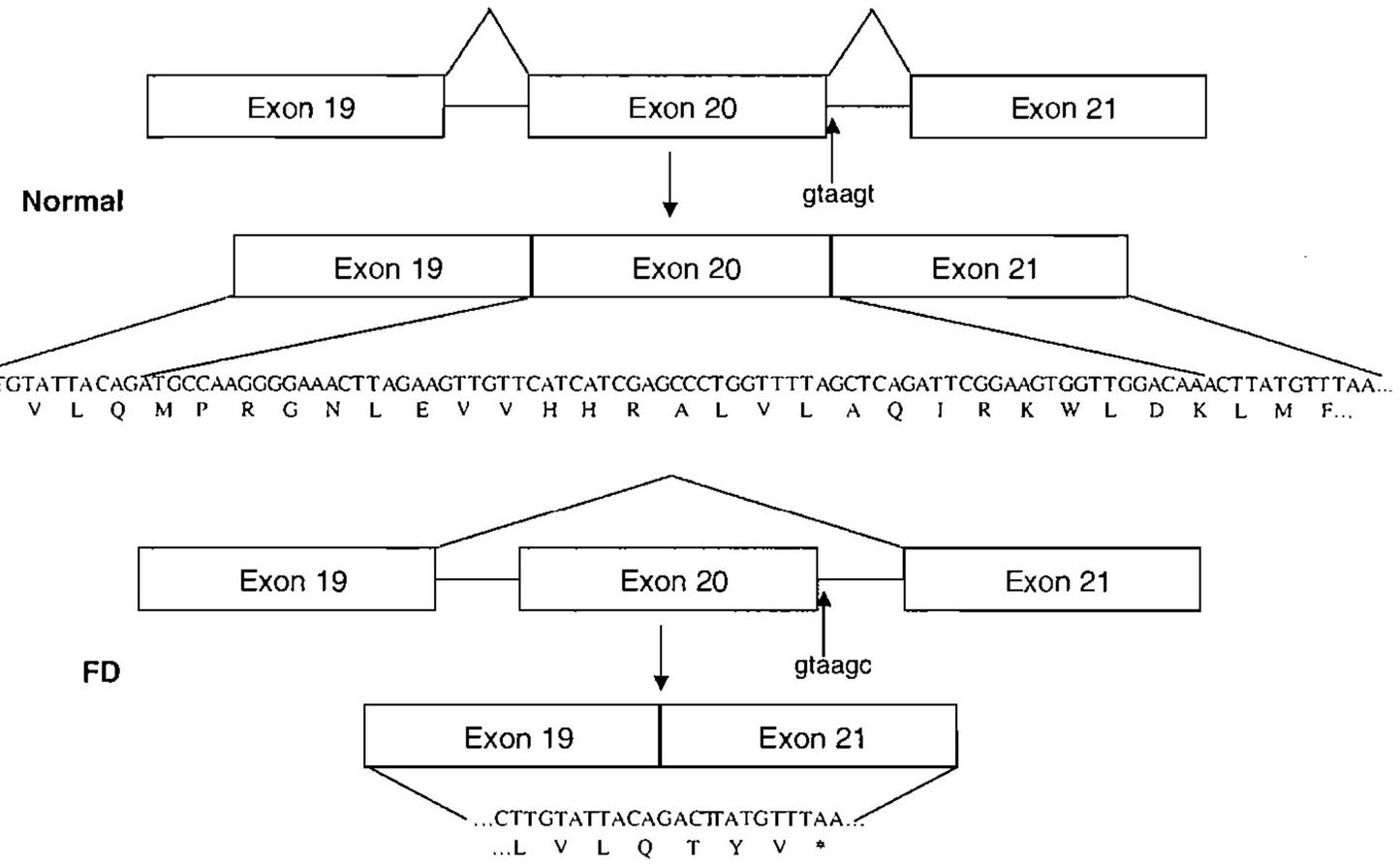

Fig. 1 The FD-causing IVS20 + 6T→C mutation in *IKBKAP* results in excision of exon 20 and generation of a transcript that encodes a truncated protein

individuals with FD continues to be studied (Svejstrup 2007).

Neuropathological Abnormalities in FD Patients

The neurological deficits in individuals with FD are due to the inadequate development and survival of sensory and autonomic neurons with the sympathetic neurons being more affected than the parasympathetic neurons. Study of neuronal presence in individuals with FD reveals that the sural nerve has a reduced transverse fascicular area, diminished numbers of myelinated axons and very few nonmyelinated axons (Aguayo et al. 1971; Pearson et al. 1974, 1975). The neuronal presence in dorsal root ganglia is markedly diminished in young patients and, while the weight of the dorsal root ganglion normally increases with age, in individuals with FD, there is a progressive degeneration of these neurons. Quantitation of the neurons in the C8 dorsal root ganglia in normal individuals reveals a count of between 42,500 and 53,600. In patients with FD, the C8 dorsal root ganglia contain between 4,090 and 8,590 neurons, with the smaller number of neurons observed in older patients. In older patients, a loss of dorsal column myelinated axons is seen in the lumbar fasciculus gracilis, cervical fasciculus cuneatus, and interfascicular fasciculus. Within the spinal cord, a severe depletion of axons is observed in the lateral root entry zones and Lissauer's tracts (Pearson et al. 1978). Sphenopalatine ganglia are reported to be less than one fifth normal in volume, and their total neuronal content is reduced to a mean of 1,510, which represents approximately 3% of that present in control subjects (Pearson and Pytel 1978a). The volume of the superior cervical sympathetic ganglia in adult FD patients is reduced to 34% of the control, the packing density is 37% of the control, and mean total number of ganglionic neurons is 120,000 as compared to 1,060,000 in controls. In addition, the number of preganglionic neurons in the first three thoracic cord segments is reduced in FD patients (Pearson and Pytel 1978b). Examination of neuronal presence in the myenteric plexus in appendices of patients with FD and control subjects reveals a ganglionic density of 10.13 and 5.01 per mm^2 in control and FD individuals, respectively. Neuronal density in the myenteric plexus was 70.12 per mm^2 in controls and 22.09 per mm^2 in those with FD (Bar-Shai et al. 2004).

Characterization of nerve fiber density and neuropeptide content in skin punch biopsies of patients with FD reveals a diminished presence of nerve fibers in the epidermis, the subepidermal neural plexus, and the deep dermis. Substance P and calcitonin gene related peptide immunoreactive protein are virtually absent from the subepidermal neural plexus (Hilz et al. 2004). Ultrastructural studies of peripheral blood vessels reveal a reduced presence or a lack of sympathetic innervation of these vessels (Grover-Johnson and Pearson 1976).

FD Symptoms

FD is characterized by a variety of symptoms including: (1) dysautonomic/hypertensive crisis; (2) dyscoordination of the gastrointestinal tract; (3) respiratory dysfunction; (4) altered sensitivity to pain and temperature; (5) cardiovascular dysfunction; and often (6) spinal curvature.

Dysautonomic Crises

Patients in dysautonomic crisis exhibit a constellation of symptoms, which include hypertension, tachycardia, nausea, vomiting, erythematous skin blotching, hyperhidrosis, and hypersalivation with drooling. Crisis is also often accompanied by personality changes that include withdrawal, irritability, and excitation (Pearson et al. 1974; Axelrod et al. 2000; Axelrod 2004).

Dyscoordination of the Gastrointestinal Tract

The dyscoordination of the gastrointestinal tract and, in particular, esophageal dysmotility, delayed gastric emptying, and poor swallowing control lead to recurrent aspirations, pneumonia, and the destruction of lung tissue (Linde and Westover 1962; Gyepes and Linde 1968; Margulies et al. 1968; Krausz et al. 1994; Maayan 2006). The reduced presence of neurons in the myenteric ganglia (Bar-Shai et al. 2004) may be responsible for the gastrointestinal dyscoordination noted in those with FD.

Respiratory Dysfunction

Respiratory dysfunction in individuals with FD can be the result of compromised lung capacity due to the damage caused by recurrent aspirations and the restrictive lung capacity resulting from scoliosis and/or kyphosis (Maayan 2006). Respiratory dysregulation is also attributable to a faulty chemoreceptor response to hypoxia and hypocapnia (Bernardi et al. 2003). In contrast to what is observed in normal individuals, exposure of individuals with FD to hypoxic conditions results in hypotension, hypoventilation, bradycardia, and potential respiratory arrest. In normal individuals, hyperventilation, which elicits hypocapnia, will result in a period of hypoventilation or apnea until the CO_2 reaches levels that stimulate ventilation. In individuals

with FD, hyperventilation induces prolonged apnea, oxygen desaturation, and a falling blood pressure.

Altered Sensory Perception

Individuals with FD exhibit diminished skin sensitivity to pain, temperature, and vibration. These deficits are often evident at birth and progress during aging (Axelrod et al. 1981). With the progression of the insensitivity to pain and temperature, unrecognized injuries and burns become more common. The ataxia-like walk common to individuals with FD is likely the result of poor sensory feedback. Despite these peripheral sensory deficits, individuals with FD have intact visceral and peritoneal pain perception (Challands and Facer 1998; Axelrod et al. 1981). The reduced sensitivity to temperature, pain, and vibration and the compromised proprioception that is responsible for the poor balance and unsteady gait observed in individuals with FD are thought to be due to the reduced presence of neurons in the dorsal root ganglion, The decrease in peripheral innervation throughout the epidermal and subepidermal layers, and the apparent absence of substance P and calcitonin gene related peptide in the skin also likely to play a role in the reduced sensitivity to temperature and pain (Hilz et al. 2004).

Cardiovascular Dysfunction

Compromised cardiovascular response and function in individuals with FD is reflected in (1) a reduced orthostatic blood pressure without reflex tachycardia and vascular resistance that occurs upon standing (Brown et al. 2003); (2) a reduced cardiac parasympathetic response (Hilz et al. 1999); (3) a muted exercise-mediated increase in pulse rate (Glickstein et al. 1993); and (4) a prolonged QT interval with a lack of appropriate shortening with exercise (Glickstein et al. 1993). The reduced presence of cervical and thoracic ganglia likely play a role in the cardiac autonomic dysfunction.

Spinal Curvature

Spinal deformities, in the form of scoliosis and kyphosis, are common in FD patients and often lead to restrictive lung disease and pneumonia. In a recent study of 123 patients who reached the age of 20 or more, 83% had spinal deformity, 56% had scoliosis only, 25% had scoliosis and kyphosis, and 2% had kyphosis alone (Hayek et al. 2000). These findings are in line with similar studies performed on smaller populations of FD patients (Yoslow et al. 1971; Albanese and Bobechko 1987). Attempts at bracing the back to halt or reverse the progression of the spinal curvature have, in general, not been successful and most patients require arthrodesis. The demonstrated association between altered proprioceptive and vibratory sensation with the development of idiopathic scoliosis (Yekutiel et al. 1981; Wyatt et al. 1986) has led to the suggestion that the disruption of the afferent sensory pathways in individuals with FD may be the cause of spinal curvature.

Catecholamine Responsiveness and Metabolism

Individuals with FD exhibit an exaggerated responsiveness to, and a dysregulated metabolism of, catecholamines. In 1964, Smith and Dancis reported exaggerated blood pressure increases in patients receiving systematic infusions of norepinephrine (NE) (Smith and Dancis 1964). Catecholamine hypersensitivity in this patient population is also reflected in the exaggerated hypotensive response to infusion with methacholine (Smith et al. 1965a); excessive meiosis following instillation of methacoline drops into the conjunctival sac (Smith et al. 1965b); and prolonged vasoconstriction in peripheral blood vessels following NE application (Bickel et al. 2002). Study of catecholamine metabolism in FD patients has demonstrated: (1) a marked elevation of plasma NE and dopamine (DA) levels in response to emotional or physical stress (Axelrod 2002); (2) elevated levels of homovanillic acid (HVA) and normal to low levels of vanillymandelic acid (VMA) in urine (Smith et al. 1963; Gitlow et al. 1970; Smith and Dancis 1967); (3) elevated levels of NE, a reduced presence of dihydroxyphenylglycol (DHPG), an elevated dihydroxyphenylalanine (DOPA)/DHPG ratio, and a normal dihydroxy-phenylacetic acid (DOPAC)/DHPG ratio in plasma (Axelrod et al. 1996); and (4) elevated plasma levels of NE and DOPA, and lower DHPG levels when in the supine position and unchanged plasma norepinephrine levels with erect posture (Axelrod et al. 1996).

Clinical Management of FD

Therapeutic regimens have until recently been supportive and focused only on the treatment of the symptoms associated with FD. Artificial tears and cautery of the tear ducts are used to increase eye moisture levels (Gold-von Simson and Axelrod 2006). In patients with a misdirected swallow, a gastrostomy is often recommended to prevent aspiration and to allow for increased caloric intake and effective hydration (Axelrod et al. 1991). Patients experiencing gastroesophogeal reflux are placed on a regimen of anti-reflux medications (Axelrod et al. 2004). Patients who fail

to respond to these medications often undergo a fundoplication (Axelrod et al. 1991). Inhalation of bronchiodialators and chest physiotherapy (Giarraffa et al. 2005) are used to improve pulmonary function. During dysautonomic crises, clonidine, a centrally-acting adrenergic agonist is used to control hypertension and diazepam, a benzodiazepine with CNS depressant properties, is used as an antiemetic (Gold-von Simson and Axelrod 2006). Postural hypotension is often treated with fludocortisone, a corticosteroid, which increases blood pressure by causing a marked retention of sodium retention, or midodrine, an alpha-sympathomimetic agent, which exhibits a vasoconstrictor effect (Axelrod et al. 2005).

Despite the use of this therapeutic approach, the prognosis for individuals with FD is dire. A recent survival study of 551 FD patients revealed that 325 of them (59%) died before reaching the age of 20 (Axelrod et al. 2002).

Recent Developments

Monoamine Oxidase A Deficiency

Monoamine oxidase A and B (MAO A and MAO B) are key isozymes that degrade biogenic and dietary monoamines. MAO A preferentially oxidizes NE and serotonin (Johnston 1968) and MAO B preferentially degrades phenylethylamine (Knoll and Magyar 1972). DA and tyramine are oxidized by both forms of MAO. Due to MAO's role in degrading neurotransmitters, MAO inhibitors (MAOIs) have been developed and used as antidepressants (Ravaris et al. 1978; Tollefson 1983). Following the ingestion of tyramine-containing foods, individuals taking MAOIs may experience hypertensive crises, which are characterized by hypertension, tachycardia, nausea, vomiting, diaphoresis, and sometimes intracranial hemorrhage (symptoms similar to those experienced by an individual with FD during a dysautonomic crisis) (Blackwell 1963; Blackwell et al. 1964; Horowitz et al. 1964; Walker et al. 1984; Lippman and Nash 1990; Anderson et al. 1993). The ingested tyramine, which is normally metabolized by MAO A in the gastrointestinal tract, is not degraded in those taking MAOIs and enters the systemic circulation (Ilett et al. 1980; McCabe and Tsuang 1982; Hasan et al. 1988). Tyramine, which is a good substrate for the high-affinity adrenergic uptake system, is concentrated in the adrenergic neurons where it causes the release of NE from the intracellular vesicle (Mayer 1980; Youdim et al. 1986). The released NE triggers a hypertensive crisis. Due to their inability to process biogenic amines, individuals taking MAOIs also exhibit hypersensitivity to a variety of sympathomimetic agents such as NE.

Table 1 IKAP, MAO-A and MAO-B RNA levels in FD-derived tissues (% of normal tissue levels)

Tissue type	IKAP	MAO-A	MAO-B
Fetal liver	18	32	108
Fetal kidney	15	34	92
Peripheral blood	17	18	nd

nd = not determined

As a reduced presence of a biogenic amine-metabolizing enzyme in individuals with FD could explain: (1) the onset of dysautonomic crises following emotional events that trigger the release of NE and DA (Axelrod 2002); (2) the hypertensive response to infusion by low doses of NE (Smith and Dancis 1964, Dancis 1968); (3) the prolonged vasoconstriction in peripheral blood vessels following NE application (Bickel et al. 2002); (4) the elevated levels of NE and reduced levels of DHPG and DOPAC present in the plasma of FD patients (Axelrod et al. 1996); and (5) the precipitation of dysautonomic crises following the ingestion of tyramine-containing foods (Anderson and Rubin 2005), Anderson and coworkers examined the levels of MAO A and B in FD-derived cells and tissues, and observed the reduced presence of MAO A mRNA, but normal levels of the MAO B mRNA (Table 1). The reduced level of MAO A in individuals with FD is likely a cause of their abnormal metabolism of and response to the catecholamines, as well as the dysautonomic crises experienced by individuals after the consumption of tyramine-containing foods. The demonstrated reduced presence of MAO A supports the reported suggestion by Axelrod and colleagues that the neurochemical anomalies observed in individuals with FD may be due to "limited oxidative deamination" of the catecholamines (Axelrod et al. 1996).

The Development of Targeted Therapeutics

Mutations that affect RNA splicing are a major cause of human genetic diseases. These diseases may occur as a result of mutations in the splice donor or splice acceptor sequences, disrupting these sites, or in exons or introns, generating cryptic splice junctions. While many of these splice-altering mutations eliminate all production of the appropriately spliced gene product, in some cases, mutations affecting splicing result in a milder or adult onset form of the disease, in which "leaky" alternative mRNA splicing is observed that produces both mutant (skipped exon) and wild-type (full-length) transcripts (Boerkoel et al. 1995; Huie et al. 1998; Beck et al. 1999; Kure et al. 2000; Svenson et al. 2001a, b). The detected presence of some exon 20-containing (wild-type) IKAP transcript and

full-length protein (Anderson et al. 2003a; Cuajungco et al. 2003) in cells and tissues derived from individuals with FD homozygous for the IVS20 + 6T→C, prompted investigators to screen large numbers of commercially available compounds and nutritional supplements, as well as compound libraries in search of substances capable of increasing the production of the correctly spliced transcript, which could be used to treat the underlying deficit in individuals with FD. Anderson and coworkers, in 2003, reported the ability of tocotrienols, a form of vitamin E, to upregulate transcription of the *IKBKAP* gene and, due to the leaky nature of the IVS20 + 6T→C mutation, to increase production of the correctly spliced transcript and normal protein in FD-derived cells (Anderson et al. 2003a). Subsequent reports have demonstrated the ability of epigallocatechin gallate (EGCG), a component of green tea (Anderson et al. 2003b), and kinetin, a plant cytokinin, (Slaugenhaupt et al. 2004) to alter the splicing process and increase the levels of wild-type transcript and full-length protein produced in FD-derived cells. In addition to facilitating the production of functional IKAP, treatment of FD-derived cells with tocotrienols and EGCG results in an enhanced production of MAO A RNA and protein (Anderson and Rubin 2005).

Therapeutic Outcomes

The ready availability of tocotrienols and EGCG and the reported safety of these compounds have allowed for their unrestricted use by individuals with FD. A study of the impact of tocotrienol supplementation in individuals with FD has revealed (1) an increased production of the full-length IKAP transcript in blood cells of FD patients; (2) an increased production of the MAO A transcript in blood cells; (3) a reduced frequency of dysautonomic crises; (4) a postexercise increase in pulse rate and an exercise-mediated shortening of the QTc interval; and (5) an increase in the level of eye moisture (Anderson and Rubin 2005; Rubin et al. 2007). This success noted with the tocotrienols represents one of few therapeutic modalities that address and attempt to directly modify the impact of a mutation that modifies the splicing process. Clinical studies evaluating the impact of EGCG on individuals with FD and preclinical studies evaluating the safety of kinetin are currently underway.

Conclusions and Future Direction

Since the gene for FD was identified in 2001, genetic screening for the general population and prenatal screening for families where both parents are determined to be carriers are now available. As a result, the number of children born with FD has begun, and is expected to continue, to decline. Research efforts currently underway are expected to improve the quality of life for those with FD and should provide insight into the function of the spliceosome and regulation of the splicing process that may be applicable to a variety of genetic disorders caused by splicing mutations.

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