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Hereditary dysautonomias: current knowledge and collaborations for the future

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Abstract The hereditary dysautonomias (H-Dys) are a large group of disorders that affect the autonomic nervous system. Research in the field of H-Dys is very challenging, because the disorders involve interdisciplinary, integrative, and "mind-body" connections. Recently, medical scientists, NIH/NINDS representatives, and several patient support groups gathered for the first time in order to discuss recent findings and future directions in the H-Dys field. The H-Dys workshop was instrumental in promoting interactions between basic science and clinical investigators. It also allowed attendees to have an opportunity to meet each other, understand the similarities between the various forms of dysautonomia, and experience the unique perspective offered by patients and their families. Future advances in H-Dys research will depend on a novel multi-system approach by investigators from different medical disciplines, and it is hoped that towards a common goal, novel "bench-to-bedside" therapeutics will be developed to improve the lives of, or even cure, patients suffering from dysautonomic syndromes.

Key words autonomic nervous system · catecholamine · neuropathy · amyloidosis

Introduction

There is increasing awareness that autonomic dysfunction, or dysautonomias, may be caused by various genetic perturbations. The hereditary dysautonomias (H-Dys) are a heterogeneous group of disorders. The better known dysautonomias are caused by genetic mutations directly or indirectly affecting catecholamine synthesis or metabolism. Several gene mutations have been identified which influence development and survival of autonomic neurons and produce variable but debilitating symptoms. Additional genetic and environmental factors affect disease severity. Although laboratory and clinical advancements have led to improved care for patients and their families, many hurdles remain. On October 3rd and 4th, 2002, in Rockville Maryland, the National Institute of Neurological Disorders and Stroke (NINDS), and the Office of Rare Diseases (ORD), National Institutes of Health (NIH), sponsored a landmark workshop on H-Dys, attended by numerous basic and clinical scientists, NIH representatives, members of advocacy groups and caregivers.

The workshop consisted of three excellent sessions, namely: (i) "Familial Dysautonomia (FD) – Genetics and Other Factors"; (ii) "Inherited Dysautonomias: Familial Dysautonomia and Beyond"; and (iii) "Lessons from Other Dysautonomias: An Overview". A comprehensive list of speakers is outlined on Table 1. This first NIH sponsored H-Dys workshop was instrumental in promoting scientific interactions between scientists and clinical investigators. It allowed researchers from different fields to discuss common themes in dysautonomia, and to experience the unique viewpoints of patients and their families. This report succinctly reviews each discussion from the workshop in more detail, summarizes the conclusions, and outlines recommendations for future directions in the H-Dys field.

Familial dysautonomia (FD)

Neuropathology and pathophysiology of FD

Riley et al. (1949) first reported 5 children of Ashkenazi Jewish descent, with central autonomic dysfunctions and defective lacrimation. Since that original observation more than 500 patients with familial dysautonomia (FD; Riley-Day syndrome) have been diagnosed [9, 10]. Although FD has a variable phenotype at both the clinical and molecular level, all patients exhibit lack of overt emotional tears, absence of lingual fungiform papillae, decreased deep tendon reflexes, and loss of axon flare following intradermal histamine injection. Patients with FD exhibit sensory dysfunction as well as peripheral and central autonomic dysfunction. Sensory dysfunction includes diminished perception of pain and abnormal temperature appreciation. The autonomic dysfunction is manifested as hypotonia, excessive sweating and salivation, skin blotching, transient severe arterial hypertension, and other peripheral sympathetic deficits [9, 10]. Patients with FD are highly susceptible to physical or emotional stress which can lead to "dysautonomic crisis." A crisis is associated with a constellation of symptoms that are consistent with central sympathetic activation. In addition to tachycardia, hypertension, increased sweating and hyper-salivation, there is irritability, withdrawal, anxiety, and occasional self-mutilation [9, 10]. Although early speculations suggested that the disease was caused by a dysfunction in neurotrans-

Table 1 A complete list of the participants in the first Hereditary Dysautonomia Workshop sponsored by the NINDS and ORD

Session 1[a]	
Felicia B. Axelrod, M. D.	Familial Dysautonomia: Historical, Neuropathological, and Clinical Overview
Susan A. Slaugenhaupt, Ph. D.	Genetics of Familial Dysautonomia – Tissue Specific Expression of the *IKBKAP* Gene Mutations
Max J. Hilz, M. D., Ph. D.	Autonomic and Sensory Assessment and Evaluation of Familial Dysautonomia with Standardized Tests
Gail E. Sonenshein, Ph. D.	Future Directions for Familial Dysautonomia
Jesper Q. Svejstrup, Ph. D.	What Is IKAP and Speculations on its Role in the Development, Maintenance and Survival of Autonomic and Sensory Neurons
William R. Kennedy, M. D.	Secondary Neurotransmitter Aberrations as Demonstrated by Immunoreactive Stains in Dermal Neurons
Yang Xu, Ph. D.	Construction of a Mouse Model to Study Mechanisms of Pathogenesis in Familial Dysautonomia
Berish Y. Rubin, Ph. D.	Moderator: Open Discussion and Q & A
Session 2[b]	
David S. Goldstein, M. D., Ph. D.	Dysautonomia in Familial Parkinson's Disease
Sonia R. Peltzer, M. D.	Setting Priorities for Basic, Disease-Oriented, and Patient-Oriented Research about Inherited Dysautonomias: A Patient Advocacy Perspective
Yukio Ando, M. D., Ph. D.	Familial Amyloidotic Polyneuropathy
Maureen K. Hahn, Ph. D.	NET Deficiency: Perspective of a Molecular Researcher
Stephen B. Liggett, M. D.	Adrenoreceptor Polymorphisms Affecting Autonomic Regulation
Italo Biaggioni, M. D.	Hypertension in Autonomic Disorders: Genetic Factors and Phenotyping
David S. Goldstein, M. D., Ph. D.	Moderator: Open Discussion and Q & A
Session 3[c]	
David Robertson, M. D.	Genetic Disorders of Catecholamine Metabolism
Linda J. Smith	NET Deficiency: Perspective of a Patient
Giris Jacob, M. D., D.Sc.	Neuropathic Postural Tachycardia Syndrome
Jens Jordan, M. D.	Genetic Factors in Blood Pressure Regulation
Daniel T. O'Connor, M. D.	Genetic Determinants of Autonomic Blood Pressure Control
David Robertson, M. D.	Moderator: Open Discussion and Q & A
Katrina Gwinn-Hardy, M. D. Quandra Scudder, B. S.	Summary of Sessions and Directions for the Future
Alan E. Guttmacher, M. D.	Moderator: Session Summary

[a] *Familial Dysautonomia (FD) – Genetics and Other Factors:* this session was chaired by Dr. Axelrod.
[b] *Inherited Dysautonomias: Familial Dysautonomia and Beyond:* this session was chaired by Dr. Goldstein.
[c] *Lessons from Other Dysautonomias: An Overview:* this session was chaired by Dr. Robertson.

mitter metabolism, neuropathological findings eventually demonstrated lesions consistent with incomplete development and early degeneration of sensory and autonomic nerves involving the peripheral nervous system structures and the vascular bed [91–94].

In addition to the peripheral nervous system (PNS), adult patients with FD show signs of progressive CNS dysfunctions. For example, MRI or CT scans reveal symmetric cortical atrophy, while SPECT studies show asymmetric cerebral perfusion, particularly in the temporal and frontal lobes [13, 15]. Generally, intelligence is within normal range [115], although some sufferers exhibit difficulty in conceptual thinking, and extrapolation of information – CNS functions attributed to the frontal cortex [13, 25]. Other CNS-related neurodegenerative changes are observed among older patients, like attention deficit, gait problems, and difficulties in performing rapid movements or turning [9]. Paroxysmal activity in the CNS has been observed in patients, but is likely secondary to fever, hypoxia or hyponatremia [14]. Indeed, it has been proposed that an FD crisis may be clinically synonymous to a "seizure" of the ANS, and may explain the therapeutic effect of pharmacologic agents such as the γ-amino butyric acid (GABA)-ergic and α-adrenergic agonists [15].

In FD, there is clinical evidence of parasympathetic, as well as sympathetic abnormality in the cardiovascular regulatory system of patients, as well as dysfunction of chemoreflex and baroreflex receptors [9, 10]. Sufferers appear to have inappropriate cardiovascular or catecholamine responses to physical stress, change of position (orthostatic intolerance) or exercise [11, 122]. Postural hypotension, without compensatory tachycardia, is a fairly consistent finding. Vascular resistance does not increase adequately in response to upright positioning or cold stimuli [11]. These vasomotor abnormalities are attributed to autonomic denervation and aberrant neurochemical modulation of the vasculature. In fact, patients with FD have high plasma levels of DOPA, while dihydroxyphenylglycol (DHPG) levels are low, resulting in disproportionately elevated plasma DOPA:DHPG ratio – an observation not present in other disorders with neurogenic orthostatic hypotension [13]. DHPG is a catecholamine metabolite that can enter the

circulation and serve as a marker of norepinephrine (NE) uptake and monoamine oxidase (MAO) activity (Fig. 1). The elevated plasma DOPA levels are consistent with the observation of increased tyrosine hydroxylase (TH) immunoreactivity in the superior cervical ganglia of FD subjects [91]. Note that during emotional crises in FD, plasma NE and dopamine (DA) levels are markedly elevated, blood pressure is in the hypertensive range, and vomiting is frequent. Not surprisingly, elevated NE is attributed to peripheral conversion of DA by the enzyme, dopamine-β hydroxylase (DBH) (Fig. 1), which reflects marked increase of urinary excretion of homovanilic acid (HVA), another catecholamine metabolite [9, 10].

Clinical diagnostic tests in FD

Classic diagnosis of FD is based upon the "cardinal" criteria of alacrima, absent fungiform papillae, depressed patellar reflexes, abnormal histamine test, and clinical recognition of sensory and autonomic dysfunctions in an individual of Ashkenazi Jewish extraction [9, 10]. Symptoms of decreased response to pain and temperature, orthostatic hypotension, periodic erythematous blotching of the skin and increased sweating, as well as gastroesophageal reflux, and delayed gastric emptying would support the diagnostic criteria. Increased frequency of spine curvature is also noted. Testing for urinary catecholamine metabolite ratios and intraocular hypersensitivity to parasympathomimetic agents provided inconsistent results, and thus is no longer used for diagnosis. Fortunately, the recent availability of genetic tests, following discovery of the gene mutations in FD [2, 104], has aided the diagnosis (see section below).

Several diagnostic and confirmatory methodologies are being employed to assess pathophysiological responses of central and peripheral autonomic function, in order to determine baseline status and then measure response to therapeutic interventions in FD. Among the techniques, quantitative thermal tests, cold pressor or cold face test, Valsalva maneuver, sinusoidal neck-suction stimulation (baroreflex response), orthostatic challenge, hypoxic/hypercapnic stimulation (chemoreflex response), and vasomotor/sudomotor functions have been successfully used in patient evaluation [10, 50–56]. By and large, data from these tests show defects in small fiber function and various autonomic regulatory systems of individuals suffering from FD. Some of these assessments may help understand differences in phenotypic expression and identify those patients with FD at greater risk for sudden death. Other specialized tests have been used to clinically quantify neuronal depletion (*e.g.* nerve biopsy). Indeed, to further understand the sensory neuropathy component of the disease, a skin biopsy method originally used for the assessment of diabetic sensory neuropathy [70] was recently employed in FD. Kennedy and colleagues (unpublished observa-

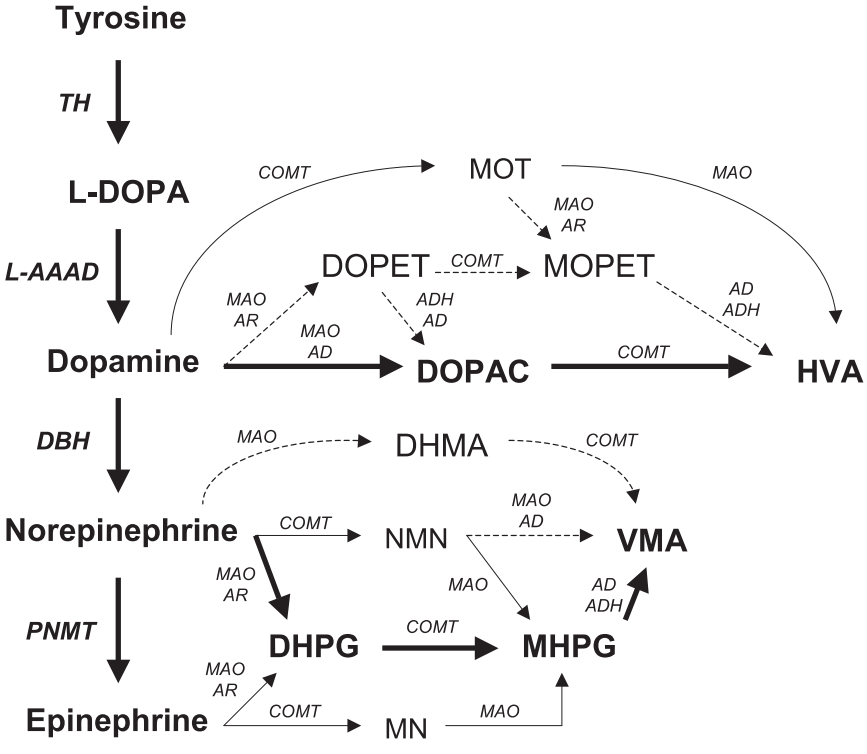

Fig. 1 Schematic representation of the catecholamine metabolism pathway. Enzymes that catalyze the conversion of major catecholamine substrates and their metabolites are shown italicized within each pathway. Neurochemicals and their substrates involved in the major metabolic pathways are in bold text; solid arrows are major enzymatic route, while dashed arrows are minor pathways. *ADH* alcohol dehydrogenase; *AD* aldehyde dehydrogenase; *L-AAAD* L-aromatic amino acid decarboxylase; *AR* alcohol/aldehyde reductase; *COMT* catechol-O-methyltransferase; *DBH* dopamine β-hydroxylase; *DHMA* 3,4-dihydroxymandelic acid; *DHPG* dihydroxyphenylglycol; *DOPAC* 3,4-dihydroxyphenylacetic acid; *DOPET* 3,4-dihydroxyphenylethanol; *HVA* homovanillic acid; *MAO* monoamine oxidase; *MHPG* 3-methoxy-4-hydroxyphenylglycol; *MN* metanephrine; *MOPET* 3-methoxy-4-hydroxyphenylethanol; *MOT* methoxytyramine; *NMN* normetanephrine; *PNMT* phenylethanolamine-N-methyltransferase; *TH* tyrosine hydroxylase; *VMA* vanillylmandelic acid. Adapted and modified from Ref. [36]

tion) found that epidermal nerve fiber density was decreased in the skin of patients with FD. Substance P and calcitonin gene-related peptide-containing nerves were absent in skin of patients. Interestingly, vasoactive intestinal peptide, normally restricted to regenerating nerves on sweat glands and blood vessels, was present in the subepidermal plexus of patients. Moreover, empty Schwann cell sheaths were present and capillary abnormalities were observed in some subjects. Taken as a whole, the skin biopsy study concluded that patients with FD have remarkable degenerative changes in sensory innervations to the skin but may also be capable of regeneration (Kennedy et al. unpublished observation). In light of this observation, the use of this technique may aid researchers in diagnostic determination of disease progression, and possibly treatment efficacy.

The advent of these modern tests has helped educate clinicians with correct diagnosis, and immediately identify treatment needs for many patients with FD. As a result, early intervention and clinical support for FD sufferers have dramatically improved over the last decades. This is evidenced by increased survival rate, enhanced quality of life, and ability to cope among patients, and their caregivers [12]. Establishment and cooperation of treatment centers in the US and in Israel has fostered the development of expertise and contributed to the improved outcomes.

The identification of the FD gene

In 1993, the FD gene was mapped to chromosome 9q31 by studying 26 families with two or more affected members [19, 20]. Detailed haplotype analysis of 441 distinct FD chromosomes narrowed the candidate interval to 471 kb [21], and nine genes were cloned from this region and screened for mutations [22–24]. Since FD is a recessive disease, the *a priori* expectation for the mutation was inactivation of one of the candidate genes; however, no homozygous inactivating mutations were identified. Therefore, the complete sequence of the candidate region was generated for both control and FD, and was compared. Slaugenhaupt and colleagues [104] identified only one DNA change unique to FD – a single T to C nucleotide change at base pair 6 of intron 20 ($IVS20^{+6T\rightarrow C}$) in the *IKBKAP* gene. The same mutation in the major haplotype was also found and reported by Anderson et al. [2]. A second mutation was identified by direct sequencing of each *IKBKAP* exon in the four unrelated individuals heterozygous for the major and minor haplotypes [104]. Similarly, Anderson et al. [2] found the same change using exon-based SSCP analysis. The minor mutation produces a single G to C change in exon 19, which causes an arginine to proline missense mutation (R696P). This mutation is extremely rare in the AJ population and has never been detected in a homozygous state, and therefore the phenotype associated with R696P homozygosity is currently unknown.

The *IKBKAP* gene contains 37 exons and encodes a 1332 amino acid protein named IKAP. Northern analysis of *IKBKAP* reveals two mRNAs of 4.5 and 5.9 kb. The 5.9 kb message differs only in the length of the 3'-untranslated region and is predicted to encode an identical 150 kDa protein [104]. The major mutation in $IVS20^{+6T\rightarrow C}$ causes tissue-specific skipping of exon 20, which results in a frameshift and consequently introduces a "stop" codon in the reading frame of exon 21. Note, however, that cultured lymphoblastoid cells from patients with FD still exhibit wild-type expression of *IKBKAP* mRNA, and Western analysis using an antibody directed against the carboxyl-terminal of IKAP [74] also demonstrates the presence of full-length IKAP protein [104]. The presence of an apparently normal IKAP mRNA and protein in FD cell line was at odds with the expectation of an inactivating mutation in a recessive disease. Further studies using various postmortem FD tissues and utilizing primers designed to amplify exclusively wild-type and mutant *IKBKAP* messages show that all FD cell types examined to date express both wild-type and mutant mRNA, albeit to varying extents [32, 104]. The demonstration that FD nervous tissues express primarily mutant *IKBKAP* mRNA, coupled with the fact that cultured FD cell lines express primarily wild-type *IKBKAP* mRNA, indicates that the major FD mutation acts by altering gene splicing in a tissue-specific manner [32, 104].

The role of IKAP in FD

IKAP is a conserved protein found in mouse, fruit-fly, mustard plant, nematode, and yeast [27, 31]. It was initially reported as a scaffold protein for the IκB kinase (IKK) complex [26], but this function was subsequently contested by Krappmann et al. [74] who reported that IKAP was in fact a member of a different cellular complex. IKAP is now recognized as the largest member of the three-subunit protein complex of core Elongator, originally identified in yeast and more recently in humans [47, 73, 89, 118]. Studies in yeast and human have revealed that core Elongator interacts with another three-subunit protein to form the six-subunit holo-Elongator complex [47, 73, 75, 78, 116, 117]. Like that in yeast, the human holo-Elongator consists of six proteins: IKAP/hElp1, hSTIP-1/hElp2, hElp3, hElp4, hElp5 and hElp6 [47]. Initial investigations in yeast have suggested that core Elongator plays a role in transcription elongation due to its observed histone acetyl transferase activity, its observed interaction with naked or nucleosomal DNA, as well as its interaction with the hyper-phosphorylated C-terminal domain of elongating RNA polymerase II during transcription *in vitro* [89, 116]. How-

ever, recent findings in yeast have called previous observations into question (*e. g.* see Refs. [75, 76, 95]). Notwithstanding the controversial observations in yeast, Kim and colleagues [73] demonstrated that human core Elongator indeed facilitates transcription by RNA polymerase II in a chromatin- and acetyl-CoA-dependent manner using purified core Elongator complex from HeLa cell extracts. Although localization studies show that each core Elongator subunit, including IKAP, is primarily detected in the cytoplasm of human [47, 57, 73, 74], or yeast cells [95], there is evidence that IKAP is also predictably detected in the nucleus, and also in the nucleoli of human cells [47, 74]. It is also interesting to note that IKAP can be isolated from cellular fractions that lacked detectable hELP3, and it was suggested that perhaps each protein in the holo-Elongator complex has multiple roles in the cell [47]. In support of this possibility, Holmberg et al. [57] reported a novel scaffolding role for IKAP in the c-Jun N-terminal kinase (JNK) signal transduction pathway due to its discerned strong physical interaction with JNK and enhancement of JNK activation. These studies suggest that IKAP may indeed have multiple roles in the cell. It remains to be determined how a decrease in cellular *IKBKAP* causes the FD phenotype and why this phenotype displays tissue-specificity.

Future studies aimed at determining how Elongator, and specifically IKAP, functions in the cell will be crucial to understanding the nature of the FD defect. Whether FD is due to defect in transcriptional elongation or JNK activation, which leads to the death and continued degeneration of specific sensory and autonomic neurons in humans remains uncertain. More research is necessary to dissect the role that IKAP plays in normal physiology and in pathology.

The FD splice mutation: creating a mouse model and the search for therapeutic agents

Gene splicing regulation can play an important role in inherited disorders, influencing phenotypic parameters such as organ specificity, disease severity, and age of onset [85]. Splicing mutations that result in exon skipping have been reported for many disorders [85]. Even though some of these mutations are associated with an imperceptible phenotype, which implies the persistence of residual normal protein activity due to low level expression of correctly spliced mRNA, it does not necessarily suggest that FD is a "mild" disease. Rather, FD is unique in that a single IVS20$^{+6T\rightarrow C}$ splicing mutation is responsible for virtually all cases of the disease, and that homozygous mutant cultured cells are capable of producing substantial amounts of WT *IKBKAP* mRNA and protein [104]. It is interesting to note that in cultured FD lymphoblasts, the ratio of wild-type to mutant *IKBKAP* message can be partly influenced by culture conditions [32], thus opening the possibility of manipulating the cell splicing mechanisms in order to prevent exon skipping and potentially correct the splicing defect in FD. Indeed, a consortium sponsored by the NINDS [48] is underway to screen for FDA approved drugs that will do just exactly that, i. e. to increase the expression of wild-type IKAP in FD cells, particularly nerve cells. Preliminary data from this consortium using cultured FD lymphoblasts show very promising results (Slaugenhaupt et al., unpublished observation). Furthermore, creation of the first FD mouse model is underway in the laboratory of Dr. Yang Xu. His team has successfully created and characterized a mouse model for ataxia telangiectasia (AT) and an AT-related human genetic disease known as Nijmegen breakage syndrome [67, 119]. Based on their accomplishments, there is no doubt that an animal model, where the IVS20$^{+6T\rightarrow C}$ mutation will be introduced into the mouse sequence, will be successfully created.

The discovery of the gene mutations that cause FD has resulted in prenatal testing and carrier screening for FD, making it possible to reduce future incidence. It is thus hoped that new therapeutic approaches will emerge from successful bench-to-bedside research in order to help the many patients currently suffering from FD.

Familial amyloidotic polyneuropathy (FAP)

Familial amyloidotic polyneuropathy (FAP) and acquired amyloidosis (sporadic) are characterized by systemic accumulation of an amyloidogenic form of variant transthyretin (ATTR) in the endoneurium of peripheral nerves and other organs [1, 69]. The TTR gene is located on chromosome 18 and is 7 kb long, with four exons of ~200 base pairs each and three introns. Among the genotypes of FAP ATTR-related amyloidoses, a point mutation in exon 2 of the TTR gene (Val30Met) is the most common [1]. Portugal is the main endemic area of FAP; however, other TTR gene mutations have been recently discovered in Sweden, Finland, France, Britain, USA, and Japan [4, 5,16, 33, 49, 58, 59, 96, 97]. Haplotype studies using TTR intron polymorphisms suggest more than one founder for the mutations [97]. Note however that fragments of gelsolin or apolipoprotein A1 have been reported as other causal factors for FAP [49, 64, 84]. Likewise, wild-type TTR can also deposit as amyloid in the heart of the elderly, termed systemic senile amyloidosis (SSA), which indicates the intrinsic amyloidogenic properties of the protein [28, 29]. Thus, the current classification of FAP is based on the nature of the precursor plasma protein that causes amyloid deposits, as well as the genetic analysis of patients [1].

Clinical presentations, diagnosis, and treatment strategies for FAP

Nerve biopsy usually allows the diagnosis of amyloid neuropathy to be established by identifying amyloid deposits in the endoneurium and always shows a progressive axonal neuropathy. As in Alzheimer's disease (AD) and amyloidopathies, amyloid protein deposits in FAP also stain with Congo red, demonstrating green birefringence under polarizing microscopy. The size and number of amyloid deposits vary: aggregates can be histochemically undetectable, so that their absence does not exclude the diagnosis. Diagnosis via nerve biopsy has been replaced recently by genetic studies in patients with a positive family history of FAP [1].

Amyloid neuropathy is a slowly progressing degenerative disease. Patients with FAP exhibit certain cardiovascular, gastrointestinal, and hematological (anemia) abnormalities. They also suffer from various dysautonomic symptoms, including dysuria, erectile dysfunction, orthostatic hypotension, alacrima, and dyshydrosis [1]. FAP has been classified into 4 stages: (i) the first shows mainly sensory neuropathy in the lower limbs; (ii) stage 2 occurs after a mean interval of 5.6 years and is marked by sensory neuropathy with motor signs in the lower limbs, progressing to the upper limbs; (iii) stage 3 occurs after a mean interval of 10.4 years, whereupon pain and temperature sensation is absent overall, except in the head and neck area, and patients are non-ambulatory; and (iv) in stage 4 patients experience full scale autonomic failure [1].

TTR is synthesized by the liver, choroid plexus and retinal epithelium which are also clinically affected, although dysautonomia is the most prominent manifestation in FAP. Ocular vessel abnormalities, including vitreous opacities, and intraocular pressure that results in blindness, are quite commonly observed [3]. Because most of the TTR protein is produced by the liver, progression of the disease can be halted by liver transplantation. Recently, orthotopic liver transplantation has appeared to be an effective therapy for FAP [6, 7]. In fact, there is evidence that transplantation therapy reduces production of variant TTR, and improves some but not all autonomic dysfunction [6]. A promising future therapy is gene repair using single stranded oligonucleotide (SSO). Using an FAP transgenic mouse model, Ando and colleagues will attempt to repair the TTR mutation through intrahepatic injection of SSO. Recent data show a modest number of TTR genes were repaired using this technique (Ando et al. unpublished observation). However, there is no current means of prevention of amyloid aggregation and deposition in the amyloidopathies, possibly because the mechanism of amyloid fibril formation in these disorders, including FAP, remains unknown. In Alzheimer's amyloidosis, several pharmacologic agents in both *in vitro* and *in vivo* model systems have been tested in an attempt to target directly and prevent amyloid-β fibrillogenesis (reviewed in [30]). Although fibril formation is a common mechanism among various types of amyloidosis, subtle differences that exist between these disorders imply that one therapy that works for one disorder such as AD, may not necessarily work for another amyloid-mediated disease like FAP. Further research is needed to identify the mechanism behind amyloid deposition in FAP to devise an effective therapy for this devastating disease.

Familial Parkinson's disease (FPD) and parkinsonism

Parkinson's disease (PD) is a debilitating movement disorder clinically characterized by resting tremor, bradykinesia, rigidity, and postural instability that respond to dopamine replacement therapy, at least initially in its course (reviewed in [68]). The pathophysiology of PD involves degeneration of dopamine (DA) neurons in the substantia nigra pars compacta, with formation of intraneuronal inclusions termed Lewy bodies. PD can be either idiopathic (sporadic) or familial (FPD), the latter being increasingly recognized. FPD is classified based on the mode of inheritance and the presence or absence of Lewy bodies in the substantia nigra [45, 83]. Genetic loci that have been linked to FPD include *SNCA* (α-synuclein), *PARK2* (parkin), *MAPT* (microtubule-associated protein tau), *UCHL1* (ubiquitin carboxyl-terminal esterase L1) and several other less characterized locations [45, 83]. Recently, mutations in the gene *NR4A2* (a nuclear receptor protein, also known as *NOT*, *TINUR*, *RNR-1*, *HZF-3* and *NURR1*) was identified to be associated with FPD [77].

Dysautonomia in FPD

Patients with sporadic or familial PD not uncommonly have symptoms or signs of autonomic failure, including orthostatic intolerance and hypotension; loss of respiratory sinus arrhythmia; urinary frequency, urgency and incontinence; erectile dysfunction; and chronic constipation [38, 40, 42, 101]. Orthostatic hypotension (OH) is defined by a progressive decrease in blood pressure by more than 20/10 mm Hg, when going from supine to standing position within a few minutes [42]. Recent observations suggest a direct association between OH in PD and sympathetic neurocirculatory failure [37, 39, 40]. Using cardiac sympathoneural PET scanning and cardiac spillovers of norepinephrine (NE) and related neurochemicals, Goldstein and co-workers have shown that there is a significant decrease in synthesis, release, reuptake, and turnover of NE in the hearts of PD patients with OH – an observation directly attributed to

sympathetic noradrenergic denervation [39–41, 43]. Results to date in FPD with OH have suggested essentially the same pathophysiologic mechanism as in sporadic PD with OH. In particular, FPD from mutation of the gene encoding α-synuclein (the Contursi kindred) is associated with OH from sympathetic neurocirculatory failure, a subnormal orthostatic increment in the plasma NE level, and cardiac sympathetic denervation [40, 41].

PD patients without OH often also have evidence of cardiac sympathetic denervation, whereas in multiple system atrophy (MSA), a neurodegenerative disease that is difficult to distinguish from PD, cardiac sympathetic innervation remains intact [37, 39, 40]. Analogously, recent findings in FPD without OH have indicated cardiac sympathetic denervation (Goldstein et al. unpublished observation).

In both sporadic PD and FPD, cardiac sympathetic denervation can occur early in the disease, and seems to progress gradually over years, from denervation localized to left ventricular free wall or apex, to diffuse denervation that includes the interventricular septum and base of the heart. Cardiac sympathoneural scanning may therefore prove useful not only for differential diagnostic purposes and to track disease progression, but also for early detection and monitoring of responses to experimental treatments in FPD [38, 40].

Dysautonomia caused by impairments of neurotransmitter metabolism

Orthostatic intolerance syndrome

Normally, an increase in heart rate upon standing (orthostatic posture) is a reflexive autonomic compensatory response for the effect of gravity, in order to maintain adequate blood pressure and cardiac output. This autonomic orthostatic response is mediated through the brainstem centers that control the cardiovascular system. Orthostatic intolerance (OI) is an abnormal response of the cardiovascular system upon postural change [62, 65, 98]. The normal response for orthostatic change is a heart rate increase of about 10 to 15 beats per minute. An increase in diastolic pressure of about 10 mm Hg is observed, but with only a slight change in systolic pressure. Robertson and colleagues [98] diagnose OI by the following 4 clinical criteria: (i) symptoms of excessive sympathetic activation on upright posture; (ii) orthostatic tachycardia greater than 30 beats per minute; (iii) less than 20/10 mm Hg change in blood pressure on standing; and (iv) upright plasma NE levels greater than 600 pg/mL. The symptoms of OI include lightheadedness, dizziness, blurred vision, palpitations, and nervousness. OI occurs more commonly in women than in men and is typically seen in younger patients [98]. OI is different from orthostatic hypotension (OH), even though both entities may share some symptom similarities. In OH there is a greater than 20/10 mmHg decrease in blood pressure of patients upon changing into an upright posture. However, in OI, sufferers show a remarkable increase in heart rate when standing, but their blood pressure does not drop significantly upon standing.

There are many names physicians have given to the entity of OI. These designations all probably represent the same clinical syndrome. They include: (i) orthostatic tachycardia syndrome; (ii) postural orthostatic tachycardia syndrome (POTS); (iii) postural tachycardia syndrome; (iv) idiopathic hypovolemia; (v) neuropathic postural tachycardia syndrome (NPTS); and (vi) mitral valve prolapse syndrome [61–63, 98, 109]. It is interesting to note that NPTS is an orthostatic syndrome that has clinical overlaps with POTS, but is also manifested by partial sympathetic denervation of the lower limbs [62].

Etiology of orthostatic intolerance (OI)

It has been hypothesized that alterations in catecholamine metabolism are a causal factor for some cases of OI [36, 61, 98]. Indeed, some genetic defects of catecholamine metabolism have cardiovascular phenotypes [36, 61, 98]. One of them is an abnormality of NE clearance in the synapse. Most of the NE released into the synapse is cleared through reuptake of NE back to the presynaptic nerve terminal, while about 10–20 % of the NE "spills over" into non-neuronal tissue or the circulation [34]. The process of NE reuptake is accomplished by a protein in the presynaptic membrane known as the NE transporter (NET), a protein with 12 transmembrane domains (TMD) [36].

A patient that presented with OI syndrome became a unique candidate for NET gene impairment when various tests revealed that the patient had significantly: (a) blunted plasma NE increase with tyramine administration (tyramine is taken up by NET and releases cytosolic NE); (b) reduced systemic NE clearance; and (c) decreased relative DHPG levels compared to NE measured in plasma (NE taken up by NET is recycled, but is mostly converted to DHPG by MAO; see Fig. 1). Hence, these findings reinforced that idea the a genetic defect in NET is central to the disorder in some patients. Not long after the search, Robertson et al. [98] identified a genetic mutation in the NET gene (*SLC6A2*) which causes NET deficiency. Direct sequencing of the *SLC6A2* gene showed a G237C missense mutation, which results in coding alteration of alanine to proline (A457P) within a highly conserved region of TMD-9. This finding thus established that NET deficiency (NETD) is due to the abnormal function of the NET protein. In fact, *in vitro* studies have shown that cells transfected with NET-A457P mutation reveals marked impairment of its up-

take activity [98]. This has been shown to be due to a defect in the biosynthetic processing and insertion of NET-A457P protein into the plasma membrane of the cell, as well as the inability of NET-A457P protein to bind normally and transport NE [46, 90]. Furthermore, NET-A457P is a dominant-negative mutation that diminishes the function of the wild-type transporter through a process that may rely on physical association of transporters in complexes [46].

The observations of elevated supine heart rate, elevated plasma NE associated with relatively low plasma DHPG, reduced NE response to tyramine and reduced systemic NE clearance all indicate that NET dysfunction could explain a number of OI symptoms. Note, however, that the NET-A457P mutation does not explain all cases of OI – no more than 2% in a series of patients. Interestingly, therapeutic tricyclic antidepressants, or illicit drugs (cocaine and amphetamine) that have the ability to inhibit NET [35], produce symptoms of tachycardia related to OI, and elevated plasma catecholamines [98] which further lends support to the role of NETD in OI and related disorders.

Etiology of neuropathic postural tachycardia syndrome (NPTS)

Several previous reports have shown that some patients with OI have peripheral autonomic dysfunction. These patients often have high plasma NE concentrations, hypovolemia, excessive pooling of blood while standing, and exaggerated orthostatic hypovolemia [63, 109]. NPTS patients are slightly $\alpha 1$ and $\beta 1$ adrenoreceptor hypersensitive. They also exhibit sympathetic nerve loss in their skin as evidenced by abnormal sweating, galvanic skin response and quantitative sudomotor axon reflex test (QSART) [79, 109]. These observations suggest that sympathetic denervation of the mesentery and legs may be the underlying mechanism in NPTS [109]. Jacob and co-workers [62] confirmed this by measuring the response of the sympathetic nervous system (SNS) to three distinct stimuli (cold pressor test, sodium nitroprusside, and tyramine administration). They reported that these stimuli, each of which causes SNS activation by different mechanisms, increased NE spillover in the arms of both the patients with OI and the normal subjects, but no NE spillover increase was seen in the legs of patients with OI [62]. Note also that without stimulation, systemic NE spillover in patients with OI was normal despite the impaired spillover in the legs. According to Jacob et al. [62] such a result could be explained by the presence of intact sympathetic neurons in other organs (*e. g.* the mesentery contributes more than one third of the systemic NE spillover in normal subjects) [8]. To sum up, the reduced clearance of NE in the legs of OI patients without similar reduction in their arms is likely due to abnormal NE release and reuptake mediated by damage to nerve terminals located primarily in the lower limbs. In light of these observations, Jacob et al. [62] proposed that patients with NPTS may benefit from treatments used in pure autonomic failure (PAF; a complete peripheral autonomic neuropathy).

Dopamine-β hydroxylase deficiency (DBHD)

The catecholamines are important neurotransmitters in the peripheral and central nervous systems. NE plays an important role in autonomic disorders. NE is synthesized by DBH (Fig. 1) and is the main neurotransmitter utilized by the sympathetic postganglionic nerve terminals. The rate-limiting step in NE synthesis is the enzyme tyrosine hydroxylase (TH), which is regulated by catecholamine levels through feedback inhibition. DBH is released into the synaptic cleft and can be measured in blood and cerebrospinal fluid.

Dysautonomia due to NE deficiency has been reported to be caused by abnormal β-hydroxylation of DA [36]. Patients with DBH deficiency (DBHD) exhibit normal parasympathetic and sympathetic cholinergic functions, but sympathetic noradrenergic response is absent [100]. DBHD patients experience severe orthostatic hypotension, noradrenergic failure, ptosis, and intermittent presyncope or syncope [36, 113]. Adult DBHD patients suffer from profound orthostatic hypotension and respond to high doses of tyramine with an increase in DA rather than NE [100]. Treatment for DBHD patients has been successful using L-threo-3,4-dihydroxyphenylserine (DOPS), an artificial amino-acid precursor to NE that is converted by endogenous L-DOPA decarboxylase to yield NE. The administration of DOPS results in dramatic increase in blood pressure and restores plasma and urinary levels of NE towards normal levels [100].

The hunt for a causal mutation in the *DBH* gene was therefore the logical next step. The *DBH* gene maps to chromosome 9q34, and four potentially pathogenic *DBH* mutations were found in two unrelated DBHD subjects [72]. One of these was a missense mutation in exon 2 (D100E) accompanied by single copies of three variants in intronic regions ($IVS1^{+2T\rightarrow C}$, $IVS3^{+8C\rightarrow T}$, and $IVS10^{+415G\rightarrow A}$), while the other was a single copy of the $IVS1^{+2T\rightarrow C}$ intronic mutation, and two additional missense mutations in exons 1 (V87M) and 6 (D331N). Both patients also carried the previously recognized polymorphism in the 5'-UTR region ($-1021^{C\rightarrow T}$) [72]. This allele is associated with low plasma DBH activity [121]. It is interesting to note that when transfected into cells, the $IVS1^{+2T\rightarrow C}$ mutation located near the consensus donor splice site of intron 1 produces both normal and aberrant DBH mRNA [72]. However, DBHD patients have no DBH protein in plasma [99] suggesting that this muta-

tion may not be solely responsible for DBHD, and that other variations in the DBH gene [72] may additionally be necessary to produce the DBHD phenotype.

The findings of causal mutations in DBHD and NETD, both of which are directly associated with dysautonomic symptoms (*e. g.* OH and OI, respectively) indicate that other genetic factors that directly or indirectly influence the activity of ANS (*e. g.* baroreceptor, and chemoreceptor functions) will be eventually identified. Indeed, the next discussion will focus on the search for gene modifiers in baroreceptor reflex control and blood pressure regulation, areas of research that are rapidly growing and producing meaningful data.

Autonomic control and dysfunction of the cardiovascular system: susceptibility genes and genetic modifiers

Adrenergic receptor polymorphisms

The targets for catecholamines are the adrenergic receptors of which there are nine subtypes. At least seven of the nine receptors have been shown to be polymorphic in their coding regions, resulting in deletions or substitutions of the amino acid sequence. In transfected cell studies alterations in ligand binding, function, or regulation have been found with many of these polymorphisms [105, 106]. Multiple studies have shown that these polymorphisms modify cardiac and vascular responses or diseases; or are risk factors for hypertension and heart failure (reviewed in [106]). Combinations of polymorphisms in several adrenergic receptors may be synergistic for some phenotypes [107]. With these polymorphisms now being identified and characterized, their potential role in modifying dysautonomias, which may account for variation in clinical characteristics, needs to be explored.

Dysautonomia in hypertensive patients

Autonomic reflexes in the cardiovascular system help control the heart rate and arterial blood pressure. One essential example of these is the baroreflex, which serves two important functions. One is the maintenance of perfusion of vital organs, and the other is protection against excessive blood pressure changes. The baroreflex receptors are stretch-sensitive receptors in the walls of major arterial blood vessels, especially the internal carotid arteries and the aortic arch. The baroreflex afferents of the ANS to the solitary tract nucleus (NTS) and baroreceptors are activated upon acute changes in blood pressure. The signal is then transmitted to the brainstem NTS and the information is integrated with signals from higher areas in the brain subsequently adjust the sympathetic and parasympathetic activities to the heart and vasculature. Alteration in the baroreceptor reflex afferents to the NTS results in a lack of buffering changes in blood pressure resulting in labile hypertension. Unfortunately, there are individuals who suffer damage to baroreflex afferents to the neck as a consequence of trauma, surgery, stroke, malignancy, or hereditary paragangliomas [17, 18, 71]. This causes severe labile hypertension, coupled with abnormal increases in heart rate, blood pressure and plasma NE. Labile hypertension is usually triggered by stress or pain due to the absence of the baroreflex circuit modulation [17].

Autonomic failure is characterized by severe OH with classic symptoms of presyncope within seconds of standing. OH can occur due to secondary autonomic neuropathy, for example, as seen with diabetes mellitus, amyloidosis (*e. g.* FAP), FPD, or it can manifest as a primary disorder due to pure autonomic failure (PAF) and multiple system atrophy (MSA) [17, 18, 71, 102, 103]. PAF leads to isolated ANS dysfunction, while MSA is also associated with other neurological difficulties including movement disorders. While PAF and MSA patients experience OH, approximately 50 % of patients with either PAF or MSA are also hypertensive when supine [17]. Biaggioni and colleagues have been studying this seemingly paradoxical observation. They have observed that PAF sufferers have SNS-independent hypertension, but MSA patients have SNS-dependent hypertension. It is worth mentioning that PAF patients have almost complete degeneration or destruction of sympathetic nerve cells, but MSA patients have some sparing of peripheral noradrenergic nerves [17]. Studies on these autonomic failure patients provide unique models of sympathetically-driven and sympathetically-independent hypertension that will be useful in further dissecting the role of the ANS in blood pressure control, for understanding other forms of hypertension, and for development of efficacious pharmacotherapy.

Genetic factors in the baroreflex circuit regulation of blood pressure

Per above, an important mechanism regulating blood pressure is the baroreflex, which buffers blood pressure changes. Recent advances have allowed direct measurement of changes in the autonomic response of the cardiovascular system [66]. For example, the overall buffering function of the baroreflex can be determined by comparing its sensitivity to vasoactive medications before and during complete ganglionic blockade [66]. Changes in heart rate and sympathetic vasomotor tone are plotted against changes in blood pressure that occur spontaneously, or are elicited by pharmacological or physiological stimuli. The resulting baroreflex curves have a sigmoidal shape. The curves have several proper-

ties, such as an operating point, maximal steepness (referred to as baroreflex sensitivity), range, etc. Each measure influences baroreflex function.

Several methods can be used to assess the contribution of genes in normal baroreceptor reflex function. For example, evaluation of those with secondary baroreceptor or adrenergic nerve terminal dysfunction (due to trauma or degeneration) may provide some insight into the genes that are necessary for baroreflex control (*e. g.* through differential microarray analysis). Twin studies are useful and straightforward approach. This approach allows estimation of how much the variability of a certain biological marker is influenced by genetic factors, since monozygotic twins share 100 % of their genes, while dizygotic twins share only 50 % of their genes. Thus, anything that is influenced by genes is more similar in monozygotic twins. Data on baroreflex function in twins are published [44, 112]. These studies show that baroreflex heart rate regulation is strongly influenced by genetic factors. This estimation was maintained after adjustment for variables that influence baroreceptor reflex function including body weight, blood pressure, age, and physical activity. Other studies suggest that the operating point of sympathetic vasomotor baroreflex regulation is influenced by genetic factors [114]. Potential candidate genes such as the BK channel β1 subunit gene [44] and aldosterone synthase gene [120] give us some insights on their role in baroreflex activity. Furthermore, known disorders like FD (*e. g.* IKAP mutation [2, 104]), and William's syndrome (*e. g.* elastin mutation [108]) reveal pathophysiological symptoms attributed to baroreflex dysregulation, which lend support to the idea that baroreceptor function is influenced by genetic modifiers. The fact that there are already known genes that either directly or indirectly shape the activity of the baroreceptor circuit imply that we will find more genetic interactors that, once identified, may serve as ideal candidates for therapeutic manipulation.

Genetic determinants of autonomic blood pressure control

Studies in catecholaminergic storage vesicles isolated from pheochromocytomas have given further insight into their roles in the autonomic control of the cardiovascular system. Pheochromocytoma is a neoplasm of the adrenal medulla, which results in excessive secretion of catecholamines and consequently, high blood pressure [60]. One interesting observation from chromaffin cells is that their catecholaminergic vessel cores contain pro-hormones known as chromogranins. Two well-characterized pro-proteins are chromogranin A (CGA) and the higher molecular weight chromogranin B (CGB). CGA and CGB are cleaved to form biologically active peptides; catestatin and vasostatin from CGA and secretolytin from CGB [86–88]. CGA is exocytosed with catecholamines from storage vesicles of chromaffin cells and postganglionic sympathetic axons, and its peptide, catestatin has been found to be a potent vasorelaxant [110, 111]. Catestatin inhibits catecholamine release by acting as an antagonist for nicotinic acetylcholine (ACh) receptor ($IC_{50} \cong 200$ nmol) and blocks agonist-induced receptor desensitization [80, 81, 86, 87]. It has been proposed that catestatin functions as an autocrine homeostatic negative feedback controller of catecholamine release [81], perhaps by preventing over-secretion and overload of neurotransmitter in synaptic clefts.

Extensive studies on the function and structure of catestatin, including homology modeling, empirically-derived and nuclear magnetic resonance-derived structure, have revealed that a loop strand containing arginines with cationic side chains is an important site of action of this peptide [82]. A mutation in any one of the 3 arginines in the loop strand confers significant decline in its catecholamine release inhibition. There is also evidence that catestatin is active *in vivo*, whereby it dampens a rise in blood pressure following central sympathetic stimulation. In fact, O'Connor et al. [87] recently found that catestatin is diminished early in the course of development of hypertension, including in the normotensive offspring of patients with the disease. Furthermore, they reported that low catestatin predicts augmented adrenergic pressor responses (*e. g.* cold pressor test), suggesting a possible mechanism whereby diminished levels of catestatin might increase the risk for later development of hypertension [87]. In light of these promising observations, research is underway to further identify allelic diversity of CGA in the population that may be involved in adrenergic responses to stress, to dissect gene modifiers that influence the function of CGA, and further elucidate the pathophysiologic effect of catestatin in hypertension.

Goals and future directions

The Dysautonomia Foundation, Inc. (www.familialdysautonomia.org), the Familial Dysautonomia Hope Foundation (www.fdvillage.org), and the National Dysautonomia Research Foundation (www.ndrf.org) (listed in alphabetical order) have been dedicated to helping patients and their families cope with dysautonomia. These organizations advocate patient, family and physician education, as well as provide funding to clinical and basic science researchers worldwide. In fact, many clinicians and scientists who attended the first H-Dys conference are funded by one or more of these organizations, and/or have been supported in the past. Furthermore, their valuable insights, assistance, and encouragement were essential to the conceptualization and actualization of the H-Dys Workshop.

Each session identified common clinical and laboratory science goals. First, it was agreed that there is a need to expand clinical research endeavors including developing outcome measures, improving both clinical diagnosis and prognostic indicators, as well as therapeutic development and testing. It was generally agreed that researchers in all areas need to continue to advance their *in vitro* or *in vivo* observations to accommodate both forward and reverse translational research. Thirdly, developing animal models and characterizing the pathophysiological roles of genetic variants responsible for a number of H-Dys and dysautonomia-related disorders was stated to be a priority. Also, there was a general agreement that genotype-phenotype correlations in many of these disorders warranted additional attention; it was suggested that core facilities for the collection and sharing of resources to aid scientific research was worthwhile. Towards these common goals, it is imperative to create standards for data collection, management and sharing by local, national, and international dysautonomia researchers.

In summary, research in the field of dysautonomias is rather difficult, because the disorders involve interdisciplinary, integrative, and "mind-body" connections. Future advances in H-Dys research will thus depend on a novel multi-system approach by investigators from different medical disciplines. Therefore, it was proposed that establishment of cooperative centers would be especially productive in this field. Other key issues proposed in the workshop were (i) expansion of clinical and basic science research, with emphasis on forward and reverse translational studies; (ii) development of clinical and scientific resources that will aid research and improve treatment strategies; (iii) creation of collaborative broad scale efforts in the form of multi-project and multi-site applications; (iv) promotion of collaborative efforts to enhance data collection, management and sharing; (v) joint cooperation between patient advocacy groups and medical institutions to advance patient advocacy, improve public awareness, and nurture research; and (vi) encouragement of additional broad-based scientific meetings in the field of H-Dys.

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