

5 look at the cardiovascular continuum of autonomic and
6 blood pressure abnormalities, and if they did that they
7 would come up with a slide something like this, where the
8 great majority of people have normal blood pressure and
9 heart rate control.

10 And so the vast majority of us are here in the
11 middle, but there are some people at the upper end who
12 have high blood pressure or hypertension, maybe 10 percent
13 of the population, a lot of people, certainly, and then
14 there are some people in the middle that are called
15 borderline hypertension, or labile hypertension, and they
16 would be in a group that are kind of on the borderline but
17 often go into this area at some point in their illness.
18 This is a small end of the continuum, in that the numbers
19 of people here at the low end of the cardiovascular
20 continuum in terms of blood pressure, they are relatively
21 limited in numbers, and there is the severe ones that are
22 most comparable to the hypertension that have low blood
23 pressure every time they stand, and it's pretty severe
24 every time they stand.

25 So that is one end of the continuum, and then

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1 there is something analogous to this here that we call
2 orthostatic intolerance. That's a term, a very old term,
3 a nonspecific term that we often use for these people.
4 There's a newer term that has been advanced especially by
5 Philip Lowe at the Mayo Clinic, but it is such a
6 compelling term, POTS, postural orthostatic tachycardia,
7 but that many patients come and tell me now they have
8 POTS, and they don't mean tuberculosis of the bone when
9 they say that, either, so this is I think the nearest
10 thing to this, and so not all of the autonomic disorders
11 fit neatly into this scheme, but this is something that
12 many people understand.

13 Now, if you look at this population here of the
14 people who have tachycardia when they stand, and usually
15 not hypertension, there are about a 1/2 million Americans
16 who we are estimating have that. The estimates go from as
17 low as 100,000 Americans to as high as 3 million
18 Americans, and that thirtyfold difference is driven by how
19 people define this, whether they define orthostatic
20 tachycardia broadly, or narrowly, and then the people who
21 have low blood pressure every time they stand, they have
22 orthostatic hypertension. There are about 100,000
23 Americans who seem to have a pretty severe case of that.

24 By a remarkable coincidence, we actually know a
25 great many of the -- at least in a superficial way we know

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1 the disorders that characterize much of this. We don't
2 know the pathophysiologies to characterize most of these
3 people.

4 There's one other big difference between the two
5 ends of the spectrum. People at the high end of the blood
6 pressure spectrum are asymptomatic, so when people are
7 treated for high blood pressure the goal is not to make
8 them feel any better. They already feel fine. The goal
9 is to prevent complications 20 years into the future, but

10 both these categories here are quite symptomatic and most
11 autonomic disorders are very symptomatic, so the goal
12 here, the therapeutic goal here is the here and now.
13 We're trying to make people feel better today. We'd like
14 to prevent complications far into the future, but we have
15 very little data in most of these entities to know if
16 we're able to do that or not.

17 (Slide.)

18 DR. ROBERTSON: In this slide I just wanted to
19 remind people how dysautonomias teach us about physiology
20 and pathophysiology, and they do teach us a lot. There
21 are two things they do. First, there is amplification of
22 signal in the absence of buffering reflexes. Things that
23 cause tiny perturbations in blood pressure in you and
24 me -- most of us are healthy in this room -- in people
25 with dysautonomias of various sorts there may be great

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1 amplification of that signal, and we may discover things
2 because of that. For example, only in the last 5 years
3 has the effect of water, drinking water on blood pressure
4 been discovered.

5 You know, this is something you would think
6 might have occurred in the 17th Century, but it didn't.
7 It occurred in the late 1990's, and it occurred because of
8 the amplification of signal in people with dysautonomias.
9 16 ounces of water raised blood pressure 40 millimeters of
10 mercury within 30 minutes in this population, and then
11 when we went carefully and looked to normal subjects, and
12 I guess Dr. Jordan in particular was the one who made
13 these crucial observations, we found that there was also a
14 pressor effect of water in normal human subjects that just
15 never got into the physiology textbooks.

16 Then, once you discover a phenomena like that
17 through the different models of dysautonomias and seeing
18 how different people, different kinds of patients react to
19 water, you might be able to go a long way toward deciding
20 where the mechanism is located and that's a process that
21 people are going through now, so these teach us a lot
22 about phenomena, they teach us a lot of phenomenology, and
23 they teach us a lot of mechanisms.

24 Now, today I want to talk about dopamine
25 betahydroxide deficiency.

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1 (Slide.)

2 DR. ROBERTSON: It was a syndrome initially seen
3 by us in a 33-year-old woman who had had severe low blood
4 pressure every time she stood throughout her life, and
5 only was able to stand about 30 seconds without losing
6 consciousness, and so had spent her life seated in chairs
7 or on the floor almost all the time, and she was able to
8 walk some distance, but I think very hard to get from this
9 podium to the door for her under most circumstances.

10 The other thing about her is, she had ptosis of
11 the eyelids, as others with this disease have had. She
12 had nasal stuffiness when she lay down at night, that's a
13 sign of autonomic impairment, and there was a complicated
14 perinatal course. She could not open her eyes when she

15 was born. The physician felt she was going to die, and
16 told the family to be prepared for that, but she survived
17 because she was placed in an incubator and so was able to
18 overcome the hypothermia, and I think the reason we see
19 these people now and not before incubators were around is
20 that crucial element of heat control, so she survived all
21 of this, and survived to be seen.

22 Male subjects with this disease, I just put down
23 here at the end, have retrograde ejaculation also as a
24 symptom.

25 (Slide.)

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1 DR. ROBERTSON: So in thinking about this
2 person, the way we analyzed her was, we asked if the
3 pressor reflexes were intact. We can check that by having
4 a patient place their hand in ice water, half ice, and
5 half water, for 60 seconds. If you do that, and you're
6 normal, you will raise your pressure about 20 or 25
7 millimeters of mercury in 60 seconds, your hand in ice
8 water, but clearly, this woman did not do that. If
9 anything, her pressure fell, so that indicated that her
10 nonadrenergic nerves were not doing their job to raise
11 pressure in this situation.

12 There are several things you can do to
13 understand what's going on in a patient like this.

14 (Slide.)

15 DR. ROBERTSON: One thing you can do is to draw
16 blood and look at norepinephrine and its metabolites and
17 precursors, and another thing you can do is to actually go
18 into the neuron that is releasing norepinephrine and
19 measure the electric activity. It is in fact not all that
20 difficult to do this in human subjects. The perineal
21 nerve is available, the lateral aspect of the knee. You
22 can enter that and there are many fibers there that are
23 not nonadrenergic, but you can find the nonadrenergic ones
24 because they fire in a sequence that is tied to the heart
25 rate, so if you find a nerve that is beating in sequence

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1 to the heart in the leg, it's pretty much got to be a
2 nonadrenergic nerve, and there's a lot of evidence for
3 that that I won't go into.

4 And so we thought maybe this woman has no
5 nonadrenergic nerves at all, so we did the test of
6 microneurography. In a normal person, going from supine
7 to upright there's about a doubling of the burst per
8 second of nerve activity in a nonadrenergic neuron, and
9 interestingly enough, in our patient, not only was there
10 an appropriate level of electrical activity in her
11 nonadrenergic nerve, but perhaps even a little more than
12 most people might have, perhaps because the pressure was
13 low, and in response to upright posture her activity of
14 her nonadrenergic nerve went up just as it should do.

15 Another thing there could have been is a problem
16 with the receptors for norepinephrine not being there.

17 (Slide.)

18 DR. ROBERTSON: That could be tested by given
19 alpha 1 or beta 1 or beta 2 agonists, and when we did that

20 we found that not only were these present at 100 percent
21 activity, as they are in all of us, but actually they were
22 sixfold hypersensitive, so not only were the receptors
23 there, but they were there in abundance, and very, very
24 hyperfunctional, so the electrical activities in the nerve
25 and the receptors are there, so the problem must be some

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1 other place.

2 (Slide.)

3 DR. ROBERTSON: Just looking at the
4 nonadrenergic synapse, normally dopa is made into dopamine
5 in the cytoplasm then pumped into the neuron, or pumped
6 into the vesicle where dopamine beta hydroxylase is
7 located and makes it into norepinephrine, and that
8 eventually gets packaged and released into the synaptic
9 cleft.

10 So a problem anywhere along this pathway could
11 lead to difficulties, and so we looked to see if the
12 patient had norepinephrine in her plasma, and we found
13 that the patient here had no norepinephrine in her plasma,
14 whereas normally people would have 250 picograms per mil.

15 On the other hand, most people who are healthy
16 have very little dopamine in her plasma, but this patient
17 had very high levels of dopamine, and just eyeing these
18 two relative to each other, the dopamine and
19 norepinephrine, you might wonder, is there stoichiometric
20 replacement of norepinephrine by dopamine in these
21 neurons.

22 We can test that by giving a drug called
23 tyramine, which was alluded to yesterday by Maureen Hahn.
24 Tyramine releases neuronal catechumen stores, and although
25 when we gave Tyramine no norepinephrine appeared in the

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1 blood from neurons, a lot of dopamine did, and that
2 indicated that the neurons were filled with dopamine
3 rather than norepinephrine, so it looked to us like the
4 DBH was probably -- since this was undetectable and this
5 was high, and this was somewhat high, it looked to us like
6 DBH was the likely villain.

7 (Slide.)

8 DR. ROBERTSON: The genetics, we have been able
9 to work out the genetics of it in the last year, and we
10 find that there are basically three haplotypes that are
11 involved in the cases that have been studied so far, so
12 you've got to have two bad copies of DBH to get this
13 disease. This is the most interesting one of them.

14 (Slide.)

15 DR. ROBERTSON: I will just show you this, which
16 indicates that whereas exon 1 ought to end appropriately
17 here, because of the change in nucleotide at this point
18 actually it doesn't end there, but a whole lot of intron 1
19 gets tied onto that, or remains tied onto that, and so
20 that presents great difficulties for the patients, and we
21 initially reported the lowest levels of norepinephrine
22 that had ever been reported, but as our assays become
23 better we think we overestimated then, and we're not sure
24 there is even one molecule of norepinephrine in the body

25 of these patients, so basically it's dopamine in the
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1 synapse coming out of the nonadrenergic neuron. No
2 norepinephrine, just dopamine.

3 (Slide.)

4 DR. ROBERTSON: And so one thing that I thought
5 was extremely interesting was that these patients had
6 complete absence in blood, CSF in urine and
7 norepinephrine, epinephrine, noremetanephrine,
8 metanephrine, DHPG, and VMA, and yet I think that anyone
9 in this room could talk to this person while seated for a
10 half-hour and not detect any cognitive or psychiatric
11 difficulties.

12 (Slide.)

13 DR. ROBERTSON: This is an example of where you
14 may be able to do something dramatically helpful if you
15 really understand mechanisms, so when we knew the problem
16 and we knew all of the things that the patient had that
17 were workable we were able to take a drug, norepinephrine,
18 with a carboxyl group attached which is called LDOPS and
19 which was mentioned yesterday by Dave Goldstein and some
20 others during the conference -- when we gave that drug to
21 the patients, whereas the norepinephrine was undetectable
22 on placebo, LDOPS gave normal blood levels of
23 norepinephrine, so we chose this drug -- we chose this
24 drug because the carboxyl group would help us get it
25 absorbed from the stomach and get it past the liver.

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1 It would still be a substrate for the
2 norepinephrine transporter, so it would get into the
3 neuron, and there's plenty of dobowdecarboxylase in the
4 neuron to decarboxylate this and target the norepinephrine
5 right where it was needed, and you can also see that this
6 standing time, the amount of time the patient could stand
7 motionless without losing consciousness rose from about 30
8 seconds to 600 seconds. 600 seconds is 10 minutes, and in
9 our clinic that is considered a cure. If you can stand 10
10 minutes, that is all we ask of anyone, so if you can stand
11 10 minutes you can walk for an hour because of the
12 muscles, and so this is really great.

13 And notice also that the actually the dopamine
14 actually went down, perhaps because of feedback inhibition
15 of the tyrosine hydroxylase.

16 (Slide.)

17 DR. ROBERTSON: One of my teachers, Otto Kuchel,
18 when he heard us report this disease said, why do you
19 think the blood pressure is low in this patient? He said
20 that I believe with a straight face, and I said, well, you
21 know, this is the lowest norepinephrine ever seen in a
22 human being. I was leaning toward that as the reason for
23 the low blood pressure, and he said, what about the
24 dopamine, don't you think that dopamine could be involved
25 in that, and so we discussed it and we decided that

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1 dopamine could be involved.

2 (Slide.)

3 DR. ROBERTSON: So we decided to give the drug

4 metyrosine. Metyrosine, which basic scientists frequently
5 call alphanethyl P tyrosine in the older literature. The
6 tyrosine blocks tyrosine hydroxylase and reduces dopa,
7 dopamine, norepine and epine, and since norepinephrine
8 dominates blood pressure in virtually all of you, in just
9 everyone who had been given this drug it had always
10 lowered blood pressure, but if you didn't have
11 norepinephrine and you only had dopamine, and if that
12 dopamine were lowering blood pressure, then metyresine
13 ought to paradoxically raise your pressure, which it had
14 never done in anyone before, and so Dr. Kuchel and I
15 agreed that was the thing we would do

16 (Slide.)

17 DR. ROBERTSON: And much to my surprise at the
18 time, but -- and possibly even to his surprise we found
19 that the norepinephrine was low before and low after
20 metyrosine. That's no surprise the dopamine was high
21 before metyrosine. We gave it to lower dopamine, and it
22 did lower dopamine, but look what happened to systolic
23 supine pressure. It went from 110 to 165, quite a large
24 blood pressure increase, perhaps the highest blood
25 pressure this patient had ever had. We discontinued the

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1 drug, because we were worried about how her blood vessels
2 would respond to that kind of pressure, never having had
3 it.

4 Moreover, the patient's weight rose 1 kilogram,
5 suggesting that dopamine in this patient was functioning
6 perhaps as an endogenous diuretic, so I think it
7 reemphasized the great importance dopamine may have, and
8 in my next-to-the-last slide --

9 (Slide.)

10 DR. ROBERTSON: -- let me just suggest to you
11 that I still think that -- I've never been able to
12 persuade anyone, other than perhaps Dr. Axelrod of this,
13 but certainly I've not been able to persuade foundations
14 for support for this, but I still think that dopamine
15 being raised during crises and familial disautonomia, I
16 think we cannot ignore that, because we know dopamine can
17 cause a diureses and hypobulimia, and that you can get a
18 hypobulimic labile hypertension.

19 We know that it can cause vasodilatation in the
20 coronary arteries, the venal arteries, the masseteric
21 arteries, and the cerebral vasculature. We know that in
22 the CNS it can alter mentation, and we know that through
23 the area postrema, even at the edge of the blood brain
24 barrier can cause nausea and vomiting, so I think this is
25 one thing that I hope will get tested in familial

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1 dysautonomia by someone in the future.

2 (Slide.)

3 DR. ROBERTSON: So in conclusion, autonomic
4 disorders lead us to the discovery of new phenomena and
5 mechanisms. They are very heterogeneous. There are
6 probably hundreds of genetic and acquired
7 pathophysiologies out there to be discovered, especially
8 in the group with orthostatic intolerance, or POTS. Many

9 but not all of these disorders should one day be as
10 amenable to drug therapy as DBH deficiency.

11 Thank you.

12 (Applause.)

13 DR. ROBERTSON: Our presentations this morning
14 are going to be diverse, and I think we will probably take
15 questions at the end of the morning if that's okay, Dave.
16 We have a number of presentations this morning that I want
17 to just introduce you to briefly before I turn to our next
18 speaker. Two of these relate to orthostatic intolerance,
19 POTS-type entities. The first of these is NET deficiency,
20 by Linda Smith. The second will be neuropathic disorders
21 that Giris Jacob will present, and finally Jens Jordan
22 will be talking about genetics factors and Dan O'Connor
23 about the genetic determinants of autonomic blood
24 pressure.

25 Let me start by introducing Linda Smith. The
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1 power of the presentation by Dr. Peltzer yesterday I think
2 will come into play again, because there is nothing as
3 shaking to all of us as the experience of a real patient,
4 or a real mom who has to deal with these devastating
5 disorders, and Linda Smith is a great hero to all of us in
6 the autonomic field, because she has been able to in spite
7 of her illness, do a great deal, I think. Her web site
8 has established the National Dysautonomia Research
9 Foundation and the web site has I think become a major
10 clearinghouse for genetic autonomic disorders and the way
11 people find a way to expertise around the country.

12 She is the sort of person who can call up the
13 White House and get the President to agree to come and
14 speak at her conference. Not many of us have the gumption
15 to even undertake that.

16 But I will just stop here and introduce to you
17 Linda Smith.

18 NET DEFICIENCY: PERSPECTIVE OF A PATIENT

19 LINDA J SMITH, EXECUTIVE DIRECTOR/FOUNDER,
20 NATIONAL DYSAUTONOMIA RESEARCH FOUNDATION, REDWING, MN

21 MS. SMITH: Thank you, Dr. Robertson. I'd like
22 to begin by thanking the National Institutes of Health and
23 Dr. Hardy for inviting me to speak with you today. I have
24 been asked to speak on the subject of the norepinephrine
25 transporter deficiency, not as a researcher or as a
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1 physician but as a lifelong patient of autonomic
2 dysfunction due to a genetic mutation of the
3 norepinephrine transporter.

4 My talk today will focus on three areas. First,
5 I will let you know how the norepinephrine transporter
6 deficiency has affected myself from early childhood,
7 adolescence, and adulthood. I will then give you a better
8 understanding of the National Dysautonomia Research
9 Foundation and its role in providing patients with
10 autonomic, or with information and support on autonomic
11 disorders, and finally I will share some observations and
12 possible changes that could help to see improvements in
13 access to quality health care and improved opportunities

14 for research into dysautonomias.

15 The norepinephrine transporter deficiency, which
16 I will now refer to as the NET, was identified as a
17 genetic mutation in my family in 1999. My mother, three
18 sisters, including my twin sister, one brother, and one of
19 our children all carry this genetic mutation. 50 percent
20 of my family have been diagnosed with this condition.

21 Although I and other members in my family
22 suffered from symptoms often seen in orthostatic
23 intolerance and neurocardiogenic syncope, the type and
24 range of symptoms are quite variable. All of us are
25 impacted with changes in heart rate and blood pressure.

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1 However, each of us is impacted in a different way.

2 Each of my family members lead fairly normal and
3 healthy lifestyles. Despite the belief of many physicians
4 that we're confused by the symptoms prior to the discovery
5 of the NET mutation, none of us suffer from depression or
6 are addicted to drugs. We are not hypochondriacs, and we
7 don't fixate on our symptoms. In fact, my family members
8 all have a tendency to ignore or dismiss our symptoms as
9 nothing more than an inconvenience, or that everyone else
10 in the world must have the same problem.

11 My early childhood included very infrequent
12 symptoms of syncope, high heart rates, nausea, vomiting,
13 migraine headaches, fatigue, motion sickness, fevers,
14 severe joint pain, and gastrointestinal problems. During
15 my adolescence, my symptoms started to change. Growing up
16 on a huge dairy farm we led an extremely busy life. Our
17 chores began at 4:00 a.m. every morning 7 days a week, 365
18 days a year. Whenever I didn't feel well, I would
19 convince myself that this was due to not enough sleep or
20 not eating for hours at a time.

21 By the time I turned 13, I began to notice that
22 my headaches became more severe, and I had more trouble
23 concentrating or keeping my focus. I was able to keep up
24 with the other members of my family and, in fact, likely
25 benefitted from the effects of the NET deficiency. On

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1 many occasions I found it easy to keep pace, and had a
2 higher level of energy than the majority of my family and
3 my friends, but then there were also times that I had
4 difficulty lifting my plate, or walking from one room to
5 the next, and the simple task of breathing seemed
6 difficult.

7 Then during a routine physical exam in 1980 at
8 the age of 17 I was told by a local physician that he
9 thought I might have coronary artery disease. The
10 followup tests ordered by the physician were all normal.
11 We were thrilled, and we moved on with our life.

12 About a year later, I moved to Los Angeles. I
13 noticed changes in my health once again, and I was
14 directed to see a neurologist to investigate what might be
15 causing my new symptoms. I was experiencing an increase
16 in headaches, dizziness, syncope, visual distortion,
17 cognitive impairment, and body tremors. Again, all
18 tests, including a CAT scan, revealed normal results. My

19 doctor in L.A. was concerned about my symptoms, but was
20 very confused as to how to proceed. I solved his problem
21 by moving again, this time to Florida.

22 My move to Florida brought about some new
23 problems. I noticed that the heat played a major role in
24 my episodes, yet I didn't know why. At times, my standing
25 period was under 30 seconds. I learned to avoid outdoor

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1 activities but did not see a doctor for my symptoms until
2 several years later, when my husband noticed the high
3 heart rates with very little physical activity. I
4 maintained a very extensive exercise schedule, yet my
5 heart rate rose rapidly just walking across the room.

6 My physician ordered a holter monitor, but later
7 canceled the order upon learning I was pregnant. She
8 believed that it would be best to have the obstetrician
9 follow me during my pregnancy, and assumed that this was
10 the likely cause of my many heart rate changes.

11 During my pregnancy, my obstetrician found that
12 my blood pressures were abnormal and told me that I had
13 toxemia poisoning. I was confined to bed rest after my
14 fourth month of pregnancy and several months later
15 delivered a beautiful baby girl by Cesarean section.
16 Unfortunately, I again experienced complications that
17 caused the anesthesiologist some serious concerns. My
18 blood pressures during recovery began to rise rapidly, and
19 became so high that the anesthesiologist spent over 3
20 hours with me during my recovery. He later told my
21 husband that after spending 30 years treating patients, he
22 had never seen a response like mine to an epidural. He
23 emphasized to my husband that we should follow up with my
24 doctor regarding this unusual response once I was released
25 from the hospital.

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1 After the birth of my daughter, I began to
2 notice even more change. I developed more chest pain,
3 periods of slurred speech, dysfunctional sleep, trouble
4 keeping my balance and walking. I had burning pain in my
5 arms and legs. Sometimes this burning pain would feel
6 like a thousand bee stings. I also began to have fainting
7 episodes that forced me back to my family doctor. A visit
8 to my family physician revealed some unusual findings, and
9 he made arrangements for a referral to a well-respected
10 nephrologist in Clearwater, Florida. The nephrologist was
11 positively certain that my symptoms were caused by a
12 pheochromocytoma.

13 Again, countless tests were performed. This
14 time, an MRI showed three small spots, which she believed
15 were from the mild strokes and was the cause of the
16 slurred speech, hesitant gait, memory and swallowing
17 difficulties, and the headaches. The holter monitor
18 recording showed highly variable blood pressure and heart
19 rates that once again perplexed my doctor.

20 After referrals to several neurologists and
21 cardiologists, I was sent to a team of
22 electrophysiologists. They realized I was having some
23 type of autonomic regulatory dysfunction. They worked

24 closely with me and provided referrals to a famous
25 specialty center in an attempt to find a solution and

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1 restore me back to health.

2 I had one of the first tilt table exams on the
3 West Coast of Florida, and eventually had a pacemaker
4 implant in an effort to control my heart rate changes. By
5 the time I reached the age of 30, I had seen over 25
6 physicians, had been hospitalized over a dozen times, and
7 had countless tests and procedures. I had MRI's, PET
8 scans, colonoscopies, echocardiograms, EKG's, EEG's,
9 laparoscopies, EPS studies, a pacemaker implant, and
10 countless blood tests.

11 I had been seen by general practitioners,
12 neurologists, cardiologists, electrophysiologists,
13 nephrologists, and gastroenterologists. I had been
14 diagnosed with vasodepressor syncope, coronary artery
15 spasms, epilepsy, Renaud's Phenomenon, Syndrome X, Sub-
16 Clavian Steel Syndrome, and panic attacks. I was
17 prescribed multiple medications: Atropine, lasix,
18 scopolamine, calcium channel blockers, beta blockers,
19 nitroglycerine, tegretol, dilantin, and countless other
20 medications.

21 It seemed as though every doctor visit would
22 result in being referred to another specialist, or to
23 another facility for yet more tests. At the age of 32, a
24 relocation for my husband resulted in a move to a new town
25 in Florida. During the first month in our new town, I had

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1 a major episode. I called my husband to tell him I was on
2 a riding lawnmower mowing the yard when I started to feel
3 crushing pain in the middle of my back, my chest, and my
4 left arm, and the left side of my face was completely
5 numb. I had great difficulty walking into the house.
6 When I did get into the house, I went into the bathroom to
7 take a cool shower, believing that maybe I was suffering
8 from heat exhaustion, or that maybe I had damaged one of
9 the pacemaker leads by mowing the yard.

10 I looked into the bathroom mirror. Both of my
11 eyes were extremely dilated and glassy, and the entire
12 left side of my face was drooping. I had a difficult time
13 breathing, and my heart rate was in excess of 150 beats a
14 minute. My husband called my previous cardiologist, who
15 in turn called the EMS to our home. By the time the EMS
16 crew arrived I was in a fetal position and had no feeling
17 from my neck down.

18 By the time I reached the hospital at 6:30 p.m.,
19 I was seen by one ER nurse. More than 5 hours later a
20 physician came into the room to examine me. My sister-
21 in-law, a critical care nurse of 25 years, was present
22 with my husband. My sister-in-law was not happy that I
23 was not being monitored or examined, and asked if it would
24 be possible for me to be given fluids. At 3:00 a.m., more
25 than 10 hours after the episode began, I was finally given

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1 fluids and was admitted to the hospital.

2 The following morning, I met a new doctor who

3 spent under 5 minutes with me and asked me three
4 questions, 1) how many children do you have, 2) what does
5 your husband do for a living, and 3) what insurance
6 company do you have? He took no family history or blood
7 pressures, ordered more than \$10,000 in additional tests,
8 and then had a nurse give me discharge instructions that
9 were as follows: return home and take a multivitamin with
10 iron.

11 My husband had not spoken with a doctor and was
12 concerned as to what we should do. We were in a new
13 community, and did not know who to turn to. My husband
14 asked the nurse to please have the physician contact him
15 and give him some idea of what may have happened. When
16 the physician did contact my husband, he informed him that
17 he sees these types of symptoms in many young women that
18 come into the ER, and he was very surprised that I didn't
19 ask for drugs.

20 Then he proceeded to ask my husband if I had any
21 psychological problems. When my husband asked, why would
22 he state this, his response was, well, your wife didn't
23 look me in the eye. Had he taken the time to ask, he
24 would have discovered that when I am symptomatic I get
25 blurred or double vision, so I focus on stationary things,
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1 things that don't move, not to mention keeping eye contact
2 is not a priority when you're feeling like you've just
3 been hit by a truck.

4 After speaking to several people, I learned of a
5 local neurologist who is treating a well-known tennis
6 player for symptoms that were similar to mine. I was seen
7 in his office, where he told me that medicine involves
8 some key concepts that are not always adhered to. At this
9 point, he told me that he would take me off all
10 medications, and that I would have to keep a daily
11 journal. He also told me that it might take some time to
12 find a cause for my symptoms, and he asked me to be
13 patient with him as we worked together to find a solution.

14 After 1 month of journal entries, and a study to
15 measure the blood flow to the brain, I was informed that
16 my symptoms were indicative of autonomic dysfunction. I
17 then NORDD, the National Organization for Rare Disorders,
18 and was given the contact information for Vanderbilt
19 University's Autonomic Dysfunction Center.

20 I proceeded to give Vanderbilt a call. Within 2
21 hours, I was in conversation with a very pleasant
22 physician from Israel. He recommended I be evaluated at
23 their facility. Within 2 weeks of our conversation, I was
24 diagnosed with orthostatic intolerance. I was told to
25 increase my salt and fluid intake, and to modify my
0027

1 lifestyle to accommodate the symptoms. After more than 30
2 years of countless tests, physicians, and
3 hospitalizations, I finally had a diagnosis and a
4 treatment plan that was beginning to work.

5 Soon after my diagnosis of orthostatic
6 intolerance, we relocated to a much cooler environment,
7 Minnesota. This seemed to make all the difference in the

8 world. In retrospect, my symptoms were always the worst
9 during the warmer Minnesota summers as a child, and when I
10 had moved to California and Florida. My symptoms today
11 include periods where I am relatively symptom-free,
12 followed by periods of flare-ups. Some episodes are worse
13 than others, and some require hospitalization.

14 I have developed more gastrointestinal problems,
15 and I also have erratic changes in body temperature. My
16 sleep pattern is more dysfunctional, and I also experience
17 more breathing problems, but at least now I have some
18 ability to manage my own health care and can enjoy a much
19 greater quality of life, and the knowledge of knowing that
20 my condition is life-altering, but not likely life-
21 threatening, has been extremely reassuring.

22 Although the discovery of the NET has not
23 resulted in any new therapy at this point in time, the
24 knowledge that there is hope for future improvements based
25 upon this discovery is especially important to me as a

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1 mother who has a child with this genetic mutation.

2 My ordeal in getting a correct diagnosis and the
3 tremendous cost to my family was staggering. By the time
4 I was diagnosed with norepinephrine transporter
5 deficiency, we had spent hundreds of thousands in medical
6 bills, lost two homes, suffered the loss of three
7 pregnancies, my job, and many of our closest family and
8 friends, but despite all this, the worst aspect of my
9 ordeal was in trying to convince everyone that I was not
10 making these things up. It is difficult enough dealing
11 with chronic symptoms, but when no one believes you, or
12 attempts to tell you that you really need to see a
13 counselor, it makes it that much harder.

14 It is especially hard to accept when it is based
15 upon a completely subjective opinion based upon a 5 or 10-
16 minute conversation with someone you have never met
17 before, and who knows absolutely nothing about you.
18 Unfortunately, I have learned that my experience was not
19 the exception in this area but, rather, the rule. I
20 realized that someone needed to do something to help
21 improve awareness of these conditions and help patients
22 like myself find better access to quality health care.
23 What if others could benefit from my ordeal?

24 In April of 1997, after spending nearly three
25 and a half decades looking for answers and trying to get

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1 help, I started the National Dysautonomia Research
2 Foundation, the NDRF. With help from my husband, Dan, and
3 several leading autonomic experts we can provide
4 information and support to thousands of individuals and
5 their families worldwide, and will continue to advocate
6 for all forms of autonomic involvement.

7 Our web site is visited by as many as 18 to
8 22,000 visitors each month. Many of these individuals
9 contact us frustrated, angry, and scared. Some have
10 voiced that it would be easier to suffer in silence than
11 to keep trying to get help. Our foundation provides
12 educational material aimed at the patient and their

13 families, as well as information for medical
14 professionals. We hold patient conferences, bringing
15 together individuals with all forms of autonomic
16 involvement.

17 We have held CME sessions for physicians, and
18 have mailed information about dysautonomias to over 25,000
19 physicians in the United States. We recently published a
20 new book, the NDRF handbook for patients with
21 dysautonomias. With the help of Dr. David Goldstein, this
22 book provides a patient with a layman's guide to the
23 autonomic nervous system, the disorders that can develop,
24 as well as diagnostic test and treatment options.

25 The book also contains some practical

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1 information on coping with chronic illness, as well as
2 other tools that can help patients and families. The book
3 is available from our web site as a free download. Please
4 feel free to inform your patients of this most valuable
5 resource.

6 I would like to share a story with you on the
7 importance of education and support in living with
8 dysautonomia. A woman recently contacted me and told me
9 she was on the brink of institutionalizing herself after
10 all three physicians recommended that she seek
11 psychological help. This was due to her inability to
12 recall her more severe episodes. When she visited with
13 her psychiatrist, he told her to NDRF web site. It was
14 there that she learned that her symptoms were real and
15 that countless others felt the same way she did. She was
16 also able to find a physician within an hour of her home
17 who was able to help her.

18 Today, with a change in diet and a small amount
19 of medication, she lives a perfectly healthy lifestyle.
20 She is back working full-time, her marriage is doing well,
21 and she was just informed she's going to have a baby in
22 late spring. She has never felt better or been happier in
23 her life.

24 Of course, not all forms of dysautonomias are so
25 easy to handle and, as many of you know, some have

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1 devastating and life-threatening or life-ending results.
2 NDRF also acts as an advocate to help find ways to improve
3 health care and advocate for equity in research funding
4 for this population, but how do we improve the current
5 system? This last July, NDRF held a patient symposium in
6 our Nation's capital. Patients and family members
7 representing various autonomic disorders spoke to key
8 Members of Congress. Our message was a simple one, that
9 the more than 1 million Americans impacted with
10 dysautonomia deserve an opportunity to find adequate
11 health care, and that more funding needs to be made
12 available for medical research.

13 Our congressional representatives heard from
14 parents of children with FD, from an individual who is
15 suffering from MSA, and from a young woman who had her
16 quality of life stolen by orthostatic intolerance. We
17 spoke to several key congressional leaders about the need

18 to improve awareness of autonomic disorders and to improve
19 access to the health care system. We also discussed the
20 need for medical schools to spend more time studying
21 disorders that affect one of the most important systems in
22 the human body, the autonomic nervous system.

23 We discussed the overwhelming number of women
24 and children impacted with these disorders, and the fact
25 that gender bias may play a role in keeping autonomic

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1 disorders low on the priority list.

2 We discussed centers of excellence, and the
3 great need to have facilities established throughout the
4 United States, not just for one form of autonomic
5 involvement, but for each and every form of autonomic
6 involvement. These centers would also help provide more
7 opportunities for research efforts and reduce the cost for
8 treating patients with these disorders.

9 Keep in mind that, due to the lack of
10 understanding within the medical community, dysautonomias
11 are often not recognized and, as such, insurance companies
12 often refuse to cover the cost of the patient's treatment.
13 Centers of excellence will vastly improve this.

14 Our visits on Capitol Hill met with great
15 success. We have commitments from key Senators from both
16 the Health and Appropriations Committees to help us in our
17 efforts. Senator Wellstone has made a commitment to us to
18 find funding for centers of excellence, and to make an
19 amendment to include dysautonomias within the Social
20 Security Administration. We will be working with these
21 Members of Congress over the course of the next several
22 months to reach these goals, and we look forward to your
23 support and help through this process.

24 Finally, I close with this. When I was in elementary
25 school my teacher asked a small boy in our class to lift a

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1 giant trampoline. Obviously, he couldn't lift it. Then
2 he asked all of us to gather around the trampoline and
3 lift together. To our amazement, we were not only able to
4 lift the trampoline, but we were also able to move it
5 clear across the room. All were shocked at how light the
6 trampoline was when it was lifted together.

7 My experience in finding a diagnosis for my form
8 of dysautonomia has been at times a great test of my inner
9 strength, but it has also taught me how light the burden
10 can be when both the physician and the patient work
11 closely together. It is my hope that all of us lifting
12 together can help improve the lives of all of those
13 impacted with dysautonomias.

14 Thank you.

15 (Applause.)

16 DR. ROBERTSON: Thanks very much, Linda. That's
17 very helpful, and maybe you could just stay there in
18 preparation for questions a little bit later. I should
19 call attention to the fact that she's greatly helped by
20 Dan Smith, who may want to stand back there, her husband.

21 (Applause.)

22 DR. ROBERTSON: I know that also there's another

23 advocacy group here representing the patients with Shy-
24 Drager Multiple System Atrophy. Don Summers, do you want
25 to stand?

0034

1 (Applause.)

2 DR. ROBERTSON: And I hope, if there are any
3 other patient advocate groups that are here that have not
4 had attention called to them, please feel free to stand up
5 and tell us who you are. We don't want to overlook
6 anyone. Okay, let me now go to the third presentation,
7 and that is by Dr. Giris Jacob on Neuropathic Postural
8 Tachycardia Syndrome. Dr. Jacob.

9 NEUROPATHIC POSTURAL TACHYCARDIA SYNDROME
10 GIRIS JACOB, M.D., D.SC., RAMBAUM MEDICAL CENTER,
11 HAIFA, ISRAEL

12 DR. JACOB: It is very impressive, and it says
13 how science and humanism goes together without politics.
14 Sorry about saying that, but thank you for the invitation
15 for giving this talk, but neuropathic postural tachycardia
16 is one of the facilitating diseases that I have met in my
17 training, and the name is neuropathic postural
18 tachycardia, just recently introduced, and we're going
19 through this history, the long history, beginning with the
20 POTS.

21 (Slide.)

22 DR. JACOB: POTS, Postural Orthostatic Syndrome
23 is a chronic form of orthostatic intolerance. Orthostatic
24 intolerance is defined when a patient has orthostatic
25 symptoms occur in the absence of a major drop in blood

0035

1 pressure.

2 (Slide.)

3 DR. JACOB: Many names have been hung on this
4 disease, and many pathologies it seems to me, and these
5 syndromes, Postural Orthostatic Tachycardia Syndromes is
6 the mainstream name now, and they're mostly used by
7 physicians and introduced in the textbooks lately.

8 Hyperadrenergic orthostatic hypertension is said
9 about the status of these patients in terms of bioclinical
10 status and clinical status. That's meaning they have
11 hyper and high catecholamines often encountered.
12 Hyperdynamic beta adrenergic state was introduced by
13 Freulich in the sixties, hydrophatic hyperbulimia in the
14 eighties, mitrovalve prolapse, Barlev's Syndrome formally,
15 has disappeared from textbooks. Vasiloregulatory asthenia
16 also is an old name, irritable heart, or Soldier's Effort
17 Syndrome, and some of the patients with chronic fatigue
18 syndrome meet this criteria of autostatic intolerance.

19 That means they are confused, they are swimming
20 in a big, big swimming pool with a lot of these names and
21 lots of these allergies, but we are going to focus on a
22 small part of these patients. We defined, we all defined
23 POTS, postural orthostatic tachycardia as the autostatic
24 syndromes that chronically occur at least for 6 months.
25 It has a remarkable increase in heart rate when standing,

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1 more than three beats per minute, and the blood pressure

2 does not drop that much significantly with standing, and
3 the normal or high plasma norepinephrine, and for
4 instance, no associated illnesses.

5 (Slide.)

6 DR. JACOB: The epidemiology, as David stated
7 before, this disease affects mostly young women. The
8 incidence earlier is a 1/2 million Americans and at least
9 1 percent or 2 percent of the population. It occurs
10 mainly after a viral infection. Some of them, they report
11 EPV infections. Some of them, they claim that they have
12 trauma delivery, blood transfusion before the occurrence
13 of the disease, although most of the patients, they don't
14 recall any of these.

15 The symptoms seems to be, or the disease seems
16 to be disappearing with aging. We are not going to argue
17 about this finding.

18 (Slide.)

19 DR. JACOB: Here is visually what happens to
20 these patients when they stand. When they are lying down
21 they are perfectly normal, and once they are standing they
22 begin with lightheadedness, dizziness, blurred vision, and
23 then palpitations, nervousness, and in most of them they
24 don't let the syncope occur because they know the symptoms
25 and can lie down and avoid the occurrence of such an

0037

1 event, and many of them, they have minorities of the
2 patients they have reduced quality of life and avoidance
3 of long standing, and they quit from their job for years
4 and months.

5 (Slide.)

6 DR. JACOB: Here is the marker of the disease.
7 The marker of the disease, how we stated before, look at
8 the normals when they're lying down they have 65, 67 of
9 heart rate, and when they stood they increased the heart
10 rate by 15 beats per minute and maintained 1 hour.

11 Our patients, the POTS patients, they have a
12 little bit higher heart rate when they are lying down, but
13 when they're standing a remarkable increase. Some of them
14 to 150, 180 at the beginning, and maintain that if they
15 can stand. These patients at least stand for 1 hour, some
16 of them, and they maintain this heart rate consistently.

17 (Slide.)

18 DR. JACOB: This is how we stated also before
19 that blood pressure, the mean arterial blood pressure when
20 we put them on the tilt table for a while at 15 degrees,
21 30 degrees, 45, up to 75 degrees, the blood pressure
22 remained and changed along the green line and also not
23 differently from the controls.

24 (Slide.)

25 DR. JACOB: Also, because of the disease, also

0038

1 the orthostatic intolerance is called hyperadrenergic
2 hypertension, or hypertension. In these patients we found
3 that they have high catecholamines when they stand. Here
4 is the level, here is the plasma concentration of
5 catecholamines when they're lying down, 250 like they're
6 normal, sometimes they're higher, sometimes they're lower,

7 and it's doubled when they stand, or tripled when they
8 stand from the normals, meaning it's in the patients, as
9 we see it rises to levels of thousands. One of our
10 patients reached the 4,000 picogram per milliliter. It
11 sounds to be like ferachlomatsotoma, and many of them
12 mistakenly they diagnosed as ferachlomatsotoma, and just
13 the site was never found.

14 And we ask ourselves the first question, and
15 this is the question for us.

16 (Slide.)

17 DR. JACOB: Why these patients, we just simply
18 measure the norepinephrine and just simply spillover, how
19 much the spillover to the plasma norepinephrine, and we
20 found that these patients in green lines, they don't
21 produce, or don't release more norepinephrine than the
22 controls. That means that the high catecholamines in the
23 plasma, they are not due to an overprotection, or
24 overrelease of norepinephrine into the plasma.

25 (Slide.)

0039

1 DR. JACOB: Meanwhile, the clearance of the
2 norepinephrine was surprisingly reduced. We just looked
3 at the steady state here, where we reached the steady
4 state. Here the kinetic steady state wasn't stable, and
5 we just look at these two points, 20 minutes standing and
6 30 minutes standing, and the changes in the clearance.
7 The change in the clearance when we stand is reduced about
8 30 percent, and by that it is contributing to the high
9 norepinephrine when we stand.

10 Meanwhile, our patients, they reduce about 50
11 percent, they reduce the clearance of norepinephrine.
12 That means they don't have a release of norepinephrine.
13 We have decreased clearance systemically of
14 norepinephrine.

15 (Slide.)

16 DR. JACOB: We ask again the question, why that,
17 because they don't produce more norepinephrine than
18 controls. Let's give them tyramine, and we give them
19 tyramine, they require higher doses of tyramine in order
20 to increase their blood pressure by 25 units. That means
21 they require 30 percent more tyramine, more controls in
22 order to reach the same blood pressure.

23 That means these patients are tyramine-
24 resistant, and if we don't have these nerves, maybe we
25 suppose these nerves don't function very well, let's try

0040

1 to find what happened to the receptors, alpha and beta
2 receptors, and not surprisingly, others have the same
3 finding. These patients, our patients, they require less
4 dose, or a half-dose of phenylephrine in order to increase
5 their blood pressure by 25 units. That means they have
6 hypa, alpha 1 or hypa responsiveness.

7 I'm afraid to say hypa specifically, because of
8 the buffering by receptors and other systemic things
9 affecting this finding of the sensitivity, but when we
10 corrected this sensitivity, we still have alpha receptor
11 hyper responsiveness, or hypersensitivity.

12 Also, the same thing, when we gave these
13 patients those we --

14 (Slide.)

15 DR. JACOB: -- those responses to iso-beta
16 agonists we found in order to increase the heart rate by
17 25 beats per minute these patients also required half of
18 the dose that required the controls, that they are beta
19 hypersensitivity, and we know that iso activates, also
20 beta, activate the bowel reflex. Also we connected this
21 data to the bowel reflex activity, and we found the same.
22 They're still beta-1 hypersensitive.

23 (Slide.)

24 DR. JACOB: Thus, we find simply that alpha beta
25 receptor hypersensitivity existed in these patients. Beta

0041

1 receptor hypersensitivity, resistance to tyramine
2 requirement, higher doses of tyramine in order to increase
3 their blood pressure, high plasma catecholamines at least
4 on the subgroup of our patients, not to augment the
5 systemic spillover but to reduce systemic clearance.

6 (Slide.)

7 DR. JACOB: Others, they found the mild group,
8 they have found by the QSART, the sweating test, that they
9 have reduced the sweating in legs. They found by Hoeldtke
10 the normal conduction galvanic skin test in the legs but
11 not in the arms. Also Streeten has found that alpha AR
12 sensitivity in the veins of the legs, and recently
13 Stewart, Gina Stewart and Roy Freeman, they found that
14 these patients, they list, vasoconstrict the arteries when
15 they stand, but without abnormal vascular abnormality.

16 All these data, it is our life, and conduct us
17 to think about one thing to this hypothesis.

18 (Slide.)

19 DR. JACOB: Are all these data compatible with
20 partial denervation of the autonomic nervous system
21 controlling the cardiovascular system?

22 (Slide.)

23 DR. JACOB: In order to test this hypothesis

24 (Slide.)

25 DR. JACOB: -- we enrolled 10 patients that meet

0042

1 the criteria previously defined, and 10 healthy controls,
2 compared them to 10 healthy controls. These guys, all of
3 these subjects were put on place for 3 days on a
4 controlled diet, sodium and potassium, 140 milli-
5 equivalents of nitrium and 70 milli-equivalents of
6 potassium, and then we instrumentalized them with
7 inserting a brachial artery catheta, and anticubital vein
8 and femoral vein also were cannulated, and also to sample
9 blood from these sites, from the arms, from the legs
10 locally, and we test our hypothesis with the spillover as
11 introduced by Essler.

12 The one compartment model widely used and very
13 accurate, it is not so high, but acceptable specifically.
14 The legs and arms of the blood flow were measured with a
15 closive vein air plethysmography, and then they rested the
16 patients.

17 (Slide.)
18 DR. JACOB: And then we sampled three times,
19 based on the arm and from the legs, and simultaneously
20 three times before each there was -- I'm going to tell you
21 about them. The nitroprusside infusion was given in order
22 to reduce blood pressure by 20 millimeters of mercury in
23 order to have this, in order to activate the bar reflex
24 and to increase the spillover locally and systemically in
25 the arms and in the legs, as NPT represents there.

0043

1 The cold pressor test is a painful somatosensory
2 reflex, as reflexes need the cortex, need the brain stem,
3 need the psychological effect of the patient, and that all
4 affects, they affect significantly the autonomic nervous
5 system and increase the release of norepinephrine
6 spillover everywhere in the body.

7 Then, when we finish these two tests, we allowed
8 rest for at least 20 minutes between each one, and then we
9 give them systemic infusion of tyramine in order to
10 peripherally release the norepinephrine from the end of
11 synapses without central nervous system effect.

12 (Slide.)

13 DR. JACOB: Then we find our results showed that
14 the same two groups, they required the same dose of NTP.
15 They decreased the blood pressure similarly by 19, 20
16 millimeters of mercury. The CPT was, the patients tended
17 to be more sensitive, but it wasn't significantly
18 different. The tyramine dose, as we saw before, the
19 patient required higher doses of tyramine in order to
20 increase the blood pressure by 20 millimeters of mercury.

21 (Slide.)

22 DR. JACOB: Then just -- I don't want to bore
23 you with a lot of data, just, we see that here is the
24 venous norepinephrine in the legs only, and the femoral
25 vein, we see just a baseline before each stimulus. We see

0044

1 175 again 244. It was also surprisingly lower in the
2 femoral vein than in the controls in the veins, the
3 femoral veins in the controls. That is suggesting that
4 the release of norepinephrine locally may be compromised,
5 but with norepinephrine we cannot deduce these conclusions
6 because we know what is the kinetic problems of this data.

7 (Slide.)

8 DR. JACOB: Then we saw the arms and the
9 spillover baseline here. We also found the spillover in
10 the arms was lower in patients before a CPT, before the
11 NBT, before tyramine. That means baseline, before the
12 stimulus, it was significantly lower in three sampling
13 moments, significantly in these three samplings in
14 patients as compared to the control.

15 (Slide.)

16 DR. JACOB: Also, in the legs the norepinephrine
17 spillover here was more pronounced a baseline before each
18 stimulus, also that the norepinephrine spillover was lower
19 in the legs of patients as compared to the control.

20 (Slide.)

21 DR. JACOB: We perceive the arms clearance,

22 baseline clearance was pretty normal as the control. They
23 clear norepinephrine in the arms normally, that they have
24 some preserved function as the controls of clearing that
25 norepinephrine.

0045

1 (Slide.)

2 DR. JACOB: Then we see that the clearance in
3 the legs is not preserved as in the arms. That is, some
4 functional defect occurred in the legs of these patients
5 as compared to the control, that the clearance of
6 norepinephrine in the legs was compromised, again, like it
7 was the norepinephrine spillover, but when we are doing
8 the stimulus of the cold pressor chest NPT and tyramine,
9 the normal spillover in the arms was again not
10 significantly different between the controls and the
11 patients. That means the arms still preserve some
12 functionality of the autonomic nervous system. Meanwhile,
13 the legs --

14 (Slide.)

15 DR. JACOB: -- and here we were very surprised
16 that the increment of the spillover during the stimuli,
17 they don't increase at all norepinephrine. That means the
18 baseline was low, and also it was maybe the high
19 norepinephrine that the legs can release, and by that it's
20 very impressive that norepinephrine was not able to come
21 out from these nerves if they existed.

22 (Slide.)

23 DR. JACOB: Let me summarize that the baseline
24 NSO is decreased in both extremities, legs and arms. The
25 baseline clearance is decreased only in legs. The legs

0046

1 failed to increase the NSO to the various stimuli.

2 Meanwhile, in the arms it is still preserved, and we have
3 this again, that the patients required higher doses of
4 tyramine to increase their blood pressure.

5 (Slide.)

6 DR. JACOB: We have to conclude that neuropathic
7 NPTS, the name comes from this conclusion, that the
8 presence of alpha AR hypersensitivity, resistance to
9 tyramine, and reduced local norepinephrine release in legs
10 more pronounced than in arms, strongly suggests that
11 partial disautonomia does exist, and we call it partial
12 disautonomia, and neuropathic postural tachycardia
13 syndrome, as we wish, and realize the physiology of
14 idiopathic orthostatic intolerance in some of our
15 patients.

16 Thank you.

17 (Applause.)

18 DR. ROBERTSON: We have one more presentation
19 before the break, and then a presentation by Dr. O'Connor
20 afterwards.

21 Dr. Jens Jordan directs the Clinical Research
22 Center at the Franz Volhard Institute in Berlin, and he
23 and Dr. O'Connor will both describe similar and
24 complementary techniques whereby there is discovery of
25 genes involved in cardiovascular regulation.

0047

1 GENETIC FACTORS IN BLOOD PRESSURE REGULATION
2 JENS JORDAN, M.D., CLINICAL RESEARCH CENTER,
3 FRANZ VOLHARD KLINIK, BERLIN, GERMANY

4 DR. JORDAN: First of all I would like to thank
5 the organizers for inviting me. I take care of patients
6 with dysautonomias, but personally I have never seen a
7 patient with familial dysautonomia, so this meeting was
8 really very instructive for me and very interesting, and I
9 think a good opportunity to get some new ideas.

10 As Dr. Robertson already indicated, I changed
11 the title of the talk and the focus of the talk a little
12 bit so it is not on genetics of blood pressure
13 regulation -- blood pressure is regulated by many
14 different mechanisms -- but rather on the genetics of
15 baroreflex function in humans, so one important mechanism
16 to control short-term blood pressure and one mechanism
17 that I believe is very important if you would like to
18 understand genetic autonomic disorders.

19 So the purpose of the baroreflex is very simple.
20 The baroreflex buffers blood pressure changes, and serves
21 two, has two important functions. One function is
22 maintenance of organ perfusion, so the baroreflex prevents
23 drops in blood pressure so organs, vital organs can be
24 perfused properly. Another function is protection from
25 excessive blood pressure increases, another important

0048

1 function, and so how does the baroreflex do that?

2 (Slide.)

3 DR. JORDAN: This is a very simple illustration
4 of the baroreflex in the neck and in the large arteries in
5 the neck. They are stretch-sensitive receptors. When
6 blood pressure changes, the stretch of these receptors is
7 also changed, and the change is transmitted to the brain
8 via efferent nerve fibers, so the brain stem,
9 cardiovascular controls in the brain stem are informed
10 that blood pressure is changing, and this information is
11 integrated with other information from higher areas in the
12 brain and leads to adjustment of the sympathetic activity
13 and parasympathetic activity to the heart, to the
14 vasculature.

15 So as blood pressure drops sympathetic activity
16 is increased, so heart rate increases, vasculature increases,
17 and parasympathetic activity is withdrawn, which also
18 tends to increase heart rate, so the decrease in blood
19 pressure is buffered, and the opposite happens when blood
20 pressure increases. When blood pressure increases,
21 sympathetic activity is withdrawn, parasympathetic
22 activity increases, and we can measure the activity,
23 although we can measure the regulation of the baroreflex
24 in humans, and this is illustrated in this slide.

25 (Slide.)

0049

1 DR. JORDAN: So this slide shows our -- this is
2 related to heart rate and the activity of the sympathetic
3 nervous system when you change the blood pressure, so when
4 we infuse an agent that increases blood pressure, our
5 heart rate decreases. When we decrease blood pressure,

6 the heart rate increases, so the interval shortens.
7 Similarly, when we increase blood pressure, the
8 sympathetic nerves are shut off, and when we decrease
9 blood pressure the sympathetic nerves are activated.

10 And I would like to point out that when we look
11 at this curve, which is sigmoidal, there are different
12 properties that we can describe. One property is how
13 steep is this curve, and this is often referred to as
14 baroreflex sensitivity, and also where's the subject that
15 we are looking at right now, which is the so-called
16 operating point.

17 So the heart rate and blood pressure in the
18 resting stage, in the stage when we do this test, and also
19 what is the range of heart rates and the range of
20 sympathetic activity that can be regulated, and if you
21 look at most studies we don't really get all this
22 information. All we know is how steep is this curve.

23 (Slide.)

24 DR. JORDAN: Now, why should we even bother to
25 think about the genetics of the baroreflex? I think as a

0050

1 doctor I would say it should be clinically relevant, so
2 this reflex should be important enough that it makes sense
3 to understand it better, and I think the importance of
4 this reflex is illustrated by patients, so there are
5 different kinds of patients who have a very severe
6 impairment and baroreflex function.

7 (Slide.)

8 DR. JORDAN: One group of patients are
9 baroreflex failure patients, and these patients have
10 either lost their baro receptors through trauma or
11 degeneration, or they have lost the efferent nerves that
12 transmit the information from baro receptors to the brain
13 stem, so in these patients the brain does not know what
14 the blood pressure is, and so the blood pressure cannot be
15 adjusted. They cannot counterregulate. When these
16 patients are excited, or when they undergo physical stress
17 or psychological stress the sympathetic nervous system
18 activity is increased, and so they have a huge increase in
19 blood pressure.

20 When they are addressed, sympathetic activity is
21 lower, parasympathetic activity is higher, so blood
22 pressure is low, and the heart rate may be so low that
23 they have bradycardia or even a systole.

24 (Slide.)

25 DR. JORDAN: This is just one of these patients,

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1 one of our baroreflex patients. That's the resting blood
2 pressure recording, so that's beat by beat blood pressure
3 recording and heart rate in the resting stage, so this
4 patient was just lying there doing nothing, and in healthy
5 subjects blood pressure doesn't change much, but in this
6 patient, blood pressure really changes dramatically even
7 though the patient didn't do anything, so when you lose
8 the efferent function of your baroreflex, this is
9 associated with great ability in blood pressure, blood
10 pressure surges, and severe symptoms.

11 (Slide.)

12 DR. JORDAN: So another group of patients has a
13 loss of epherin function, so the brain knows, perhaps,
14 that blood pressure is changing but the brain can't do
15 anything about it because the connection through the
16 periphery is lost, which may be due to neurodegeneration
17 in the brain stem, maybe a loss of peripheral nerves or,
18 as Dr. Robertson indicated, just an inability to produce
19 or release neurotransmitters.

20 (Slide.)

21 DR. JORDAN: So these patients have completely
22 different symptoms but also very, very severe symptoms.
23 This is one of these patients, a patient with pure
24 autonomic failure, a loss of peripheral autonomic nerves
25 in this patient.

0052

1 This is again a blood pressure recording, the
2 beat by beat blood pressure recording and heart rate, and
3 the supine position in this patient, the blood pressure is
4 actually higher, but when she stood up the blood pressure
5 decreased dramatically to very low levels and she was not
6 able to stand for more than a few minutes, and other
7 stimuli also caused a great drop in blood pressure, for
8 example, food intake, exercise, so the loss of epherin
9 function of the baroreflex also has a major impact on
10 health and on quality of life, so I could conclude it
11 makes sense to look at this, so complete interruption of
12 the baroreflex arc causes very dramatic changes in blood
13 pressure and heart rate regulation. Even very subtle
14 changes in baroreflex function produced by genes perhaps
15 may have a substantial effect.

16 (Slide.)

17 DR. JORDAN: So it makes sense to look at the
18 genes that influence baroreflex function, but are there
19 any genes? Do we have any evidence to suggest that the
20 genetic effect on baroreflex function is strong?

21 One approach to look at this is doing twin
22 studies, so when you compare monozygotic and dizygotic
23 twins you can get an idea how strong a certain measure is
24 influenced by genes. Monozygotic twins share 100 percent
25 of their genes, so when a certain measure, in this case

0053

1 the baroreflex, is influenced by genes, you would expect
2 that they are very similar. Dizygotic twins share only 50
3 percent of their genes, so you would expect that they are
4 similar, but not as similar as monozygotic twins. That's
5 what we did.

6 (Slide.)

7 DR. JORDAN: That is a pretty complicated table,
8 but it shows the results of one of our twin studies, and
9 these are monozygotic twins and dizygotic twins, and as
10 indicated on here, several noninvasive measurements to
11 determine the baroreflex control of heart rate, and that
12 is just the baroreflex slope, so just one of these many
13 measures that determine how your baroreflex works.

14 Over here, that's the correlation coefficient
15 between siblings, so basically the higher this value the

16 more similar are the twins, and the lower this value, the
17 less similar they are, and very obviously, the monozygotic
18 twins have much higher values for these baroreflex
19 sensitivity measures than the dizygotic twins.

20 You can use, and you can calculate something
21 that is called heritability, which gives you an idea how
22 much of the variability in this measure is influenced by
23 genes, and it's a very strong effect, so the baroreflex
24 sensitivity is strongly influenced by genes, even though
25 we don't know the genes at this point, and when we adjust

0054

1 for other things that influence baroreflex function like
2 body weight, blood pressure, age, physical activity, this
3 effect remains strong, so there are genes that influence
4 baroreflex function in humans, and the effect is strong
5 enough that it makes sense to look at it.

6 (Slide.)

7 DR. JORDAN: There are some other data from the
8 twin studies from other groups, and unfortunately we don't
9 know whether or not the regulation of the sympathetic
10 nervous system is also strongly influenced by genes, but
11 there is some evidence. We know, for example, that
12 resting venous norepinephrine and resting venous
13 epinephrine levels are strongly influenced by genes. We
14 also know that resting sympathetic nerve traffic
15 determined by microneurography is also influenced by genes,
16 so the set point of the baroreflex is strongly influenced
17 by genes. Obviously, we would like to know more about
18 this.

19 (Slide.)

20 DR. JORDAN: Now, if it is influenced by genes,
21 what are the genes, and we have different types of studies
22 that give us some very raw ideas. We have data from
23 monogenic diseases, and we have data from larger healthy
24 populations, so-called association studies.

25 (Slide.)

0055

1 DR. JORDAN: And first I would like to point out
2 that there is really a huge number of genes that has some
3 potential to influence baroreflex function that has the
4 potential to cause dysautonomic symptoms. For example,
5 connective tissue, changes in connective tissue, genes
6 might change how the baro receptors are stretched, or how
7 the blood vessel reacts to the sympathetic nervous system.

8 Ion channels are extremely important in the
9 excitation of nerves and the excitation of blood
10 vessels, and the regulation of neurotransmitters really
11 is -- of course, neurotransmitters could be changed as
12 indicated by Dr. Robertson, neuromodulators, so many
13 substances modulate how the baroreflex is operating,
14 receptors, transporters, and so on, so there are many
15 potential candidate genes that might influence baroreflex
16 function.

17 (Slide.)

18 DR. JORDAN: Now, one disorder that we were very
19 interested in is a disorder in which affected family
20 members have high blood pressure, and blood pressure

21 increase dramatically as they get older, and they also
22 have brachydactyly, and so short fingers, and we found
23 this family in Northeastern Turkey, and the family members
24 died at a very early age, so one of our interests was, why
25 do they have high blood pressure, and so these patients,

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1 Tank and others studied these patients in Northeastern
2 Turkey in a genetic field study, and one interesting
3 finding was that when we determine baroreflex
4 sensitivities, how the baroreflex was able to control
5 heart rate.

6 We found at a very young age they had a
7 reduction in their ability to control heart rate properly,
8 and so the affected family members had lower baroreflex
9 sensitivities than the nonaffected family members, and
10 whereas at an earlier age it would be diminished, so it
11 really is very important not to just look at genes, but
12 also look at other factors that might modulate how the
13 genes were operating.

14 (Slide.)

15 DR. JORDAN: Now, in a laboratory study in a
16 small group of these patients we measured monogenic
17 hypertension, and unfortunately we didn't determine the
18 sympathetic sensitivity at this time, but we observed, so
19 these bursts are bursts of sympathetic activity in the
20 blood vessels. The sympathetic activity was not increased
21 compared with normotensive controls, or hypertensive
22 control subjects, but blood pressure was increased, so the
23 set point of the sympathetic nervous system, or the
24 sympathetic reflex had changed.

25 (Slide.)

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1 DR. JORDAN: And well, of all of these changes,
2 you would expect the buffering function of the baroreflex
3 should also be different, and that's a pretty complicated
4 method to look at the buffering function of the
5 baroreflex, but what we basically did is, we determined
6 how sensitive the hypertensive patients and the control
7 subjects were to a vasoconstricting agent, how high is the
8 dose that you need to increase blood pressure by 12.5
9 millimeters of mercury, and in the hypertensive subjects
10 at baseline we did need much lower doses to obtain the
11 same increase in blood pressure.

12 And then we got rid of the baroreflex, so we
13 infused again a blocker to interrupt the baroreflex and
14 said, how sensitive are they when the baroreflex is gone,
15 and then they were rather similar, so the baroreflex had a
16 much greater impact on how sensitive normal subjects are
17 than how sensitive these hypertensive subjects are, so
18 really all these changes are associated with a loss of
19 baroreflex function, of baroreflex buffering function, so
20 the gene that caused this syndrome influences baroreflex
21 function.

22 (Slide.)

23 DR. JORDAN: Now, there are some other things
24 that have been shown to influence the baroreflex function
25 and that is, there is one disorder that is called the

0058

1 Williams Syndrome, which is caused by an elastin mutation,
2 and so mutation that is likely to influence the mechanical
3 properties of blood vessels in the neck, and Girard and
4 colleagues looked at the sensitivity control subjects
5 using different methods, and these affected patients, and
6 they found a reduction in the baroreflex sensitivity, so
7 there seems to be another gene that is important for
8 baroreflex regulation.

9 (Slide.)

10 DR. JORDAN: Of course, these are data from
11 Hilz, and he was so kind to provide me with this data
12 yesterday. Of course, familial dysautonomia it is another
13 disorder that is associated with baroreflex control of
14 heart rate, so when the LF, which is basically a measure
15 for the heart variability caused by autonomic innervation
16 of the heart is lower, it increases the control subjects
17 compared with the FD patients. It increases dramatically
18 in healthy subjects when you stimulate the neck using a
19 neck suction device, but it does not increase in the FD
20 patients, so that's another important gene that influences
21 baroreflex function in humans.

22 So these are rare disorders, but we have learned
23 that very often the genes that cause rare disorders also
24 are found in the general population.

25 (Slide.)

0059

1 DR. JORDAN: I think, for example, looking at
2 heterozygotes would be very important. Perhaps that might
3 also have an impact on blood pressure and heart
4 regulation.

5 There are also some studies looking at larger
6 healthy populations, and one gene that has been found to
7 influence baroreflex control of heart rate is the
8 aldosterone synthase gene. I don't know, I have no idea
9 how this gene influences it, but it seems to do it, and so
10 when you have two copies of the TTLE you have much higher,
11 you have higher baroreflex sensitivity than when you are
12 heterozygous, or when you have two copies of the CCLE, so
13 that's one part in a gene that influences baroreflex
14 sensitivity in the general population.

15 We found another gene, and I have to say we
16 didn't really expect that baroreflex function was
17 influenced by it. The main reason we were interested in
18 this gene, which is the beta subcomponent of a keratin-
19 activated potassium channels, that we thought that it
20 might be very important in the regulation of vascular tone
21 and blood pressure regulation.

22 And what we found was that polymers in this gene
23 did not influence blood pressure much, but they influenced
24 baroreflex sensitivity, and these are different measures,
25 so that the baroreflex sensitivity is determined by the

0060

1 so-called sequence method, and they both show that
2 different alleles are associated with different
3 sensitivities, and the interesting part of this is that it
4 only involves very rapid changes in heart rate, so smaller

5 changes in heart rate elicited by the baroreflex were not
6 influenced by this gene, so it's really a selective effect
7 on very rapid changes in heart rate.

8 (Slide.)

9 DR. JORDAN: So I would like to conclude that
10 genes influence clearly baroreflex blood pressuring
11 buffering, genes influence baroreflex heart rate control,
12 and there's also some evidence to suggest that baroreflex
13 control of sympathetic vasomotor tone is influenced by
14 genes, but we don't have very good data on that yet.

15 This genetic effect can be selective, as shown
16 in the lab. It may just involve one aspect of baroreflex
17 function and, for example, this Turkish family, the loss
18 of baroreflex buffering function was much greater than we
19 would have expected from just looking at the heart rate
20 control by the baroreflex.

21 So certain genes may just influence a certain
22 aspect of baroreflex regulation, and there are several
23 human baroreflex genes, but there are probably many, many
24 more. One of these genes is the elastin gene, one of them
25 is the gene that causes familial dysautonomia, one of them

0061

1 is the beta subunit of the potassium channel and the
2 aldosterone synthase, and the hypertension and
3 brachydactyly gene, which hasn't been identified yet, is
4 also a gene that is likely that it influences baroreflex
5 function, and all the genes that influence baroreflex
6 function may also be important in familial dysautonomic
7 syndromes.

8 Thank you very much.

9 (Applause.)

10 DR. ROBERTSON: Thanks very much, Dr. Jordan.
11 We will in just one second take a 10-minute break. Before
12 we do break, let me ask those of you who have moderated
13 the sessions yesterday and today to be thinking about
14 summary statements or key things that might be a part of
15 the discussion of Dr. Guttmacher in an hour or so from
16 now, so let me let you go ahead and take your 10-minute
17 break and then come back for Dr. O'Connor's presentation.

18 (Recess.)

19 DR. ROBERTSON: It's a pleasure to introduce the
20 last speaker, Dr. Daniel O'Connor, who is always a little
21 ahead of the curve. He moved from the clinical end of
22 things into molecular biology and he has moved into
23 genetics now, so we know that's an important area to go
24 into.

25 It's a pleasure to introduce Dr. Daniel

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1 O'Connor, professor of medicine and pharmacology at the
2 University of California at San Diego. Dan.

3 GENETIC DETERMINANTS OF AUTONOMIC BLOOD PRESSURE CONTROL
4 DANIEL O'CONNOR, J.D., UNIVERSITY OF CALIFORNIA,
5 SAN DIEGO, SAN DIEGO, CALIFORNIA

6 DR. O'CONNOR: I think I almost know how to use
7 almost all of the gadgets, and I'm the fossil with the
8 slides.

9 (Slide.)

10 DR. O'CONNOR: So anyhow, it's kind of neat for
11 me to be invited to a meeting like this, because I spend
12 most of my time on the high end of the blood pressure, and
13 I run a hypertension program at USCSD and the VA in San
14 Diego, and don't spend as much time as I should thinking
15 about the other end of the bell curve, low blood pressure,
16 or dysautonomia, and so as not to overlap what Dr. Jordan
17 talked about in the last lecture I've changed the focus a
18 little bit to talk about some of our studies in
19 catecholamine storage vessels and, in particular, the
20 genomic basis of some alterations we've noticed in
21 catecholamine storage vessels that seem to have
22 implications for patients with high blood pressure and
23 thus might well have implications for patients with low
24 blood pressure.

25 So I've been interested in blood pressure for a
0063
1 long time, and I took a pretty strange route to high blood
2 pressure, in particular, catecholamines.

3 (Slide.)

4 DR. O'CONNOR: I got hooked on the study of
5 catecholamine storage vessels a number of years ago, and
6 this is a transmission electron micrograph obtained from a
7 tumor of a patient with a catecholamine disorder,
8 pheochromocytoma, a neoplasm of the adrenal medulla, which
9 results in excessive secretion of catecholamines and
10 therefore high blood pressure, and these are typically
11 benign tumors of the adrenal medulla which can be
12 resected, resection of which basically cures the patient,
13 and I got interested in these vesicles basically in two
14 dimensions.

15 There are these membrane-delimited particles
16 which look like disks, their electron dense core. In
17 three dimensions, of course, they would be spherical, and
18 from tumors such as this, or from normal sources such as
19 brain or sympathetic nerve or adrenal medulla we devise
20 methods for purifying catecholamine storage vesicles using
21 density gradients, isopycnic density gradients, and these
22 are isolated catecholamine storage vesicles.

23 The trivial name that they pick up in the
24 adrenal medulla is chromaffin granules, so named because
25 of their morphologic appearance, the granule, and their

0064
1 staining. They basically, with chromium salts they will
2 stain brown on light microscopic sections.

3 And if you look at these, we got kind of curious
4 about what might be inside these vesicles so as to give
5 this electron dense core appearance, and if you suspend
6 catecholamine storage vessels in distilled water they're
7 pretty good osmometers. They explode. Put them in the
8 ultracentrifuge and spin out the membranes, you're left
9 with a soluble core, and the proteins that you find in the
10 soluble core were pretty ill-described up until about 20
11 years ago.

12 One of them Dave Robertson has talked about
13 pretty extensively. It's the enzyme dopamine
14 betahydroxylase, which results in the betahydroxylation of

15 dopamine to form the neurotransmitter norepinephrine, and
16 that process takes place right inside the catecholamine
17 storage vesicle, that very last step in norepinephrine
18 biosynthesis, but what I've done here is to take the
19 proteins in the core of the vesicle and fractionate them
20 first by charge and then by size, and then Koumasy blue
21 stain them, and I think you see the major proteins in the
22 vessel core, not DBH, but this group of proteins that have
23 been called the chromogranin, so named because they were
24 initially described in chromaffin granules, the
25 quantitatively major one being chromogranin A, another one

0065

1 of higher molecular mass being chromogranin B.

2 Well, we spent a long time plucking and
3 sequencing cDNA's for these proteins, and we found that
4 they are pro-proteins, and in particular there is a region
5 within chromogranin A which is highly homologous across
6 species, which is bounded by paired basic residues, which
7 are the usual cleavage sites, recognition sites for
8 prohormone processing proteases, which liberate a peptide
9 which has an interesting biological activity, probably
10 rather interesting for this audience.

11 It's a fairly powerful inhibitor of sympathetic
12 outflow and, in particular, of catecholamine release from
13 chromaffin cells and post ganglionic sympathetic nerves,
14 and what we've done is to look at the activity of this
15 region in vitro, and what I've done here is to take
16 chromaffin cells, which are simply highly modified post
17 ganglionic sympathetic nerves, as one way of looking at
18 them, I load them with norepinephrine, in particular
19 tritiated norepinephrine to label their vesicular stores.

20 And then I trigger the release of
21 norepinephrine, and the neurotransmitter I use to trigger
22 the release is typically acetylcholine, because if you
23 think about a chromaffin cell or a post ganglionic nerve
24 sitting in apposition to the spinal cord, it's innervated
25 by the preganglionic nerves which emerge from the cord

0066

1 which utilizes their main neurotransmitter, acetylcholine,
2 which diffuses across the cleft and then interacts with
3 nicotinic cholinergic receptors of the neuronal variety,
4 which are basically extracellular elongated cation
5 channels.

6 So I trigger release with nicotine as analogue
7 of acetylcholine, the natural neurotransmitter, and then
8 when I include this peptide in the incubation I can pretty
9 completely inhibit catecholamine release from chromaffin
10 cells with an IC-50 of around 200 nanomolar, and we began
11 to call this peptide catestatin because of its ability to
12 inhibit catecholamine release from chromaffin cells and
13 sympathetic nerves, and through a variety of agonist and
14 antagonist studies, teasing out signal transduction within
15 chromaffin cells or sympathetic nerves, we've come to the
16 conclusion that the site of action of this peptide
17 catestatin is on the nicotinic cholinergic receptor
18 itself, which is, of course, the initial trigger towards
19 catecholamine secretion from chromaffin cells or

20 sympathetic nerves by virtue of responding to the
21 preganglionic nerve transmitter acetylcholine, one of the
22 major transmitters in the preganglionic nerve. Now --

23 (Slide.)

24 DR. O'CONNOR: So the conclusion we've come to
25 is that perhaps the catestatin mechanism could be an

0067

1 autocrine negative feedback break on catecholamine
2 release, thereby modulating neurotransmitter release into
3 synaptic clefts so as not to oversecrete and overload the
4 cleft in the presence of previously secreted
5 neurotransmitter, and in terms of this Larson cartoon
6 maybe the chromaffin cell and the post ganglionic
7 sympathetic nerve have finally acquired the brain whereby
8 they can modulate their secretion as appropriate, and what
9 he's saying here is almost off-scale stimulus response,
10 stimulus response, don't you ever think. Well, now maybe
11 we've discovered a mechanism whereby the cell can think.

12 (Slide.)

13 DR. O'CONNOR: We spent a lot of time on the
14 structure and function of this peptide, and by both
15 homology modeling and by empirically derived structure,
16 the nuclear magnetic resonance structure, it would appear
17 to have a looped strand structure, and at the tip of the
18 loop are position 3 argonines with their very cationic
19 side chains, which seem to be an important part of the
20 action of this peptide. If you mutate any one of these
21 three argonines the activity of the peptide as a
22 catecholamine release inhibitor declines substantially.

23 (Slide.)

24 DR. O'CONNOR: The peptide seems to be active in
25 vivo. These are some studies in instrumented rodents done

0068

1 at Mike Siegler's lab at USCSD Medical Center in which he
2 triggers graded increments of epherin sympathetic outflow
3 by central sympathetic stimulation, with consequent rises
4 in blood pressure. In the presence of catestatin these
5 blood pressure rises are blunted.

6 (Slide.)

7 DR. O'CONNOR: These are some experiments that
8 we did in our own lab. We're looking at the in situ in
9 vivo perfused adrenal gland in a rodent, in a rat, and one
10 can trigger increments of norepinephrine or epinephrine
11 release from the adrenal gland in this intact moving
12 rodent using varieties of preganglionic stimulation,
13 including electrical stimulation of the preganglionic
14 nerve, or the usual transmitter, acetylcholine, or its
15 analogue, more specific to the nicotinic receptor. In
16 each case we get triggering of catecholamine relief. In
17 each case in the presence of catestatin there's blunting,
18 so the peptide does seem to be active in vivo.

19 Now, I work on a hereditary disease. The
20 hereditary disease I work on is high blood pressure, and
21 in my clinic about 70 percent of our patients give us
22 positive family histories in a first degree relative, mom
23 or dad, or brothers and sisters. There's a clear familial
24 complement. However, it's not quite so simple as many

25 hereditary diseases in terms of its pattern of

0069

1 transmission. It's not a simple Mendelian single locus
2 disease. It's not like Huntington's disease, or cystic
3 fibrosis. It's not an autosomal dominant or recessive,
4 and in fact it's quite complicated, and likely to be
5 polygenic.

6 So we wondered if we might begin to explore the
7 catestatin mechanism in patients with high blood pressure,
8 so we measured catestatin by region-specific radioimmuno
9 assay in people with high blood pressure and in people
10 with normal blood pressure, and we also stratified them by
11 their genetic risk of hypertension, or family history of
12 hypertension. That is, the hypertensives in this group
13 all had positive family histories of hypertension. The
14 normotensives had negative family histories, and you can
15 see that there's a substantial difference in the plasma
16 concentration of catestatin, and since the job of this
17 peptide seems to be inhibition of catecholamine release,
18 then the deficiency of this peptide would be a plausible
19 protohypertensive mechanism.

20 But lo and behold, when we looked at the still
21 normotensive offspring of patients with high blood
22 pressure, that is, normal blood pressure but positive
23 family history, already these individuals displayed
24 evidence of deficiency in catestatin, suggesting that this
25 might not be a very late response in people with high

0070

1 blood pressure, but might be a very early or even
2 prehypertensive event in the long-term pathogenesis of
3 their trait or disorder.

4 (Slide.)

5 DR. O'CONNOR: To gather evidence that this
6 deficiency might, in fact, be physiological, or might, in
7 fact, have some importance in human beings in vivo, we
8 looked at the adrenal medulla secretion of epinephrine and
9 we chose the urine as basically the scavenger that handles
10 epinephrine once secreted into the bloodstream, because we
11 could look at longer term changes in epinephrine secretion
12 and integrate them over a period of several hours, and
13 when we look at the urinary excretion of epinephrine in
14 patients with high blood pressure, it's elevated as
15 compared to their normotensive counterparts.

16 And this then once again is a protohypertensive
17 mechanism, but interestingly enough, those normotensive
18 offspring of the patients with high blood pressure, as
19 long as they have a positive family history, even though
20 their blood pressure currently -- and these are typically
21 people in their twenties or thirties -- is quite normal,
22 already they displayed this elevation in epinephrine
23 secretion, and this then I think goes hand-in-glove with
24 the catestatin mechanism and suggests that perhaps
25 catestatin might be acting in vivo, as an autocrine

0071

1 suppressor of epinephrine release from chromaffin cells in
2 the adrenal medulla.

3 (Slide.)

4 DR. O'CONNOR: To further explore the
5 significance, the possible significance of this mechanism
6 in vivo, we wanted to see if we could link it with some
7 physiologic, as opposed to simply biochemical changes, and
8 there's the hypothesis that has been floating around in
9 the hypertension world for a couple of decades that
10 perhaps people with high blood pressure initially are
11 normotensive, but they're predisposed to interact with the
12 environment in adverse ways, such that with typical
13 environmental stressors, whether those be psychological
14 stressors or physical stressors or dietary stressors or
15 other kinds of maneuvers that perhaps you and I would kind
16 of brush off and not respond to terribly adversely, that
17 these individuals predisposed to develop hypertension
18 might have inordinate pressor responses to daily life
19 situations that would over the long term translate into
20 sustained pressor or high blood pressure responses.

21 In order to mimic these kinds of responses, we
22 decided to artificially induce an adrenalin rush, if you
23 will, in human beings, and we did this by basically taking
24 a hand --

25 (Slide.)

0072

1 DR. O'CONNOR: -- the nondominant hand and
2 putting it into a tank of ice water for a minute, and
3 looking to see to what extent blood pressure rose after
4 immersion of the hand in ice water, and this is the
5 baseline, and these are the increases you see, and it
6 became apparent that when we profiled people prior to the
7 study on the basis of their catestatin concentration, that
8 those who had the very lowest catestatins tended to have
9 the greatest rises in blood pressure during the sympatho-
10 adrenal stressor in the cold pressor test, and the R value
11 was in the range of about .2, and the P was fairly highly
12 significant.

13 For those of you who do human research, this is
14 not a bad correlation, so I think we have perhaps begun to
15 integrate catestatin into physiological mechanisms of
16 blood pressure control.

17 (Slide.)

18 DR. O'CONNOR: I have no idea as to how this
19 might integrate into pathophysiological mechanisms of low
20 blood pressure. That would be interesting to study.

21 To further understand how heredity might control
22 the catastatic mechanism or other mechanisms of autonomic
23 dysfunction we, like Dr. Jordan, have turned to relative
24 pairs, and we've looked at both twins, identical twins
25 being a wonderful experiment of nature in which two

0073

1 genetically identical people emerge from a single
2 fertilization, as opposed to dizygotic twins, which are
3 basically siblings that simply happen to be born at the
4 same time, or pedigrees, as a way of probing hereditary
5 mechanisms, and using twins or pedigrees --

6 (Slide.)

7 DR. O'CONNOR: -- you can basically scale traits
8 and begin to understand how much of the variability of a

9 trait is the result of gene action, and genetic
10 variability divided by total variability of a trait is a
11 fraction, therefore scaled from zero to one we call this
12 fraction heritability, and it's a nifty way of getting a
13 fix on to what extent genes determine any trait.

14 It doesn't tell you how many genes determine
15 that trait, but it tells you that genes are important and
16 are involved, and so we've measured a number of the
17 fragments of a variety of the three major members of the
18 chromogranin secretogranin family, chromogranin A,
19 chromogranin B, and secretogranin 2, in plasma of twins
20 and also in plasma of extended pedigrees, and I think you
21 can see that catestatin, which is human chromogranin A --
22 in this assay we've utilized an epitope ranging from
23 immunoacids 361 to 372 -- is rather heritable. On a scale
24 of zero to 1 it comes out in the range of about .47, and
25 it's highly significant.

0074

1 In our book this is pretty interesting, because
2 when we do similar studies on the heritability of blood
3 pressure, we find that the heritability of blood pressure
4 per se is down around .3, so blood pressure is a heritable
5 trait, but this trait is more heritable, and we sometimes
6 refer to this as an intermediate phenotype in the
7 pathogenesis of high blood pressure. That is, it is a
8 treat which is pathogenic, but has earlier penetrants and
9 higher heritability than the ultimate disease trait, once
10 again speaking from the perspective of high blood
11 pressure, and we've looked at a number of other peptides,
12 and towards the end of the talk I'll tell you about
13 another fragment called chromogranin B, 312 through 331,
14 which also displays substantial heritability.

15 (Slide.)

16 DR. O'CONNOR: Now, how many genes might affect
17 the plasma concentration of catestatin? Well, one way to
18 get a fix on this, as with any other quantitative trait,
19 is to simply look at its frequency histogram and look for
20 evidence of bimodality, bimodality being at least one
21 hallmark of a major gene effect or a Mendelian effect upon
22 a trait, and so we logged and transformed the data to
23 compress outliers, which would just by simple skewness
24 perhaps just give the artifactual appearance of
25 bimodality.

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1 And even after we do that compression, here is
2 what the distribution ought to look at if it is unimodal,
3 but here's what the distribution actually looks like, and
4 in fact when we formally test the alternative hypotheses
5 that the distributions are bimodal versus unimodal, we can
6 reject the hypothesis of unimodality in fairly high
7 confidence, and then it turns out that in this bimodal
8 distribution in this lower mode, every last one of these
9 guys, men and women is at genetic risk of developing high
10 blood pressure.

11 (Slide.)

12 DR. O'CONNOR: So there may be a major gene
13 effect. What might that gene be? Well, even before we

14 had any linkage results we went right to the locus itself,
15 which is chromogranin A on chromosome 14Q32, and one of
16 our approaches is to understand how allelic diversity
17 might intervene in determining a trait that is common in
18 the population is to resequence the living daylights out
19 of that gene in normal individuals.

20 So what we did, and this was done by Bruce
21 Hamilton and Gun Wen in a group, and they resequenced all
22 of the exons, the intron exon borders, and the promoter in
23 80 individuals, so this is an eight-exon gene spread out
24 over about, with about 2 kilobases of coding regions
25 spread out over about 12 kilobases, and we selected about

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1 80 individuals corresponding to the ethnic diversity we
2 see in Southern California, people of European ancestry,
3 sub-Saharan African ancestry, Mexican-Americans, and
4 people of East Asian ancestry.

5 Half of them had high blood pressure, half of
6 them had normal blood pressure, and in those 80
7 individuals, in those 160 autosomes and chromosomes we
8 found an enormous amount of allelic diversity, with 37
9 newly discovered SNIPS, or single nucleotide
10 polymorphoses. By the way, almost all the diversity we
11 discovered is in the form of SNIPS. We discovered one
12 microsatellite, but SNIPS are really where the action is,
13 and basically here are the eight exons in the dark.

14 I've indicated the open reading frame, starting
15 with the ATG and terminating with the termination codon,
16 and of the SNIPS there were a variety in the promoter,
17 there were a variety scattered across all the exons, there
18 were some in the introns although none right at the
19 intron-exon borders, and indeed there were two SNIPS that
20 we identified within catestatin, including a gly 364
21 serapolymerism and a pro 370 loop polymorphism.

22 This guy caught our eye because on the basis of
23 previous studies we have determined that this is a crucial
24 residue in the biologic activity of catestatin, and
25 previously I showed you a homology model. Now I'm showing

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1 you an empirically derived NMR structure of human
2 catestatin obtained in DMSO, and there's a fault here that
3 seems to be crucial for the action of this peptide to
4 inhibit nicotinic cholinergic receptors, and in recent
5 studies where we've threaded this new sequence over this
6 template it looks like this fault is warped by several
7 angstroms as a result of this amino acid substitution.

8 (Slide.)

9 DR. O'CONNOR: We previously have scrolled
10 through the entire catestatin region using a technique
11 that we call alanine scanning idiogenesis, and what we do
12 there is, we systematically take the catestatin molecule
13 and replace every residue by alanine. You might say, why
14 alanine? Well, alanine is the smallest, most generic
15 residue that retains chirality. Actually, glycine would
16 be smaller, but with glycine you lose chirality. Alanine
17 is methylglycine.

18 So we replace every single residue and look at

19 the ability of those resulting mutant peptides to inhibit
20 catecholamine release in response to nicotinic
21 stimulation, or another interesting feature of the
22 nicotinic receptor is that it desensitizes in response to
23 prolonged stimulation, then repeated stimulation results
24 in a diminished response. This is a conformational change
25 in the nicotinic receptor.

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1 Catestatin seems to have the ability of
2 inhibiting nicotinic desensitization, and when we go to
3 that mutation, this glycine, we have not yet reconstructed
4 the mutation which occurs in human beings, which would be
5 a syrene, but in the alanine scan mutagenesis, the
6 mutagenesis of that glycine results in a substantial
7 decrement in the ability of that peptide to inhibit
8 catecholamine release and a substantial decrement in the
9 ability of that peptide to protect against nicotinic
10 desensitization, so it would appear that we are detecting
11 common mutations, then, in a variety of this peptide that
12 is likely to influence sympathetic activity.

13 (Slide.)

14 DR. O'CONNOR: When we look at the frequency of
15 these alterations, these mutations, we find that about 15
16 out of the 37 are pretty common. They have minor allele
17 frequencies greater than 5 percent. Basically with SNIPS
18 you're talking about biallelic polymorphisms, and the
19 frequencies in the two alleles have to add up to 100
20 percent, and if you're talking about rare SNIPS, you're
21 talking about allele frequencies less than 1 percent.

22 If you're talking about common ones you're
23 talking about alleles whose frequency is greater than
24 about 5 percent, such that if I went through this audience
25 I would find a lot of them -- in fact, I have resequenced

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1 myself, and I have a few of these things, and they
2 basically kind of cluster in the promoter region, the
3 kilobase or upstream of exon 1, and the rest of them
4 cluster in exon 6 through 8, the common ones, and when we
5 do chi square tests looking to see whether particular
6 alleles are consistently associated with each other from
7 individual to individual, that is, whether they inhabit
8 the same chromosome, we're attempting then to reconstruct
9 haplotypes. A haplotype is an array of alleles at
10 adjacent loci along a single chromosome. It's a little
11 difficult issue to get at human beings, because we are, of
12 course, diploypes. We have two copies of each
13 chromosome, and a priori you really don't know, but
14 statistically you can reconstitute these things, and when
15 we look for patterns of association which can be referred
16 to as linkage disequilibrium, there's a cluster in the
17 promoter, there's a cluster in exon 6 through 8, and
18 there's a disrupted region between them.

19 So there seem to be two common haplotypes in the
20 chromogranin A gene, one encompassing the catestatin
21 region, the other centered on the promoter, and in the
22 promoter there are a huge number of -- there are eight
23 common SNIPS, some of which pretty clearly disrupt known

24 transcription factor binding sites.
25 We've spent a lot of time bashing the chromogram

0080

1 promoter of the seven haplotypes, about five of them
2 actually fairly common, such that we can readily find in
3 our resequenced individuals' homozygotes, and then we can
4 take these promoters and fuse them to reporters, pop them
5 into chromaffin cells and begin to test whether they
6 might, in fact, alter promoter strength, and might lead to
7 common variations in chromogranin A expressing in human
8 beings.

9 (Slide.)

10 DR. O'CONNOR: We've been using the technique of
11 comparative genomics, which actually is getting to be
12 pretty easy, based upon Eddie Rubin's server up in
13 Berkeley, the Berkeley genome pipeline, and when we look
14 at the span of human chromosome 14Q32 that harbors this
15 gene, there's exon 1, 2, 3, 4, 5, 6, 7, 8, and when we
16 look across species, the best species to look across these
17 days is the mouse, because the mouse genome is pretty well
18 completed in terms of its sequencing.

19 The exons display substantial homology between
20 human and mouse, but there's another region about six
21 kilobases upstream that's exon 1 that's tremendously
22 homologous. We've previously overlooked this. Holy
23 smoke, this might well be a transcriptional enhancer that
24 we've previously just completely stuck our head in the
25 sand and not paid much attention to, so we're now looking

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1 for polymorphism in this guy, infusing it to promoter
2 reporters to see if it might, in fact, be functional.

3 (Slide.)

4 DR. O'CONNOR: You might say, now, wait a
5 minute, why are you telling me all of this about
6 transcriptional control and the amount of chromogranin A?
7 You've already told me the active fragment is catestatin.
8 Well, there may be another important action of this
9 protein that may have a fundamental role in stabilizing
10 the core of the catecholamine storage vesicle, and in
11 particular catecholamine storage vesicles are enormously
12 chock full of catecholamines.

13 The concentrations of norepinephrine or
14 epinephrine in the vesicles in the adrenal medulla simply
15 approach .5 molar. We're talking about drastic
16 concentrations here, as opposed to the concentration in
17 plasma of norepinephrine, which is down in the range of
18 about 50 molar, so we're talking about 10 to the 15th or
19 so gradient.

20 How did those catecholamines get in there?
21 Well, there are a variety of mechanisms resulting in their
22 accumulations to these drastic concentrations. One of
23 them might be osmotic in activation by binding to
24 catecholamine storage vesicle core anions, such as the
25 chromogranins, which are, of course, quite anionic, and my

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1 colleague, Lee Iden at the NIH down the road in Bethesda
2 has shown that if you, using antisense technology, knock

3 down or knock out the expression of the chromogranin A
4 gene in chromaffin cells, catecholamine secretory vesicles
5 virtually disappear.

6 You can rescue this phenotype with
7 reintroduction of chromogranin A by transvection, so this
8 may be an important protein, and we hypothesize then --

9 (Slide.)

10 DR. O'CONNOR: -- common variations of which
11 might result in profound alterations in the ability to
12 cope with sympathetic stresses.

13 I have not looked at all in people with low
14 blood pressure, but that would be certainly an interesting
15 extension.

16 The final point I will make is that we have done
17 a fair number of pedigree studies now, twin studies and
18 pedigree studies, and there's a kind of pedigree study
19 that is fairly useful, involving huge pedigrees, and these
20 are pedigrees -- of course, in mobile urban Southern
21 California we don't really have these huge extended
22 pedigrees, but the people who run the CEPH program, in
23 particular its Utah branch, Mark Leppert and Jean-Marc
24 Labouet have been ascertaining and reascertaining the CEPH
25 pedigrees, which basically are the foundation of the human

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1 genetic map, the marker positioning that defines the
2 structure of the human genome.

3 They were originally ascertained back in the
4 eighties. They're now being reascertained for the study
5 of common traits, and Jean-Marc and Mark have been
6 interested in these pedigrees because they have an
7 enormous number of sib pairs. If you think about the
8 number of sib pairs given by a sib ship, it's N times N
9 minus 1 divided by 2, such that in this pedigree, even
10 though there are only nine siblings there are 36 sib
11 pairs, so the Utah CEPH families are just an incredible
12 resource for sib pairs, which are a nifty way of doing
13 genome scans to position loci really throughout the genome
14 that might influence any trait, and these are about the
15 most genotyped people on the face of the earth. They've
16 been genotyped at up to 15,000 polymorphisms, 15,000 loci
17 up and down the genome.

18 And I will just give you a quick peek at one of
19 the things we've been looking at. One of the proteins
20 we've looked at is a family member of chromogranin A
21 called chromogranin B, which also sits in the
22 catecholamine storage vesicle and is subject to exocytotic
23 corelease with catecholamines and chromogranin A, and
24 we're interested in this guy because he is incredibly
25 responsive to sympathetic stimulation.

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1 That is, in a chromaffin cell, if we mimic
2 efferent preganglionic stimulation coming out of the spinal
3 cord either through the neurotransmitter acetylcholine
4 acting in a nicotinic cholinergic receptor, or through the
5 cotransmitters, which in the case of preganglionic nerves
6 in mammals are PACAP and VIP, this protein jumps like
7 crazy in terms of its transcription as judged by these

8 blocks. It jumps about twofold in response to nicotinic
9 stimulation, it jumps about fivefold in response to PACAP
10 stimulation, another preganglionic transmitter. It jumps
11 fourfold in response to VIP stimulation. We think this is
12 an example of what's been called stimulus transcription
13 coupling, or stimulus secretion synthesis coupling. It
14 makes sense in that, wouldn't it be nice if the
15 neurotransmitter that provoked release also talked to the
16 nucleus and promoted resynthesis of that transmitter.

17 (Slide.)

18 DR. O'CONNOR: We have taken the promoter region
19 of chromogranin B and fused it to luciferase reporters,
20 made deletions of the promoter region, popped these guys
21 by transvection back into chromaffin cells and looked at
22 their ability to respond to either nicotine as a surrogate
23 for acetylcholine, or VIP or PACAP, the other two
24 preganglionic neurotransmitters.

25 We get brisk responses in each case. They map

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1 onto the proximal promoter, in particular a cyclogranin P
2 response element, and a GC rich region, the cyclogranin P
3 response element then having transacting factor CREB, the
4 GC rich region having the transacting factors SP-1.

5 What happens if we measure this guy in plasma in
6 the CEPH families?

7 (Slide.)

8 DR. O'CONNOR: So this is then an extremely
9 responsive read-out of epherin sympathetic stimulation.
10 Its plasma concentration is elevated in people with high
11 blood pressure.

12 In the CEPH families, using basically the CEPH
13 genotypes and the expertise of Nik Schork, a statistical
14 geneticist at UCSD, we were able to scroll through the
15 entire genome, and we found a region right at the tip of
16 chromosome 11Q, which is strongly linked to transmission
17 of this trait in the CEPH pedigrees -- this is in the
18 range of about 5.7 to 5.8, depending upon what map we use.
19 That suggests that the odds ratio in favor of linkage are
20 close to a million to one.

21 Linkage is basically cosegregation of a
22 chromosomal segment and the trait in these families, and
23 what do you suppose is there? Well, it's not chromogram B
24 itself, because that's on chromosome 20. It is not any of
25 the transacting factors. We've already torn that promoter

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1 apart. It is not CREB. It is not AP-2, it is not SP-1,
2 it is not EGR-1. Might this be a locus, the variability
3 of which controls variability in human sympathetic
4 activity? That's our working hypothesis, and we're going
5 after two loci right beneath that log peak that we think
6 are strong candidates.

7 So just to quickly summarize --

8 (Slide.)

9 DR. O'CONNOR: -- we work on chromaffin cells.
10 We work on the transmitters stored and released with
11 catecholamines. We found a biological active peptide,
12 catestatin, which seems to be a fairly powerful inhibitor

13 of catecholamine release. It does so by virtue of
14 blocking nicotinic cholinergic receptors.

15 There are common variations of catestatin that
16 are floating around in the population, and it might have
17 something to do with alterations in adrenergic responses
18 to stress, and there's another family member, chromogranin
19 B, that we think tells us about the fundamental process of
20 sympathetic stimulation and sympathetic activity that we
21 think might have led us to some major loci involved in
22 determining sympathetic activity in people, and we're
23 ripping the daylights out of those as we speak,
24 resequencing to see if we can see the allelic variance
25 that might be responsible.

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1 And I don't know anything about low blood
2 pressure, but it is fun to hear what you guys are up to.
3 Thank you.

4 (Applause.)

5 DR. ROBERTSON: If we could get the speakers
6 from this morning to pick up their name tag and bring it
7 up here, we will entertain questions and maybe we'll get
8 Katrina or Alan Guttmacher to signal me when they think it
9 is time to go to the next phase of our operation and cease
10 with questions, so let me just open up to questions and,
11 if you would, please identify yourself by name as you ask
12 your question.

13 DR. KOPIN: Irv Kopin. Your presentation was
14 great. The problem that -- well, it's not really a
15 problem. I was just wondering, you talk about the adrenal
16 medulla, and then you sort of extrapolate to the
17 sympathetic nervous system. These are sort of different,
18 the responses are different, and I was just wondering
19 whether you compare something like hypoglycemia, where you
20 have a predominantly adrenal response.

21 Does the chromogranin constituents go up in
22 plasma versus what happens with a cold pressor test, where
23 you're getting sympathetic activity, but you're not
24 getting adrenal medullary activity, at least -- or not
25 exactly the same? It would be nice to know whether or not

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1 you could demonstrate anything coming from the sympathetic
2 nervous system, if these proteins go up under those cases
3 as well, and what sort of cross-interaction there is
4 between the two.

5 DR. O'CONNOR: You make a very good point. If
6 you provoke the sympatho adrenal system in normal
7 volunteers the kinds of outflow are substantially
8 differentiated depending upon the kind of stressor that
9 you use, and stressors that are pretty good for the
10 adrenal medulla include ancillary hypoglycemia, to some
11 extent. emotional stress associated with public speaking
12 is another pretty good one for buzzing the adrenal
13 medulla. Fainting is another pretty good one for buzzing
14 the adrenal medulla, hard to systematically evoke that,
15 whereas there are other stressors that are pretty specific
16 for the sympathetic system. That is, they elevate
17 principally norepinephrine release from sympathetic

18 nerves. Exercise is a pretty good one. The cold pressor
19 test is another one.

20 It remains to be seen whether single nucleotide
21 type polymorphisms at the CGA locus really do predict the
22 response to cold pressor tests. We actually just finished
23 resequencing the CGA locus within the last, about 2
24 months, and as soon as we found these polymorphisms we've
25 set them up as single nucleotide polymorphism or SNIP

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1 assays, and now we're running them on our larger cohort.

2 Actually now we work largely with twins. We've
3 got about 140 twin pairs we've studied, and we're busily
4 testing all these polymorphisms in the twins to see if
5 they do, in fact, predict the cold stress response.

6 DR. KOPIN: The other issues is whether or not
7 the same proteins are present in the sympathetic nerves as
8 they are in the adrenal medulla. Have you done any
9 histological studies to look for this?

10 DR. O'CONNOR: It looks to us as if they're the
11 same ones, and we have not. We've done a little study in
12 human beings, a good source of post ganglionic sympathetic
13 nerves in humans is the vas deferens. A good source of
14 post ganglionic sympathetic nerves in rodents is also the
15 vas deferens. In a larger animal it would be the sperlip
16 nerve in the cow, and with the now-retired Dick Klein, who
17 spent a lot of time purifying so-called large dense core
18 vesicles and post ganglionic sympathetic nerves -- post
19 ganglionic sympathetics at the large core and small core
20 vesicles.

21 The large core vesicles are supplied with
22 peptides. The small core vesicles are electron-dense but
23 seem to have no peptides, just the catecholamines. Then
24 the large core vesicles, it would appear there is
25 authentic chromogranin A with the same molecular mass and

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1 the same immunoreactivity as the form found in the adrenal
2 medulla, so I think the protein is there.

3 DR. KOPIN: Just let me get clear what you just
4 said. Did you say that the large granules in the
5 sympathetic nerves do not have catecholamines?

6 DR. O'CONNOR: No, that the large dense core
7 vesicles are supplying both catecholamines and peptides,
8 and the catecholamine to peptide ratio is about the same
9 as it is in the adrenal medullary granules. Peculiarly,
10 the small dense core vesicles seem not to have the
11 peptides, seem only to have the catecholamines.

12 One school of thought is that these are formed
13 by membrane retrieval from the large dense core vesicles
14 after exocytosis with subsequent endocytosis. They've got
15 the right transmitters. They've got the right
16 transporters in terms of VMAT and the proton translating
17 ATPA's to really accumulate catecholamines from the situs,
18 but they've lost their peptides by virtue of exocytosis.

19 If we go to the large dense core vesicles we do
20 find chromogranin A, and interestingly enough there are
21 nicotinic receptors found all the way up to the termini of
22 post ganglionic sympathetic axons, which you could study

23 either in the bovine splenic nerve, or you could study in
24 the vas deferens. Exactly what they're doing and exactly
25 what neurotransmitter they're responding to and exactly

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1 where that neurotransmitter came from is kind of an
2 unidentified area at this point.

3 DR. ROBERTSON: Other questions for any member
4 of the panel?

5 VOICE: I was struck, David, between what's been
6 found in the DBH-deficient patients and what's been found
7 in the FD patients in terms of the hypothesized
8 cardiovascular role of dopamine. In your case or in the
9 DBH patients they have very high DOPA levels, no DHPG,
10 exactly the same sort of neurochemical pattern that
11 characterizes all patients with FD. High DOPA, if
12 anything decreased DHPG.

13 Both conditions are supposed to be associated
14 with receptor, a post synaptic receptor of regulation.
15 Both are supposed to be associated with either a relative
16 or absolute deficiency of DBH. Both are associated with
17 high dopamine level, either when a DBH deficient patient
18 stands up, or when an FD patient has a crisis, and yet the
19 hypothesized hemodynamic consequences are exactly
20 opposite, and I don't understand why.

21 In your case, in DBH-deficient patients' case
22 dopamine you think plays a role in the hypotension, and
23 therefore if you block dopamine synthesis with
24 alphamethylparatyresine the blood pressure goes up. In
25 the FD patients, when they have their crisis and dopamine

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1 levels are high, that's when their blood pressure is high,
2 and the hypothesis is that somehow dopamine is eliciting a
3 hypertensive response.

4 So I'm confused as to why these conditions,
5 which are neurochemically similar, have apparently
6 diametrically opposite hemodynamic effects of dopamine.

7 DR. ROBERTSON: Well, let me just say that we
8 only have, to my knowledge, two familial dysautonomia
9 patients in Tennessee, so I don't have a very large
10 experience in that disease. I would say that the
11 differences between the two diseases are more striking to
12 me than the similarities.

13 I agree that the pathology seems to suggest
14 there's less DBH relative to tyrosine hydroxylases. I
15 agree that DHPG and norepi, they're both reduced in both
16 diseases, but extraordinarily reduced in DBH deficiency.
17 DHPG is less than 10 picograms per mil. That's a
18 hundredfold or more reduction. That is just amazing.

19 It is not like -- it is nothing on the order of
20 magnitude in the FD to my knowledge, so I guess -- and the
21 other thing, of course, is that the dopamine levels in DBH
22 deficiency are always 200 picograms per milliliter, even
23 lying down, so even lying down they start out 24 hours a
24 day at least being five to sixfold above normal, and then
25 when people stand they become tenfold, maybe, above

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1 normal, so that is not the case in FD, if I've read the

2 data right. Sometimes the changes are modest, but
3 sometimes greater.

4 So I would just say that rather than
5 similarities between the two, I see a lot of differences,
6 but there still may be some common mechanisms that have
7 therapeutic implications.

8 I'll bet Dr. Axelrod has a comment on that.

9 DR. AXELROD: I think you're absolutely right,
10 David. I think that what happens in FD is more episodic,
11 and actually there were two papers on DBH levels, one by
12 Mina Goldstein, and which showed that the DBH levels in FD
13 patients actually to be low, they could be mid-level
14 normal, or they may be high, so it may be a modifier
15 actually, and maybe these are the patients that are really
16 more prone to having crises. We've never really looked at
17 that particular situation.

18 I know, as my graph showed, the dopamine level
19 does come down to normal at times. In the older
20 population it starts to stay persistently high.

21 VOICE: I actually wonder if the difference
22 doesn't really have to do with the source of dopamine,
23 which people have ignored in large measure. Last time I
24 read anything about it, the estimate was as much as 50
25 percent of blood dopamine could be coming from gut, and

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1 people certainly have spent a lot of time recently
2 figuring out the origin of gut dopamine.

3 If you imagine that a familial patient, an FD
4 patient lacks sympathetic nerves in large part then the
5 dopamine and norepinephrine in crises can't be coming from
6 those nerves, and could more likely be coming from gut,
7 where dopamine is secreted in response to injury.

8 VOICE: First in relation to that, if dopamine
9 is coming into the bloodstream from the gut, it's going to
10 be removed by the liver. If you can inject catecholamines
11 into the blood in circulation it will have no effect.

12 The other question is, the patients that have
13 DBH deficiency with high plasma dopamine levels do have
14 nausea.

15 DR. ROBERTSON: I would say that the nausea is
16 not particularly striking. I think that there have been
17 times when they have had vomiting, but it is not common.
18 It is not common in them.

19 VOICE: So again I think one of Mike's points of
20 where the dopamine is produced is maybe a very important
21 thing.

22 DR. ROBERTSON: And I don't want to talk about
23 people's data who aren't here, but I think that Peter
24 Hasay and some of his colleagues are reporting a dopamine
25 receptor abnormality that they are linking to

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1 hypertension. Maybe -- I don't know if anyone from their
2 group is here.

3 So even in people whose norepinephrine --

4 VOICE: I collaborated with that group, and the
5 short story is that dopamine receptor subtype knock-outs
6 often have hypertension, so the implication is that

7 dopamine if anything is playing some sort of a depressor
8 role, and presumably if there is a relationship of
9 dopamine to blood pressure regulation overall by way of
10 these receptors, then when dopamine effects are blocked,
11 that tends to produce an increase in blood pressure.

12 DR. ROBERTSON: Other questions or comments from
13 anyone?

14 (No response.)

15 DR. ROBERTSON: Okay. Let me turn things over
16 to Katrina and to Alan Guttmacher and invite our people to
17 go back to the audience.

18 DR. HARDY: Thanks very much, Dr. Robertson, and
19 thank you to all the speakers of the last session as well
20 as all the previous sessions.

21 Now I would like to turn the stage over to Dr.
22 Alan Guttmacher, who is Deputy Director of the Genome
23 Institute here at NIH. We're honored and pleased to have
24 him here to moderate the final summary session. I would
25 like to invite him to come to the podium as well as all of

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1 the moderators and the chairpeople of each of the
2 sessions, so Dr. Rubin, Dr. Axelrod, Dr. Goldstein, and
3 Dr. Robertson, if you could join us and take a seat. Then
4 we're also going to ask someone to come up and actually
5 we're going to be taking notes on a laptop that hopefully
6 will be broadcast regarding each of the summary
7 statements.

8 DR. GUTTMACHER: While the chairs and moderators
9 come up, let me make a couple of quick observations. The
10 first observation, I'm very sorry I wasn't here for the
11 whole meeting, because it seemed from the parts that I
12 could attend that it really was a wonderful meeting, and I
13 certainly hope that it is one that is only the beginning
14 of a series of such meetings that will occur over the
15 coming years, specifically looking at FD, but also some
16 other of the issues that have come up in this meeting.

17 I can tell you -- what I did here, a word on my
18 background is primarily I've done some genomics research,
19 primarily as a clinician, and as somebody who first met a
20 child with FD over 20 years ago in the pediatric ICU, it's
21 really encouraging to hear about the work that has been
22 done, and clearly the work that is very close on the
23 horizon is a very encouraging thing.

24 And then also, as the Deputy Director of the
25 Genome Institute at NIH, it again is very encouraging to

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1 hear the way that people are using genetics and genomics
2 and applying it both to individuals and families who have
3 concerns about single gene relatively rare conditions, and
4 also to more common conditions, and also, of course, often
5 by applying to the first you apply to both, as we
6 certainly know, for instance, from Goldstein and Brown's
7 Nobel prize-winning work in using familial
8 hypercholesterolemia, a relatively rare disorder, to
9 figure out things about high cholesterol in general that
10 gave birth to statin drugs that now save tens of thousands
11 of people a year, et cetera, et cetera.

12 I think we can see some of these similar kinds
13 of potential output from the kind of work we have heard
14 about in the last couple of days that will be not only
15 immensely important for the dysautonomias but also for
16 understanding other aspects of human health and disease
17 such as blood pressure, of course, which has ramifications
18 for not only a few people but for everyone.

19 So I celebrate the meeting. I think it is
20 wonderful, but I'm going to stop talking, because what we
21 really want to do now is to accomplish somewhat of a
22 summary, but particularly looking for, I would say, what
23 are the action items coming out of this? It is nice to
24 have everyone come together and to share, and I think
25 that's been well-accomplished, so what I'm going to ask is

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1 both the chairs and moderators up here to give us their
2 views of sort of how would they summarize their session in
3 five or six points, perhaps, but also ask you in the
4 audience to think about those summaries and action items,
5 what should we be doing from here, and I use that "we" to
6 be, where should the research be going? If you have any
7 particular instructions for where you think the NIH should
8 be going with this, we are certainly interested in hearing
9 those as well.

10 So with that, I think it probably makes sense
11 for us to go through this maybe chronologically, and it
12 looks as though maybe you already have something ready on
13 Power Point there for us.

14 DR. AXELROD: No, actually, Dr. Rubin, we
15 thought we would start with him, because he was a
16 moderator for our session.

17 DR. RUBIN: So what I've done is, I've
18 highlighted a few points from each of the talks which I
19 think bring a very nice story together at the end, and I
20 would like to share with you what I see as the highlights
21 for the various sessions.

22 The first talk which was given by Felicia
23 Axelrod provided us with a very nice history of the
24 syndrome, spoke about the progression that occurs during
25 the process of aging, and FD patients, and then through

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1 various serious interventions has been able to extend the
2 life span and the quality, and improve the quality of FD
3 patients, and I thought that summarized that talk, and
4 obviously Dr. Axelrod will be able to hop in and
5 contribute further on that.

6 Sue Slaughaupt presented some very nice work
7 on the differential expression of the various of the two
8 forms of IKAP in FD patients in various tissues, and
9 demonstrated that both the mutant and the normal form
10 could be detected in tissues of FD-affected individuals.
11 The level of expression of the wild type versus the mutant
12 transcript in vitro seemed to be affected by environmental
13 conditions, which has prompted her to begin screening a
14 panel of drugs to look for drugs which can enhance the
15 expression of the normal ICAP transcript in FD patients as
16 well as hopefully eventually lead to the use of these

17 drugs and compounds in FD-affected individuals.

18 I actually joined the talk in my mind of Dr.
19 Hilz and Dr. Kennedy, because I saw both of them as
20 looking at the physiology and the histology of FD-affected
21 individuals, and I think it provides an excellent baseline
22 of information that is now available on FD patients.

23 I think it would be valuable to make these
24 observations over a period of time in the same patients.
25 I think it would be interesting to see whether any of the

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1 changes can be correlated with severity of disease, and I
2 think that provides for a very, very unique base so that
3 if and when Dr. Slaugenhaupt identifies compounds that can
4 be used in therapy, one can see whether or not those
5 compounds are going to have an effect on the physiology
6 and the histology, so I thought that that was a very nice
7 aspect, and I think it ties the biochemistry, the
8 molecular biology and the physiology and the histology all
9 together.

10 Dr. Sonenshein gave a very nice overview of the
11 research that was funded by the Dysautonomia Foundation.
12 I thought her words on collaboration and cooperation were
13 important, and I commend her on her efforts in that
14 regard, and then she provided us with a nice overview of
15 the JNK signalling pathways and the role they may play in
16 FD as well as their association with IKAP.

17 Dr. Syejstrup discussed his studies in both
18 yeast and now in human with regards to the elongated
19 complex. He has now been able to characterize the LP-5
20 and 6. They are interesting new genes, and he made the
21 suggestion that I think everybody here is thinking about,
22 about the possibility that there may be mutations in these
23 other components of the elongated complex that may also be
24 playing a role in the FD-like diseases.

25 And finally Dr. Xu presented a very nice

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1 strategy that he is using to generate NOK in mice, which
2 will have either of the two mutations that have been shown
3 to cause FD, and he has suggested the kinds of analyses
4 that would be done when these mice are available.

5 And I think that summarizes those talks. I hope
6 it does.

7 DR. AXELROD: I think that is a very
8 comprehensive and excellent outlining of what was
9 discussed in the workshop, and I think it shows how the
10 request for collaboration was being fulfilled, at the same
11 time the concept of translational research, and the fact
12 that we did present both clinical material as well as
13 basic science material within the one workshop so that the
14 two areas would be dialoguing with each other and setting
15 up efforts that would be collaborative for the future.

16 DR. HARDY: In terms of action items, what could
17 we identify?

18 DR. GUTTMACHER: What do we need to accelerate
19 the field? What do we need to move these kinds of things
20 forward?

21 DR. AXELROD: I think that the fact that we were

22 able to establish some background clinical work that has
23 been done, I think we need to expand on that to continue
24 to document as baseline assessment in FD patients what is
25 actually wrong, so that when the time comes to give

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1 definitive treatments we will be able to measure
2 effectiveness.

3 At the same time, I think we have to go further
4 with the genetic research and identifying these IKAP
5 mutations at elongator changes -- would you agree with
6 that, Dr. Rubin? -- and then eventually go forth with
7 definitive therapies.

8 DR. RUBIN: The only thing I would add, which
9 has already been said, which is, is I think the fact that
10 there can be modulation of the efficiency of splicing
11 requires that we seriously examine a panel of drugs as has
12 been done to see whether there are any compounds that are
13 currently FDA-approved which can be used to facilitate the
14 production of the correct IKAP in these patients.

15 DR. GUTTMACHER: Let me just, in followup to
16 that, mention one thing particularly in reaction to that
17 comment, I think which is a very good one, that I can tell
18 you there is talk certainly that I see at the NIHI and I
19 know at other NIH institutes about seeing whether one of
20 the things that we might be able to do in the next few
21 years that would be useful to move a lot of science
22 forward is to create more access for academic researchers
23 to libraries of small molecules and other kinds of
24 potential targets that would to some degree expand what's
25 available sometimes now in fairly small ways, sometimes

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1 only to people who are in industry, and try to make those
2 tools more available to researchers in general in academia
3 and outside of academia.

4 We see that as a potential -- and I am seeing
5 heads nodding -- for the people who might use those
6 things, not surprisingly, so that's something that we have
7 on our plate.

8 Another thing that I heard both of you talking
9 about that made me think a little bit about is, I know our
10 institute is looking very carefully at the idea of in the
11 not too distant future creating a new type of K-23 award
12 that would support researchers for 5 years, basically, who
13 are clinician researchers. This is really research not
14 just involving human specimens, that would actually have
15 to involve human beings, but there would be clinical
16 researchers who would be trying to apply the tools of
17 genomics and genetics to diseases with a genetic
18 component, and I could certainly see some careers here
19 perhaps involving familial dysautonomia, for instance, as
20 being very appropriate for that.

21 So a couple of things that I know the NIH is
22 thinking about seriously at the moment that might help
23 move this forward. Other thoughts?

24 VOICE: I applaud the concept. There are
25 enormous numbers of people who would use these. I think

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1 the NIH needs to start thinking now about the downstream
2 product of that effort, which is that in the near term,
3 yeah, you will get research reagents, but currently the
4 process of regulatory approval of drugs and putting them
5 into patients is really designed around large populations
6 affected by disease, and leaves out these rare disorders
7 where there just aren't enough patients, even though
8 they're very severely affected, to overcome the regulatory
9 hurdles necessary to test drugs, and some kind of a way of
10 dealing with that is going to have to be done if these
11 libraries that produce compounds are ever going to produce
12 a drug.

13 DR. GUTTMACHER: You're absolutely correct.
14 There are conversations ongoing about this, and you're
15 absolutely correct.

16 VOICE: I would also say that we have the animal
17 models that are being developed, and I think they should
18 be listed as one of the important tools that will be used,
19 and also I think that other people have discussed possibly
20 doing things such as finding downstream targets or ways of
21 modulating the function of the IKAP as another -- not
22 another level, but also the function possibly through
23 phosphorylation of blocking junk, if that's how it
24 functions, so I think those other things would be
25 necessary.

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1 DR. GUTTMACHER: Any other thoughts for session
2 1 before we move on to session 2? Okay. If you have
3 other thoughts about session 1 we can put them up later.
4 That's the nice thing about doing Power Point here. But
5 let's move on and talk about session 2, then,
6 Dr. Goldstein.

7 DR. GOLDSTEIN: Well, I think the structure and
8 the content, and I think the implications of the second
9 session were quite different from the first, and one clear
10 message from the second session I believe, and also the
11 third, is that dysautonomias are common, and I think the
12 view that dysautonomia means a rare disease, very rare
13 disease that essentially is localized to a particular
14 ethnic group is actually counterproductive in terms of the
15 development of science and even effective treatment of FD.

16 And I think the talks in the second session and
17 the third illustrate how common abnormalities of the
18 autonomic nervous system are, and that therefore this
19 points, I think points out an obvious discrepancy between
20 how much support is given for research on autonomic
21 disorders and how frequent those disorders actually are.

22 A second point that comes from all the sessions
23 I think, and especially from the talk of Linda Smith this
24 morning, is that dysautonomias are real. These are not
25 psychosomatic disorders. This is not panic, or anxiety,

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1 or some sort of medical-legal attempt at obtaining
2 disability compensation. These are real.

3 And the third message I think is that
4 dysautonomia research is hard. It is hard for three
5 reasons. First -- and I think it is clear from what the

6 patients had to say and also from the discussions, first,
7 dysautonomias are multidisciplinary. They don't fit well
8 within the structure of the usual funding mechanisms,
9 where you have a study section that deals with
10 gastrointestinal disorders or neurologic disorders or
11 cardiovascular disorders or endocrine disorders and so
12 forth. These are multidisciplinary, and because they're
13 multidisciplinary, I think the subject matter suffers.

14 Second, dysautonomias are integrative disorders.
15 I think this was especially made clear by Jens Jordan's
16 talk. There are abnormalities of systems, the feedback-
17 regulated systems, where it's possible that an abnormality
18 will not be identified in any particular locus in that
19 homeostatic loop, but perhaps in multiple loci, and that
20 requires thinking in terms of systems, something that
21 again does not fit with the usual NIH mold, which is if
22 there's a disease, look at the rare one, come up with a
23 gene and then work backwards from there.

24 You miss all the important compensatory changes,
25 adaptations and changes in sensitivity and so forth that

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1 really contribute importantly to the clinical
2 manifestations and even the treatment from the point of
3 view of the patient.

4 And the third complication that makes
5 dysautonomia research hard is that to a large extent they
6 are mind-body diseases, mind-body disorders, and this is
7 tough because of the Cartesian view that either you have a
8 psychiatric disorder, that that is your problem, or you
9 have a disease, a medical disease, and that is the problem
10 that physicians are trying to deal with, and the fact of
11 the matter is that this sort of dualism doesn't work well
12 for the research or for the patients. The emotional storm
13 in FD can be viewed from a psychiatric point of view just
14 as well as from a medical one, and the idea that, well, a
15 patient with orthostatic intolerance must have a panic
16 disorder, and a patient with a NET deficiency, well, that
17 person shouldn't have a psychiatric problem, it just
18 doesn't jibe. It doesn't work.

19 So as I see it, the problems with dysautonomia
20 research are, it's multidisciplinary, it's integrative,
21 and it's mind-body, and none of those things are really
22 served by the type of reviews and funding mechanisms that
23 exist.

24 The last thing that I want to mention, that
25 comes from the talks in my session and also from the

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1 session this morning, deals with translation. People, I
2 think, in NIH decisionmaking circles view translation as
3 by and large unidirectional. In other words, someone
4 working at the level of the bench dealing with some
5 molecular genetic subject matter comes up with an idea
6 which could translate into a clinical trial, and what's
7 done in the patients is kind of plug-in, does it actually
8 work, but the intellectual process is at the level of the
9 bench.

10 You discover, as an example, this very

11 interesting business of the ratio of wild type to mutant
12 gene-splicing. That's a very interesting idea, and I can
13 see how eventually there would be a clinical trial where
14 basically from the point of view of the clinical
15 investigator there's no intellectual contribution. You
16 just do the clinical trial, and you come up with the
17 various dependent measures, and that's it.

18 But I think it's clear that there's reverse
19 translation when it comes to autonomic research, and there
20 should be, and when I say reverse translation I'm talking
21 about studying groups of patients in particular centers so
22 that clinical investigators can appreciate commonalities
23 that in turn are the driving force for the intellectual
24 process, and I think the talk of David Robertson about
25 DBH, and the talk about NET deficiency by Maureen, for

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1 instance, in my session illustrate the importance of
2 reverse translation.

3 Careful, intelligent information-processing by
4 the clinician leads to testable scientific hypotheses
5 which in turn can drive what's happening at the bench, and
6 when you have translation going in both directions, that's
7 when you really move science along, so what that means in
8 terms of recommendations as I see it is centers of
9 excellence, because the only way you can really allow
10 clinical investigators to appreciate these commonalities
11 is to have concentrations of the patients, not just for
12 the purpose of clinical trials, but also for a better
13 understanding of what the disease really is, and I think
14 in my talk that became clear when it came to Parkinson's
15 disease.

16 I think familial Parkinson's disease is not just
17 a movement disorder. It's also a dysautonomia, and unless,
18 until this is appreciated as a consistent finding in a
19 large group of patients all together, that sort of idea
20 that there's a link between the autonomic nervous system
21 and the movement disorder in Parkinson's disease would not
22 really have come to the fore.

23 So those are my sort of summary statements about
24 the session and what I think the future should show.

25 DR. GUTTMACHER: Very good. Other ideas from

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1 the audience about that session, and particularly things,
2 action items that should come out of the session? The
3 centers of excellence certainly is one, along with the
4 others that I think are already up there. Any other
5 thoughts from the second session?

6 VOICE: I would like to reemphasize the
7 cooperation-collaboration between the medical centers and
8 to remind that a third of the population lives in Israel,
9 and a lot of medical centers are interested, especially in
10 clinical trials, and I hope cooperation between the
11 American side and the Israeli side will be fruitful and
12 may enhance treatment for our children.

13 DR. GUTTMACHER: So international cooperation
14 clearly is a point, and one that's already been achieved
15 to some degree by this group. Very good. Well, let's

16 move on to session 3. Again, we can always come back to 1
17 and 2. Dr. Robertson.

18 DR. ROBERTSON: Well, one of the advantages of
19 being third is that most of the things have already been
20 said, and I think I agree with virtually everything I've
21 heard. I would just make a couple of comments about the
22 presentations in my session.

23 I think the presentation of the DBH deficiency
24 was meant to point out that there can be great success
25 stories and therapeutic success stories, but they are few
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1 and far between, and it is worth remembering that when I
2 was a medical student, not to put too fine a point on it,
3 but some years ago we already knew the genetic defect in
4 the sickle cell disease, and we also, because of the work
5 in Britain we knew the three-dimensional structure of the
6 hemoglobin molecule, and yet here we are 32 years or so
7 later, and we don't have a genetic answer for that disease
8 yet, so we have to rely on these other things.

9 And I was very pleased to see that immediately
10 after the gene is being discovered efforts are being made
11 to find small compounds that can have an effect perhaps
12 through the splicing of the gene to have favorable effects
13 long term, and I think that's important, because we don't
14 want to put all of our eggs in one basket. I think in our
15 scientific lives as well as our financial lives a balanced
16 portfolio is a great think.

17 In relation to the wonderful presentations we
18 heard from Linda Smith and from Dr. Peltzer, we need to
19 hear those things, and we need to hear those frequently.
20 Just about everything that has been found at the clinical
21 level in autonomic research has been told to us by
22 patients, and patients told me for many years that water
23 raised blood pressure, and I explained for many years
24 about, it was sodium really that raised pressure, and they
25 drank water, and then they wanted salt, and the salt

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1 raised the pressure.

2 But the patients were right about that. It was
3 the water, it wasn't salt, so I think that we have to
4 listen carefully to what people say, and people,
5 articulate people can tell you an awful lot about where to
6 go in your research.

7 Two rather striking things to me about research
8 in the autonomic fields and others and how they've changed
9 over the past decade, we've gone from an information-poor
10 field of research, clinical research, to an information-
11 rich field. I can tell you that at the time DBH
12 deficiency was discovered the investigators involved in
13 that initial patient carried around in their head every
14 number relevant to the assays, et cetera, about their
15 patient. We could reconstruct from memory all the aspects
16 of data that related to that patient. It's no great feat.
17 There just wasn't much data. It was very information-
18 poor, but it was sufficient.

19 Now, we have extraordinary information-rich
20 disciplines emerging such as you heard from Jens Jordan,

21 the twin studies, the amount of information that goes into
22 finding the associations he described and that Dr.
23 O'Connor is finding, those are amazing. They're just
24 unbelievable. It's not a hundredfold more information,
25 it's a thousandfold, and sifting through it is now

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1 becoming one of the most difficult things of all in
2 finding out what all that data means.

3 And then my final comment, I think, is that 15
4 years ago I think most of us would have expected major
5 problems and major neurotransmitter defects to emerge from
6 psychiatry clinics and neurology clinics, but they really
7 emerged from observation of cardiovascular and renal
8 variables, and I think it is the striking vividness of the
9 autonomic phenotypes that causes that to occur, and I
10 think that vividness is due to several things.

11 Number 1, there is tremendous -- it is the
12 ubiquity of the autonomic nervous system. It's
13 everywhere. Every organ is a potential site of a read-
14 out of an abnormality, so there are many places you can
15 look and quantitate abnormalities, and secondly, I think
16 that paradoxically the limited plasticity of the autonomic
17 nervous system may also be a reason that some of these
18 things emerge in the autonomic area.

19 If your noradrenergic neurons in the brain don't
20 work when you are 1 year old, the brain may have ways to,
21 through plasticity, bring other nerve types in to fill the
22 gap, but when you only have a noradrenergic neuron going
23 down to intubate your lower extremity, there really is not
24 much plasticity and dependence on other cell types that
25 can occur, so I think that's another reason that the

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1 autonomic nervous system would be a place that
2 psychiatrists and neurologists will want to continue to
3 watch for abnormalities that might lead them to find
4 subtle things in the brain.

5 So I think we are in a growth phase where a
6 balanced portfolio is going to be important, and I'm very
7 excited to hear what, Alan, you say about the Genome
8 Institute and the K-23 awards. The K-23's and K-24's are
9 extraordinarily important to patient-oriented
10 investigators, and I've been very disappointed that more
11 institutes have not embraced the K-23 than did. Only a
12 few have initially embraced it, and I'm delighted that
13 your institute has, because there's an enormous amount of
14 clinical research that needs to be done for the genome
15 project.

16 DR. GUTTMACHER: NINDS already has them, of
17 course.

18 All right, other thoughts about session 3, other
19 action items emerging from session 3. If not, let me just
20 throw it open for other points. You think, how could
21 somebody walk away from this couple of days without
22 realizing that "blank" came out of this meeting, you know.
23 How could you all have missed this kind of thing, or how
24 could you all have missed, boy, you know, when we come
25 back in 5 years or whatever it might be, this is the stuff

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1 that we should have been working on during that period.
2 So any other just sort of key thoughts that emerge for
3 anyone here, researchers, nonresearchers, et cetera?
4 Anyone brave enough to go up to the microphone anyway?
5 Good, there's a couple of brave souls.

6 VOICE: I would say that it's nontrivial to do,
7 and there are lots of roadblocks in the way, but it seems
8 to me that if you're ultimately going to take advantage of
9 the human genome project in the best way possible and
10 really affect this disorder, the critical aspect of that
11 is relating genotype to phenotype, and the problem is it
12 takes a long time to get phenotype and it takes a long
13 time to get patient resources in place, and some kind of
14 consortium at the present time aimed at really good
15 phenotyping and banking of samples that covered more than
16 a single center so that you really captured the population
17 would allow you to design really good genetic studies when
18 the haplotype map comes along and when additional
19 resources come along, but you really have to prepare for
20 those in advance, and it's very difficult for any single
21 center to really prepare properly.

22 DR. GUTTMACHER: That's a very good point, and I
23 would add to that that we've spent so much focus recently
24 gathering genotypic information that we've taken
25 phenotypic information a little bit for granted. I think

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1 it's going to become clear, even when one is talking about
2 single gene disorders, that the expression of those genes
3 and why everything within the same family, the same
4 genetic illness can vary incredibly has something to do
5 with modifying genes, but it also has clearly to do with
6 environmental factors which, that of course covers a lot
7 of stuff.

8 We've gathered the DNA that we continue to
9 investigate, but we haven't always gathered the best
10 really broad and deep phenotypic information, particularly
11 about environmental exposures, et cetera, of individuals,
12 or subjects, patients, et cetera, that we may want to have
13 in years to come, and so I think as one thinks about
14 pooling this kind of information we shouldn't only, of
15 course, focus on the genotype as you said, but also think
16 about the phenotype that we're gathering.

17 DR. AXELROD: I would just like to comment on
18 that. I think at the same time, even though you want
19 multiple cooperation, you have to also have a certain
20 amount of experience and consistency as one documents
21 phenotype. I think Dr. Myan and I have both had the
22 experience where in our hands even doing a histamine test
23 is more accurate than another center or another individual
24 doing a histamine test, and that it could be
25 misinterpreted, but I think that the phenotype, and I

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1 think that the collection of the data really has to be
2 very consistent.

3 VOICE: Yes. Let me clarify what I'm talking
4 about. I consider the initial genetic study to be solved,

5 and now it is the detailed phenotyping done by the
6 experts, but I think you've got this whole other area of
7 autonomic nervous system dysfunction that's a black box of
8 which individual phenotypes are going to emerge connected
9 to genotype, and it's not trivial to approach that in the
10 way that it was with dysautonomia, where you have a clear
11 ethnic bias, a finder of fact, and nice recessive
12 inheritance. It's much more complex.

13 It's going to involve many different genes, and
14 you know, the concept of not doing it via a single center
15 is because there are going to be variations around the
16 world in what's seen, and if people are talking to each
17 other, they're going to have more consistency in their
18 phenotyping than if they're all doing it on their own.

19 DR. AXELROD: Absolutely, and I think what we
20 talked about today, also in the earlier sessions, in some
21 of the sessions was the consistency in autonomic testing,
22 and that's one of the roles and the goals of the American
23 Autonomic Society, which is looking to try and set
24 standards so that everybody is assessing patients the same
25 way and providing guidelines so that the phenotyping will

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1 be consistent.

2 VOICE: It's also worth pointing out that the
3 patients studied by all of the groups involved in linkage
4 and ultimately positional cloning of the FD gene were
5 recruited in large measure and the recruitment paid for by
6 private organizations, not the Government.

7 People in the outside world tell me that it's
8 incredibly difficult to get funding for phenotyping and
9 collection of families, that study sections often react to
10 requests for such funding by saying, show us the gene and
11 then we'll fund your downstream research, and I think
12 that's a flaw in the funding system right now. It's
13 something we don't suffer from quite as much in the
14 intramural program, but people in the outside community
15 shouldn't have to suffer from it either.

16 Second, to the extent that there are tissue
17 banks right now, a lot of the funding also comes from
18 private organizations, and these tissue banks downstream
19 getting genes are incredibly useful, and I think probably
20 more Government support, or at least thought about
21 providing more support should be given to this as well.

22 DR. HARDY: By tissue, could you clarify?

23 VOICE: Well, in the case of FD I think we need
24 very well-annotated and well-collected tissues for
25 translational research at this point. Brain, spinal cord,

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1 blood wouldn't be quite as useful, certainly, but
2 certainly a great set of banked brain samples at this
3 point would have been useful, and they don't really exist.

4 DR. GUTTMACHER: In one of the talks yesterday
5 morning we talked about the value of having this.

6 DR. RUBIN: May I just follow up on Mike's
7 comment with regard to private funding? Private funding
8 is also the mechanism by which screening is being done for
9 a large part of the Jewish community, and attempts at

10 obtaining funding through Federal agencies has really
11 presented a significant challenge, so I think all aspects
12 of this kind of investigational as well as outcome
13 research has presented a challenge.

14 VOICE: One of the other problems is, it's very
15 hard to publish clinical articles when the number of
16 patients is very small, so you're almost looking by
17 definition at anecdotal reports rather than complicated
18 double-blind control studies. That means that a lot of
19 the research that is being done is not being published
20 because it may not be acceptable in terms of numbers, et
21 cetera.

22 For instance, there have been studies with
23 secretin and other various drugs in dysautonomia where the
24 results have not been made available, relevant negative
25 results, positive results, and we need to have some

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1 mechanism where we can release the information in a way
2 that can be shared among researchers. This I see as a
3 very big problem, because it is an international disorder,
4 and studies are being carried out in various centers, and
5 we're not able, particularly in Australia, for instance,
6 to get that information and so it remains purely
7 anecdotal. It is not open to scrutiny or review.

8 DR. GUTTMACHER: That's one of the concepts that
9 led to something still being worked on kind of at the NIH
10 and other places, the idea of a central kind of thing that
11 would allow for wider publication of things, particularly
12 negative results, but also clearly the kind of things
13 you're talking about. It's definitely a problem.

14 DR. WEIL: My name is Miguel Weil from Tel Aviv
15 University, but the point is now I was a father of an FD
16 son, and I don't want to make any remarks on the
17 scientific point of view now, but I would like to talk
18 about my personal feeling, what I felt coming into here.

19 I came here just by myself. I am not related
20 with any foundation whatsoever. I happen to have a kid
21 with familial dysautonomia, and I am in contact with the
22 center in Israel. I am really very -- we manage very well
23 by ourselves. The point is that I feel this day or the
24 last 2 days that there's really very good, positive
25 energy. Everybody means very well, have very good ideas.

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1 The projects are fine. I'm very happy that this is going
2 to expand the interest-taking on getting a grip and going
3 for it. Two or three groups are doing basic science. It
4 is very good.

5 The problem is, what I really feel is that
6 people are not talking that much between each other. My
7 concern is, let's put all this positive energy together.
8 Let's communicate more. Let's make all this, make
9 physical for everybody, for more groups, for other people
10 to come in and give the positive energy, because time is
11 ticking.

12 My concern is really everybody of us that we
13 have a dysautonomic child, we have 100 percent of a
14 problem. Every day we are dealing with these things, and

15 I understand, and I believe that all of this effort will
16 do good, but the foundation, people that are in charge of
17 deciding where the money should go, I agree you have a
18 very good advisory board, everything is done fantastically
19 well, but I feel, my personal feeling is that things, our
20 communication between yourself is not going well, that we
21 need to all talk to each other, enable to make the best
22 effort, all of this money pumped in to make the things
23 faster and more efficient for everybody for the sake of my
24 children, for everybody.

25 That is what I feel, and this needs to be said.

0122

1 Everybody means very well. Everybody is positive. I've
2 spoken with everybody in the foundation, in FD Hope.
3 Let's come together. Finish with -- I don't know, I
4 really -- I'm not political. I don't know what the rules
5 of these problems were, but things need to get done.
6 Everybody has the communication. Everybody has the
7 information. Let's share it. Come on. It is very
8 important.

9 Thank you.

10 DR. GUTTMACHER: One point about information-
11 sharing that we haven't got up there yet, that was well-
12 made yesterday morning, was the idea of an e-mail
13 listserve, so that should certainly I think give us
14 something to do .

15 DR. HARDY: And everybody's e-mail was
16 distributed.

17 DR. GUTTMACHER: It sounds like it's already
18 been done.

19 Yes.

20 MS. LIEBERMAN: My name is Lynn Lieberman, and
21 I'm the parent of a child with FD from upstate New York,
22 and I just wanted to address maybe underneath my concern
23 is what was just expressed, which is a feeling of urgency
24 to try to make the best use of the resources we all have
25 together, and I've been listening and thinking about this.

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1 I don't know all the terminologies and centers
2 of excellence, what actually the definition of that is,
3 but I have a feeling that there could be a distinction
4 between a clinical center that provides treatment and care
5 and approaches, maybe multidisciplinary approaches, a
6 distinction between that type of center and an overall
7 center that is a data collection agent.

8 I would like to see some of the expertise shared
9 with centers around the world so that anyone could
10 competently do a histamine test. I had a histamine test
11 done in Philadelphia that was laughable, and unfortunately
12 that was true, but probably a couple of days of training
13 or whatever it takes, any one of you who is a clinician
14 could do a test, could replicate some of the data
15 collection procedures that are now being done in New York
16 City so that our children -- those of us who aren't
17 especially in the New York City area can't come in and
18 don't have the same relationship to the center in New York
19 and to the center in Israel.

20 So that's all I'm proposing, is that there be
21 some thought to a distinction between clinical, providing
22 clinical care and using the resources we have around the
23 country and all the different fields and data collection,
24 and that would require us to collaborate. It would
25 require information-sharing. It would require sharing

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1 of -- I don't want to be snide, but it would require that
2 we all agree that for the good of the situation, that it's
3 okay to be the second name on the paper, or you know --
4 there are a lot of issues that I think need to be put
5 aside in order for us to get the care that we need for our
6 children.

7 Thank you.

8 DR. GUTTMACHER: I think we have time for one
9 more, then we'll have to wrap up.

10 VOICE: Just one additional comment that I think
11 we can all agree upon to put on our list, and that is
12 regular and frequent followups to this meeting, that this
13 meeting was truly -- it really did accomplish an awful
14 lot. There were wonderful discussions, and I think
15 everyone is going to leave this week with a lot of
16 enthusiasm, and I think that the next step will be to have
17 another meeting so that we can hear from Dr. Slaugenhaupt
18 and Dr. Gusella about what FDA-approved drugs are making a
19 difference, and we can hear from all of these other
20 researchers on their research and see where the next step
21 has been, and then even come even further together.

22 DR. GUTTMACHER: That's not a bad last comment.
23 We'll give you the \$5 later, I think, but the earlier
24 comments were also worth remembering, of course, that
25 there clearly are problems out there, but I personally,

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1 what I'm taking away from this is -- and I'm looking back
2 over a couple of decades kind of thing, but even, there
3 has been incredible progress, and I would think that the
4 next time this meeting is held, and it sounds like a good
5 idea to me, to keep having them, there will be exciting
6 progress, and for those of you who have kids right now who
7 are affected, it's clearly going to come too slow.
8 However fast it comes it will be too slow, but it is
9 coming, and that is an important thing.

10 And the other thing that no one spoke about
11 that's self-evident is, one of the ways that this meeting
12 worked well was to have the researchers, the clinicians
13 and the families together, and that is a very powerful --
14 that's the way the work goes forward. It is the way a
15 conference like this works best.

16 So I'm appreciative to be included in this, in
17 even a small part. It has really been a wonderful
18 conference. I think you will look back on it for years to
19 come as a kind of, I hope, seminal event in terms of
20 moving these things forward, and I personally would thank
21 Katrina very much for doing the organizing of this.

22 (Applause.)

23 DR. GUTTMACHER: Would you like to have the last
24 word?

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DR. HARDY: Just as a last word, I would like to

1 say that we do have a writer writing the summary of this
2 meeting. I don't have a time frame on that, because we
3 are taping the meeting, but hopefully he will get a
4 transcript of that and be able to put that together so
5 hopefully we will see some document emerge summarizing the
6 content scientifically, and hopefully some of the human
7 side of what was discussed today.

8 I want to thank everyone who came. I want to
9 thank all of you for being open, honest, and expressing
10 exactly what you think both scientifically and from the
11 human standpoint, and thanks for everyone's support and
12 encouragement. Now let's go out and do lots of great
13 science and cure some of these diseases, or at least get
14 them some treatment.

15 Thanks very much.

16 (Whereupon, at 12:05 p.m., the meeting
17 adjourned.)

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