European Journal of Human Genetics (2015), 1–7

www.nature.com/ejhg

ARTICLE

Asymmetry of parental origin in long QT syndrome: preferential maternal transmission of *KCNQ1* variants linked to channel dysfunction

Hideki Itoh*[,1,2,3], Myriam Berthet[1,2,4], Véronique Fressart[1,2,4,5], Isabelle Denjoy[1,6], Svetlana Maugenre[1,2,4], Didier Klug[7], Yuka Mizusawa[8], Takeru Makiyama[9], Nynke Hofman[10], Birgit Stallmeyer[11], Sven Zumhagen[11], Wataru Shimizu[12,13], Arthur AM Wilde[8], Eric Schulze-Bahr[11,14], Minoru Horie[3], Sophie Tezenas du Montcel[15,16] and Pascale Guicheney[1,2,4]

Transmission distortion of disease-causing alleles in long QT syndrome (LQTS) has been reported, suggesting a potential role of *KCNQ1* and *KCNH2* in reproduction. This study sought to investigate parental transmission in LQTS families according to ethnicity, gene loci (LQT1-3: *KCNQ1*, *KCNH2*, and *SCN5A*) or severity of channel dysfunction. We studied 3782 genotyped members from 679 European and Japanese LQTS families (2748 carriers). We determined grandparental and parental origins of variant alleles in 1903 children and 624 grandchildren, and the grandparental origin of normal alleles in healthy children from 44 three-generation control families. LQTS alleles were more of maternal than paternal origin (61 *vs* 39%, $P<0.001$). The ratio of maternally transmitted alleles in LQT1 (66%) was higher than in LQT2 (56%, $P<0.001$) and LQT3 (57%, $P=0.03$). Unlike the Mendelian distribution of grandparental alleles seen in control families, variant grandparental LQT1 and LQT2 alleles in grandchildren showed an excess of maternally transmitted grandmother alleles. For LQT1, maternal transmission differs according to the variant level of dysfunction with 68% of maternal transmission for dominant negative or unknown functional consequence variants *vs* 58% for non-dominant negative and variants leading to haploinsufficiency, $P<0.01$; however, for LQT2 or LQT3 this association was not significant. An excess of disease-causing alleles of maternal origin, most pronounced in LQT1, was consistently found across ethnic groups. This observation does not seem to be linked to an imbalance in transmission of the LQTS subtype-specific grandparental allele, but to the potential degree of potassium channel dysfunction.
European Journal of Human Genetics advance online publication, 16 December 2015; doi:10.1038/ejhg.2015.257

INTRODUCTION

Long QT syndrome (LQTS) is an inherited arrhythmogenic syndrome with QT interval prolongation, syncope, and risk of sudden death from polymorphic ventricular tachycardia or ventricular fibrillation. Thirteen candidate genes are implicated, of which two potassium channel genes (*KCNQ1*, LQT1 [MIM 192500] and *KCNH2*, LQT2 [MIM 613688]) and a sodium channel gene (*SCN5A*, LQT3 [MIM 603830]) are the major genetic subtypes, accounting for ~80% of all genotyped LQTS patients.[1]

We previously reported transmission ratio distortion (TRD) in comprehensively genotyped European LQTS families, with an excess of variant carriers and female predominance.[2] TRD is defined as a significant deviation from expected Mendelian ratios of allele inheritance, that is, a preferential transmission of a variant allele of particular parental origin. In consequence, this may lead to an unexpected higher frequency of disease in offspring. The mechanisms underlying TRD in LQTS are unclear. Whereas siblings should randomly receive grandparent origin alleles, Naumova *et al*[3] reported grandparental transmission distortion in normal subjects for imprinted regions such as the 11p15.5 locus, where *KCNQ1* is located. They suggested that the TRD in LQT1 could be linked to a locus-specific distortion over three-generation transmission.[4] Accordingly, in this study we compared the grandparental origin of normal alleles in control families from the Centre d'Étude du Polymorphisme Humain (CEPH) with that of variant alleles in LQTS families.

On the other hand, several studies have demonstrated the expression of *KCNQ1* voltage-gated potassium channels in the ovaries, in granulosa and trophoblastic cells.[5–7] Thus, the voltage-gated potassium channel may have an important role in zygote maturation.

[1]INSERM, UMR S1166, Paris, France; [2]Sorbonne Universités, UPMC Univ Paris 06, UMR S1166, Paris, France; [3]Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Otsu, Japan; [4]Institute of Cardiometabolism and Nutrition, ICAN, Pitié-Salpêtrière Hospital, Paris, France; [5]AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Service de Biochimie Métabolique, UF Cardiogénétique et Myogénétique Moléculaire et Cellulaire, Paris, France; [6]Cardiology Department, AP-HP, Hôpital Bichat, Paris, France; [7]Hôpital Cardiologique de Lille, CHRU, Service de cardiologie A, Lille, France; [8]AMC Heart Center, Department of Clinical and Experimental Cardiology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [9]Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan; [10]Department of Clinical Genetics, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [11]Department of Cardiovascular Medicine, Institute for Genetics of Heart Diseases (IfGH), University Hospital Münster, Münster, Germany; [12]Division of Arrhythmia and Electrophysiology, Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, Suita, Japan; [13]Division of Cardiology, Department of Internal Medicine, Nippon Medical School, Tokyo, Japan; [14]Interdisciplinary Centre for Clinical Research (IZKF) of the University of Münster, Münster, Germany; [15]Biostatistics Unit, AP-HP, Groupe Hospitalier Pitié-Salpêtrière Charles-Foix, Paris, France; [16]Sorbonne Universités, UPMC Univ Paris 06, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique (IPLESP UMRS 1136), Paris, France
*Correspondence: Dr H Itoh, Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Seta Tsukinowa-cho, Shiga, Otsu 520-2192, Japan. Fax: +81 77 543 5839; E-mail: itohhide@belle.shiga-med.ac.jp
Received 29 March 2015; revised 30 October 2015; accepted 15 November 2015

We followed maternal and paternal transmission of LQT1–LQT3 variant alleles in a large number of families from Europe (France, Germany and the Netherlands) and Asia (Japan), and explored the link between parental origin asymmetry and the severity of channel dysfunction.

MATERIALS AND METHODS

Subjects

Genotyped LQT1–LQT3 families with known disease-causing variants, of at least two generations, were collected from three European centers (Paris, France; Münster, Germany; and Amsterdam, The Netherlands) and three Japanese centers (Shiga University of Medical Science, Kyoto University Graduate School of Medicine, and Suita National Cerebral and Cardiovascular Center). Genotyping in each center followed written informed consent and the ethical rules of each country. The subjects included in the study were as follows: LQTS patients carrying one variant, obligate carriers with or without documented cardiac sudden death, and non-carriers who were descendants of LQTS patients. If both parents carried a LQTS variant, their descendants were excluded. The cohort comprised 679 families (2748 carriers and 1034 non-carriers), of which 164 European families were analyzed previously[2] and 515 families were new additions, which included the entire Japanese cohort. The variants have been submitted to the Leiden Open Variation Database (LOVD 3.0, www.LOVD.nl/*KCNQ1*, *KCNH2*, and *SCN5A*) with IDs given in Supplementary Tables S1 and S2 (LQT1, 49 181–49 512; LQT2, 49 515–49 793; and LQT3, 47 336–47 343, except 47 341, 47 469–47 495, and 49 027–49 060). A total of 83 sudden cardiac deaths occurred before the age of 40 years, 44 in LQT1 (19 male and 25 female), 28 in LQT2 (7 male, 21 female), and 11 in LQT3 (8 male and 3 female). The cases who died but not from sudden death were 8 females and 2 males.

Origin of the variant alleles

We determined the parental origin of the allele transmitted to proband and all carriers from each family without regard to phenotype. When Mendelian transmission is respected, variant alleles are transmitted from mothers and fathers in a 1:1 ratio. Furthermore, we evaluated the grandparental origin of variant alleles in three-generation LQTS families and the grandparental transmission of *KCNQ1*, *KCNH2*, and *SCN5A* loci markers in three-generation control families, to determine whether grandparental transmission distortion occurs. We then compared the grandparental origin of LQTS variant alleles with that of normal alleles. Figure 1 shows allele transmission in a large LQT1 family.

Genotypes in control families

We studied 44 three-generation CEPH families, including the 31 families analyzed by Naumova *et al*[3], and 13 additional kindreds (1353, 1354, 1356, 1357, 1358, 1447, 1451, 1454, 1458, 1459, 1463, 1477, and 1582). Markers within or near each of the genes were genotyped: D11S4088 and D11S1318 for *KCNQ1*, D7S2461 for *KCNH2*, and D3S1260 and D3S3521 for *SCN5A*. For some families, we also used data from the CEPH genotype database as shown in Supplementary Figure S1. Haplotypes allowed us to determine the grandparental origin for 2025 alleles including 712 *KCNQ1* alleles, 703 *KCNH2* alleles, and 610 *SCN5A* alleles (Supplementary Tables S3–S5). Statistical analyses were performed on the 31 families in Naumowa *et al*,[3] on all the families, and after exclusion of some families when the origin of the four grandparental alleles could not be determined (for *KCNQ1* locus, see Supplementary Table S6). Three families were excluded for *KCNQ1*, four for *KCNH2*, and eight for *SCN5A*.

Variant categories

To determine whether transmission distortion was linked to channel dysfunction, we classified *KCNQ1* variants according to location and type.[8] For channel

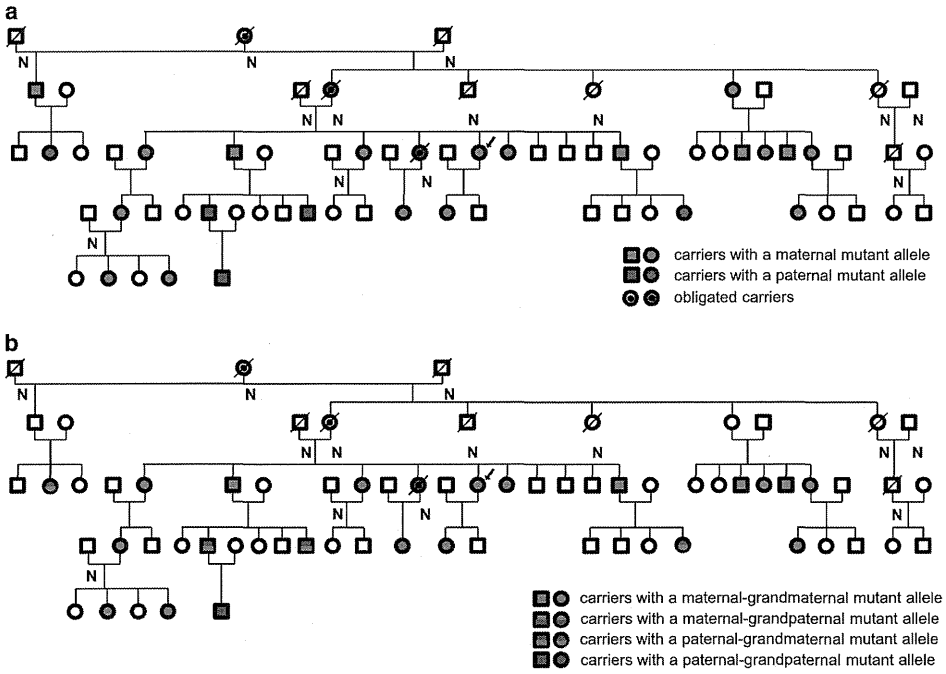

Figure 1 Parental and grandparental origin of variant alleles in a LQT1 family. In this pedigree, there are 25 carriers (18 women) of the R555C variant including three obligate carriers. The classification of the variant carriers is illustrated by colored symbols. (**a**) Parental origin of LQTS alleles transmitted to carriers. (**b**) Grandparental origin of the LQTS alleles transmitted to the carriers. N, not tested. Proband is indicated by an arrow, and open circles and squares are non-carriers.

dysfunction, missense variants were categorized according to either a dominant (>50% reduction in I_{Ks} current; $n=24$) or a non-dominant negative effect ($n=9$) on wild-type subunits, according to published heterologous expression studies. Other non-missense variants such as nonsense ($n=6$), splice site ($n=7$), and frameshift variants ($n=22$) with known or assumed loss of function were grouped as haploinsufficiency variants (Supplementary Table S1). The last group comprises mostly variants with unknown function including many missense ($n=76$), some splice site ($n=7$), and in-frame deletion variants ($n=7$).

For *KCNH2*, variants of the S5-pore-S6 caused the most severe phenotypes, mostly due to a dominant negative effect,[9] and we therefore categorized *KCNH2* variants into four classes: N-terminus, S1–S4, S5-pore-S6, and C-terminus (Supplementary Table S2). We also classified LQT2 variants according to variant type, as for LQT1. We did not analyze LQT3 variants, as the size of the group did not allow subdivisions and there is no evidence of risk stratification for *SCN5A* variants.

Statistical analysis

Differences between two groups were analyzed by the χ^2-test or Fisher's exact test where appropriate. Two-tailed exact binomial tests were used to assess whether the ratio of parental or grandparental alleles differed from 0.5 or 0.25, respectively. The odds ratios (ORs) with 95% confidence interval (CI) were estimated as a measure of association. Statistical significance was considered as $P<0.05$. Ages at sudden death were compared using an unpaired Student's *t*-test.

RESULTS

Table 1 shows analysis of the genotyped cohort according to ethnicity, LQTS genes, carrier or non-carrier status, and gender, demonstrating female predominance among LQTS carriers (1563 LQTS females of 2748, 57 *vs* 43% males) but not among non-carriers where the sex distribution was nearly equal (524 females of 1034, 51 *vs* 49% males; $P=0.0004$). This was due to a larger number of female carriers in LQT1 and LQT2, but not in LQT3 families. The results were similar in both Japanese and European samples.

Preferential maternal transmission of LQTS alleles

Among the 479 LQTS probands, 61% inherited the variant from their mothers (maternal *vs* paternal: 292 alleles *vs* 187 alleles, $P<0.0001$). When all the carriers were considered, we observed the same asymmetry, with 61% alleles of maternal origin (1155 of 1892 variant alleles, $P<0.0001$). Sixty-six percent of LQT1 carriers received the variant from their mothers (602 of 910 carriers, $P<0.0001$), highly significant for children of both genders for the whole cohort, and in the subgroups from France, Germany, and Japan, but not from the Netherlands (Figure 2 and Supplementary Table S7). The preferential maternal transmission was less impressive for LQT2 carriers and for LQT3 carriers, but still significant (LQT2: 56%, $P=0.0008$ and LQT3: 57%, $P=0.036$), mostly due to a preferential transmission to female offspring (Supplementary Table S7). This results in a genotype-specific significantly larger maternal variant allele ratio among LQT1 carriers than for LQT2 and LQT3 (OR: 1.5, 95% CI: 1.3–1.9, $P<0.0001$ and OR: 1.5, 95% CI: 1.1–2.0, $P=0.011$).

Grandparental-allele transmission in CEPH and LQTS families

We determined the grandparental origin of alleles transmitted to grandchildren for the three LQTS genes in CEPH families. The allele ratios from each grandparent varied from 0.23 to 0.27, not significantly different from the expected value of 0.25 (Figure 3 and Supplementary Table S8). In contrast, comparison of grandparental LQTS allele ratios to control alleles for each locus, showed significant distortion for allele transmission in three generation LQT1 and LQT2 families (LQT1, $P<0.0001$ and LQT2, $P<0.025$, but not in LQT3 families ($P=0.25$; Figure 3). In LQT1, transmission to grandchildren of both genders was distorted, with an excess of maternal grandmother alleles (39%) and a reduction of paternal grandfather alleles (13%; $P<0.0001$), whereas in LQT2 families a significant discordant transmission was only found for female grandchildren compared with that for CEPH families ($P=0.036$; Supplementary Table S8).

Parental origin asymmetry and variant classification

We aimed to determine whether the observed distortion was dependent on ion channel dysfunction. We thus classified LQT1 variants into subgroups according to type, location, and their functional effect, (i) either leading to haploinsufficiency, (ii) with a proven dominant negative or (iii) non-dominant negative effect on

Table 1 Cohort according to LQTS genes, gender and countries

	Total				*France*			*Germany*			*The Netherlands*			*Japan*		
	n	*(%)*	P-value[a]	P-value[b]	n	*(%)*	P-value[a]	n	*(%)*	P-value[a]	n	*(%)*	P-value[a]	n	*(%)*	P-value[a]
Families																
All	679				199			147			85			248		
LQT1	332				126			74			19			113		
LQT2	279				68			66			34			111		
LQT3	68				5			7			32			24		
Carriers female/all (% of female)																
All	1563/2748	(57)			487/871	(56)		373/650	(57)		301/557	(54)		402/670	(60)	
LQT1	763/1314	(58)	<0.0001	0.0045	317/559	(57)	0.0015	178/308	(58)	0.0062	72/133	(54)	0.34	196/314	(62)	<0.0001
LQT2	647/1135	(57)	<0.0001	0.066	158/286	(55)	0.076	174/311	(56)	0.037	141/247	(57)	0.026	174/291	(60)	0.0008
LQT3	153/299	(51)	0.68	0.47	12/26	(46)	0.84	21/31	(68)	0.07	88/177	(50)	0.9	32/65	(49)	0.94
Non-carriers female/all (% of female)																
All	524/1034	(51)			215/404	(53)		138/258	(53)		119/259	(46)	52/113	(46)		
LQT1	208/415	(50)	0.96		112/229	(49)	0.74	55/87	(63)	0.013	18/45	(40)	0.27	23/54	(43)	0.17
LQT2	253/486	(52)	0.36		97/165	(59)	0.024	78/158	(49)	0.87	55/116	(47)	0.88	23/47	(49)	0.57
LQT3	63/133	(47)	0.54		6/10	(60)	0.75	5/13	(38)	0.58	46/98	(47)	1	6/12	(50)	0.54

[a]χ^2-Test when compared with a female ratio assumed to be of 0.5.
[b]χ^2-Test when compared with female ratios in non-carriers.

wild-type alleles, or (iv) with unknown functional consequences (Figure 4 and Supplementary Figure S2). Interestingly, variants leading to haploinsufficiency such as splice, nonsense, frameshift variants, and missense variants with non-dominant negative effects, which both cause the mildest channel dysfunction, were found associated with less distortion (58% maternal transmission) compared with dominant-

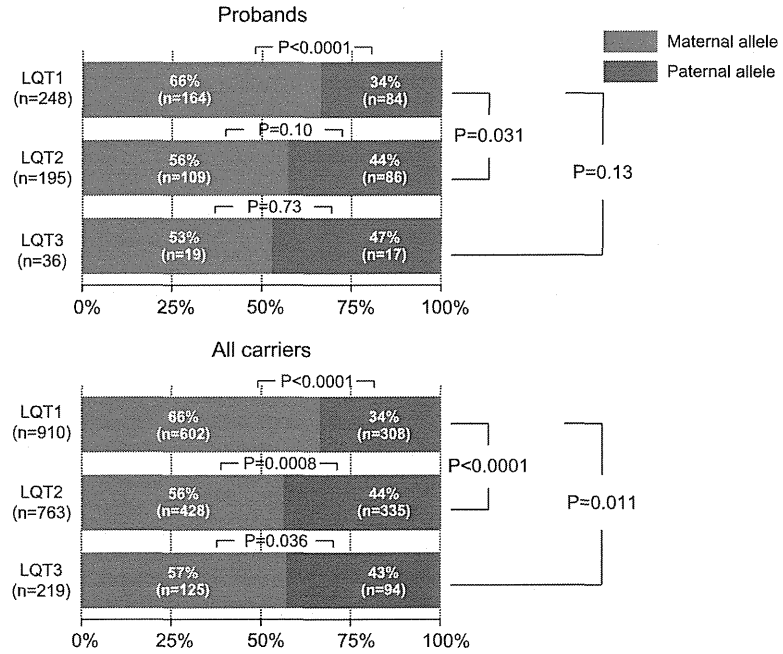

Figure 2 Parental origin of variant alleles transmitted to probands or all carriers for each LQTS subtype.

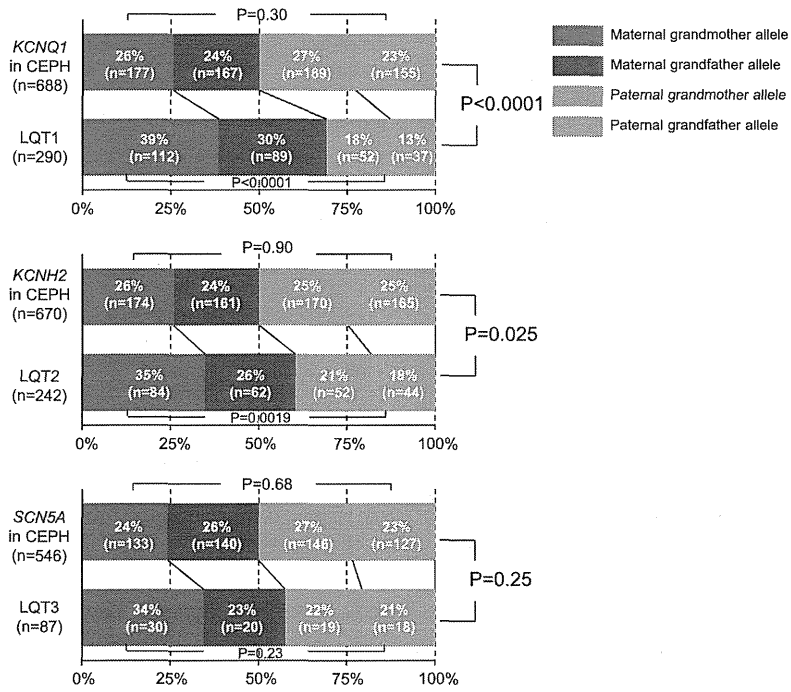

Figure 3 Grandparental origins of LQTS alleles for each LQT subtype. The upper bar shows the allele distribution of the four grandparental origins in CEPH families and the lower bar shows the variant-allele distribution of four grandparental origins in LQTS.

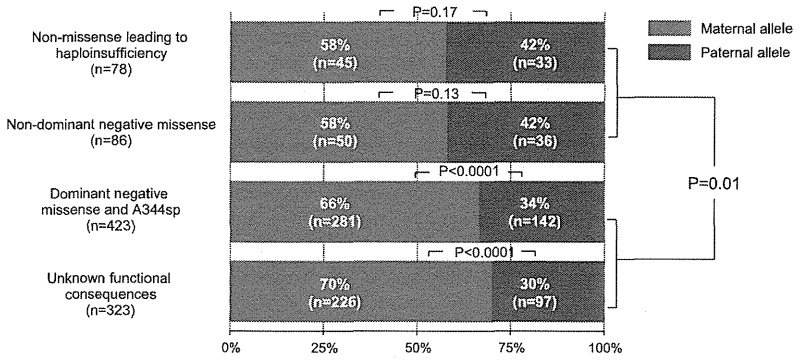

Figure 4 Parental origins of LQT1 alleles and channel dysfunction. LQT1 variants were classified as non-missense variants, leading to haploinsufficiency, non-dominant negative missense variants, and dominant negative missense variants when their co-expression with wild-type channels induced >50% reduction in I_{Ks} current according to published patch clamp analyses. The last group corresponds to variants with unknown functional consequences. The maternal transmission compared between the four function groups under the assumption that the four maternal transmission are equal to 0.5 was significantly different ($P<0.0001$). For more explanations on classification, see Supplementary Table S1.

negative missense variants, and with other variants, a heterogeneous group, which mostly includes missense variants with unknown effects (68%, $P=0.01$).

For LQT2, maternal transmission was higher than the paternal transmission only for missense variants (58 *vs* 42%, $P=0.002$). A similar distortion was observed for missense variants located in the pore region of the protein (S5-pore-S6) compared with other missense variants (Supplementary Figure S3).

DISCUSSION

This study provides information on parental and grandparental transmission in a large number of genotyped LQTS patients from different countries. We report an asymmetrical parental origin for variant LQTS alleles, with higher-than-expected maternal transmission and inheritance, especially for LQT1, in both European and Japanese families. In addition to this, we found that this is not linked to a LQTS subtype (locus-specific) grandparental origin allele transmission distortion, but probably associated with the severity of channel dysfunction. This suggests that the altered function of the potassium channel, especially *KCNQ1*, could modulate reproduction.

We had previously reported transmission distortion in LQTS families, demonstrating female predominance and an excess of carriers through segregation transmission in LQT1 and LQT2 families.[2] Our data also suggested a tendency to more frequent transmission of LQTS alleles from mothers to daughters. Furthermore, Naumova *et al*[3] reported in 2001 an asymmetrical transmission of the imprinted chromosome 11p15.5 region through meiosis in 31 CEPH families, with a significantly higher transmission rate of paternal grandfather alleles to sons and of maternal grandmother alleles to daughters. They suggested that grandparental origin-dependent transmission distortion is related to imprinting, an epigenetic factor that could be responsible for transmission distortion of several genes,[3,10] including *KCNQ1*, and thus could explain transmission distortion in LQT1.[4] To study this hypothesis, we compared grandparental allele transmission in CEPH families for the LQT1–3 gene loci. We differed on two methodological points compared with the Naumova study: we studied more families and used highly polymorphic intragenic markers or markers more closely linked to the loci of interest. We found that the grandparental allele distribution was normal for the three loci whether we included all families or only those in which the four grandparental alleles were traced. This is concordant with a study on the Framingham heart cohort, which does not report distortion for chromosome 11.[11] If we did not observe significant TRD for the *KCNQ1* locus throughout the three generations in control families, this does not exclude a TRD for other loci on 11p15.5, such as INS-IGF2 locus, which is distant of 300 kb.[12]

In contrast, distribution analysis of grandparental variant alleles to grandchildren in LQTS families showed an obvious and significant excess of maternal grandmother LQTS alleles in LQT1 and LQT2 families, and a net reduction of paternal grandfather LQTS alleles, compared with values expected from Mendelian laws and CEPH family ratios. For LQT3 families, an increased ratio of grandmaternal alleles cannot be formally excluded; however, the variations did not reach significance, owing to the limited number of cases. The marked distortion observed in grandparental LQTS allele transmission correlates well with the more frequent transmission of maternal LQT1 or LQT2 alleles in nuclear families, and results from a cumulative effect during two generations.

The mechanism resulting in the higher transmission rate of maternal LQTS alleles, highly significant in LQT1 families, is not known. A reduction in the number of male carriers of reproductive age, compared with female carriers, could induce female predominance and higher maternal transmission. However, in LQTS, even if gender differences have been demonstrated, they do not support this theory. At adult age, LQT1 and LQT2 women have longer QT intervals and a higher risk of life-threatening cardiac events than men.[13,14] By contrast, before puberty, the incidence of arrhythmia is higher and occurred at a younger age in boys than in girls,[15] but the numbers of sudden deaths are similar between men and women LQTS patients in most of the studies, as it was in our cohort, where even more females than males died from non-arrythmogenic causes. Over several decades, β-blocker therapy has been used to prevent sudden death in LQTS patients, and intrauterine or infancy deaths due to LQTS remained exceptional.[16] Even if it is unlikely that premature death in males could explain the sex imbalance and the transmission distortion among patients in our population, we cannot exclude that fetal wastage of embryos could occur at very early stage of development, as 50–75% of all human conceptions are lost before the first missed menses.[17,18] It is known that abnormal imprint resetting could lead to early death of embryos[19] and *KCNQ1* is a paternally imprinted gene. Nevertheless, it is difficult to conceive the specific loss of male *vs* female embryos.

In contrast to other imprinted genes, for which only one of the two parental alleles is expressed in tissues during all the life, *KCNQ1* paternal imprint is reversed during development, and both maternal and paternal alleles could be detected in most of the tissues. This was shown in mouse[20] and a partial erasing was reported in tissues from human embryos, especially in hearts.[21] To our knowledge, no other study was performed to determine whether the *KCNQ1* paternal imprint is fully erased or not in all human tissues. Nevertheless, it is clear that the cardiac phenotype of LQT1 sibs are similar regardless of the gender of parents with a variant in *KCNQ1* as shown for the Japanese cohort (Supplementary Table S9). This suggests a normal expression of both alleles in the heart.

We rechecked in the enlarged pool of families, the transmission to fully genotyped offspring applying correction for single ascertainment[22] as in our earlier study.[2] Our previous observation in LQT1 families that an excess of carriers is only present in descendants from affected mothers, but not from affected fathers, was confirmed (Supplementary Table S10). We postulate that the normal transmission of paternal LQT1 variants is probably due to the repression of paternal *KCNQ1* allele expression by imprinting in spermatozoids and early embryogenesis. By comparison, in LQT2 and LQT3 families, there was also an excess of carriers but without significant difference between maternal and paternal transmissions; these genes are not in imprinted loci. The excess of maternal variant alleles in LQT1 was thus probably related to the sole expression of the maternal alleles and suggested that the LQT1 variants may confer a sex-differential reproduction rate. Following this hypothesis, our objective was to determine whether the degree of ion channel dysfunction could influence preferential allele transmission, and this was clearly observed for LQT1 variants. We found a less pronounced distortion for the heterozygous variants recognized to be associated with the mildest dysfunction. This subgroup consisted of variants leading to haploinsufficiency such as nonsense, frameshift, and splice variants. Conversely, we found a maternal transmission rate of 67% for the dominant negative variants, which are associated with the most severe channel dysfunction, compared with 58% for non-dominant negative variants.[8]

Thus, the degree of transmission distortion in LQTS may vary among populations depending on variant type. This may explain the absence of significant female predominance/transmission distortion recently reported in a Norwegian population where most of the identified LQT1 variants led to haploinsufficiency and a moderate channel dysfunction.[23]

Nevertheless, there is no obvious link between the increased maternal transmission and the female predominance, these two observations may result from different mechanisms involving oocytes, spermatozoids, or other maternal tissues. As *KCNQ1* is expressed in numerous organs and in different types of cells, variants that alter myocardial repolarization might also have important effects in other tissues and at early stages of development such as fertilization in which ion channels are crucially important.[24]

Differential co-assembly of *KCNQ1* with single transmembrane *KCNE* β-subunits in different cell types gives rise to a variety of biophysical properties, thus endowing distinct physiological roles for *KCNQ1–KCNEx* complexes.[6,20,25] Some of them are well-known. In the heart, *KCNQ1* co-assembles with *KCNE1*, the major cardiac β-subunit, to form the slowly activating K+ channel sensitive to adrenergic regulation.[8,26] In contrast, co-assembly of *KCNQ1* with *KCNE3* produces a current with nearly instantaneous activation.[27] In colonic crypts, these *KCNQ1:KCNE3* channels mediate basolateral K^+ recycling required for Cl^- secretion and their expression is regulated by estrogens.[28] In the stomach, *KCNQ1:KCNE2* regulates acid secretion.[29] Interestingly, *KCNQ1* is also expressed in porcine and human granulosa cells, as well as in large follicles where it could have a role in follicular maturation.[5,30] In the ovaries, acetylcholine elevates intracellular calcium levels, thereby opening a calcium-activated potassium channel (BKCa), an action resulting in membrane hyperpolarization.[31] This latter event could allow activation of other voltage-dependent ion channels, which are linked to steroid production.[32] Besides, *KCNQ1* is regulated by acetylcholine, a novel intraovarian signaling molecule.[5] In cultured granulosa cells, inhibition of K^+ channels by a *KCNQ* channel blocker, XE991, inhibited gonadotropin-stimulated steroid production and increased cell proliferation.[5] This suggests a crucial role for *KCNQ* channels in the follicle. The effect of a *KCNQ1*-specific blocker has not been studied so far; however, if it also increases granulosa cell proliferation, this may contribute to the maturation of oocytes carrying a LQT1 variant, and thus the maternal transmission and the observed TRD in favor of carriers in the families.

Classification of *KCNH2* variants based on dysfunction remains difficult, as many missense variants affect to a variable level channel trafficking and expression when others impair biophysical properties.[33,34] We found more maternal than paternal transmission of LQT2 variants but the differences were smaller than for LQT1 variants. The ratios were of 0.58 for missense variants and 0.54 for variants leading to haploinsufficiency, and a similar distortion was observed for missense variants located in the pore region of the protein (S5-pore-S6) compared with other missense variants. The *KCNH2* channels are developmentally regulated and may also have a role in early mouse embryogenic development.[35]

It is noteworthy that although we postulate that the loss-of-function of the cardiac potassium channels could induce transmission distortion and contribute to female predominance in LQTS, a marked male predominance, up to 80%, has been observed among patients with a short QT syndrome.[36,37] This syndrome is caused by gain-of-function variants in the same channels where loss-of-function variants lead to LQTS, but only in a limited number of cases. It is tempting to suggest a possible role for channel dysfunction in this male predominance, but this must be tempered by the fact that most of the genetic background of this syndrome has not been elucidated.

Further studies on larger cohorts are needed to determine whether gain- or loss-of-function *SCN5A* variants could also influence parental transmission and to elucidate the origin of the preferential maternal transmission of *KCNQ1* and *KCNH2* variants.

Study limitations

This study is a retrospective study and has several limitations. The variability in size of the families could be a limitation. Large families were collected to study allele transmission across three generations, but small families were also included, especially those from Japan. It is interesting to note that female predominance among carriers and preferential maternal transmission were both observed in this Asian population for LQT1 and LQT2. Most of the LQT3 families, generating a smaller cohort than for LQT1 and LQT2, were from the Netherlands and harbored the same founder variant, c.5385_5387dup (p.(Tyr1795_Glu1796insAsp)). The Netherlands LQT1 and LQT2 cohorts were too small to show true differences from those of the other countries.

CONCLUSIONS

From our multi-national cohort of LQTS families, there was an excess of grandmaternal and maternal variant alleles with the strongest effect

for LQT1. We favor the hypothesis that the dysfunction of potassium channels could have a major role in preferential maternal transmission in LQTS.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We are grateful to Dr Graham Macdonald and late Professor Josué Feingold for helpful discussions; Dr Megumi Fukuyama for the collection of Japanese LQTS families; Dr Sven Zumhagen and Dr Birgit Stallmeyer for support in German LQTS family sampling; and Dr Rachel Peat for article proofing. ES-B was supported by the Fondation Leducq, Paris, and the DFG (Schu1082/3-1 and 3-2) and the Interdisciplinary Center for Clinical Research (IZKF; Schu1/011/12) of the Medical Faculty of the University of Münster. This work is also supported by a Grant-in-Aid for Scientific Research from JSPS (HI).

1 Cerrone M, Priori SG: Genetics of sudden death: focus on inherited channelopathies. *Eur Heart J* 2011; **32**: 2109–2118.
2 Imboden M, Swan H, Denjoy I *et al*: Female predominance and transmission distortion in the long-QT syndrome. *N Engl J Med* 2006; **355**: 2744–2751.
3 Naumova AK, Greenwood CM, Morgan K: Imprinting and deviation from Mendelian transmission ratios. *Genome* 2001; **44**: 311–320.
4 Naumova AK: Long-QT syndrome. *N Engl J Med* 2007; **356**: 1680, author reply 1680.
5 Kunz L, Roggors C, Mayerhofer A: Ovarian acetylcholine and ovarian KCNQ channels: insights into cellular regulatory systems of steroidogenic granulosa cells. *Life Sci* 2007; **80**: 2195–2198.
6 Mistry HD, McCallum LA, Kurlak LO, Greenwood IA, Broughton Pipkin F, Tribe RM: Novel expression and regulation of voltage-dependent potassium channels in placentas from women with preeclampsia. *Hypertension* 2011; **58**: 497–504.
7 Luo Y, Kumar P, Mendelson CR: Estrogen-related receptor γ (ERRγ) regulates oxygen-dependent expression of voltage-gated potassium (K+) channels and tissue kallikrein during human trophoblast differentiation. *Mol Endocrinol* 2013; **27**: 940–952.
8 Moss AJ, Shimizu W, Wilde AA *et al*: Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. *Circulation* 2007; **115**: 2481–2489.
9 January CT, Gong Q, Zhou Z: Long QT syndrome: cellular basis and arrhythmia mechanism in LQT2. *J Cardiovasc Electrophysiol* 2000; **11**: 1413–1418.
10 Dean NL, Loredo-Osti JC, Fujiwara TM *et al*: Transmission ratio distortion in the myotonic dystrophy locus in human preimplantation embryos. *Eur J Hum Genet* 2006; **14**: 299–306.
11 Liu Y, Zhang L, Xu S, Hu L, Hurst LD, Kong X: Identification of two maternal transmission ratio distortion loci in pedigrees of the Framingham heart study. *Sci Rep* 2013; **3**: 2147.
12 Eaves IA, Bennett ST, Forster P *et al*: Transmission ratio distortion at the INS-IGF2 VNTR. *Nat Genet* 1999; **22**: 324–325.
13 Sauer AJ, Moss AJ, McNitt S *et al*: Long QT syndrome in adults. *J Am Coll Cardiol* 2007; **49**: 329–337.
14 Conrath CE, Wilde AA, Jongbloed RJ *et al*: Gender differences in the long QT syndrome: effects of beta-adrenoceptor blockade. *Cardiovasc Res* 2002; **53**: 770–776.
15 Locati EH, Zareba W, Moss AJ *et al*: Age- and sex-related differences in clinical manifestations in patients with congenital long-QT syndrome: findings from the International LQTS Registry. *Circulation* 1998; **97**: 2237–2244.
16 Crotti L, Tester DJ, White WM *et al*: Long QT syndrome-associated mutations in intrauterine fetal death. *JAMA* 2013; **309**: 1473–1482.
17 Edmonds DK, Lindsay KS, Miller JF, Williamson E, Wood PJ: Early embryonic mortality in women. *Fertil Steril* 1982; **38**: 447–453.
18 Rushton DI SJ, Jenkins DM, Goodhart CB: Letter: Where have all the conceptions gone? *Lancet* 1975; **1**: 636–637.
19 Huang LO, Labbe A, Infante-Rivard C: Transmission ratio distortion: review of concept and implications for genetic association studies. *Hum Genet* 2013; **132**: 245–263.
20 Gould TD, Pfeifer K: Imprinting of mouse Kvlqt1 is developmentally regulated. *Hum Mol Genet* 1998; **7**: 483–487.
21 Lee MP, Hu RJ, Johnson LA, Feinberg AP: Human KVLQT1 gene shows tissue-specific imprinting and encompasses Beckwith-Wiedemann syndrome chromosomal rearrangements. *Nat Genet* 1997; **15**: 181–185.
22 Li C: *Human Genetics: Principles and Methods*. McGraw-Hill: New York, 1961.
23 Berge KE, Haugaa KH, Früh A *et al*: Molecular genetic analysis of long QT syndrome in Norway indicating a high prevalence of heterozygous mutation carriers. *Scand J Clin Lab Invest* 2008; **68**: 362–368.
24 Shukla KK, Mahdi AA, Rajender S: Ion channels in sperm physiology and male fertility and infertility. *J Androl* 2012; **33**: 777–788.
25 Lundquist AL, Turner CL, Ballester LY, George AL: Expression and transcriptional control of human KCNE genes. *Genomics* 2006; **87**: 119–128.
26 Goldenberg I, Thottathil P, Lopes CM *et al*: Trigger-specific ion-channel mechanisms, risk factors, and response to therapy in type 1 long QT syndrome. *Heart Rhythm* 2012; **9**: 49–56.
27 Schroeder BC, Waldegger S, Fehr S *et al*: A constitutively open potassium channel formed by KCNQ1 and KCNE3. *Nature* 2000; **403**: 196–199.
28 Alzamora R, O'Mahony F, Bustos V *et al*: Sexual dimorphism and oestrogen regulation of KCNE3 expression modulates the functional properties of KCNQ1 K^+ channels. *J Physiol* 2011; **589**: 5091–5107.
29 Grahammer F, Herling AW, Lang HJ *et al*: The cardiac K+ channel KCNQ1 is essential for gastric acid secretion. *Gastroenterology* 2001; **120**: 1363–1371.
30 Mason DE, Mitchell KE, Li Y, Finley MR, Freeman LC: Molecular basis of voltage-dependent potassium currents in porcine granulosa cells. *Mol Pharmacol* 2002; **61**: 201–213.
31 Kunz L, Thalhammer A, Berg FD *et al*: Ca2+-activated, large conductance K+ channel in the ovary: identification, characterization, and functional involvement in steroidogenesis. *J Clin Endocrinol Metab* 2002; **87**: 5566–5574.
32 Kunz L, Richter JS, Mayerhofer A: The adenosine 5'-triphosphate-sensitive potassium channel in endocrine cells of the human ovary: role in membrane potential generation and steroidogenesis. *J Clin Endocrinol Metab* 2006; **91**: 1950–1955.
33 Shimizu W, Moss AJ, Wilde AA *et al*: Genotype-phenotype aspects of type 2 long QT syndrome. *J Am Coll Cardiol* 2009; **54**: 2052–2062.
34 Anderson CL, Delisle BP, Anson BD *et al*: Most LQT2 mutations reduce Kv11.1 (hERG) current by a class 2 (trafficking-deficient) mechanism. *Circulation* 2006; **113**: 365–373.
35 Winston NJ, Johnson MH, McConnell JM, Cook DI, Day ML: Expression and role of the ether-à-go-go-related (MERG1A) potassium-channel protein during preimplantation mouse development. *Biol Reprod* 2004; **70**: 1070–1079.
36 Giustetto C, Schimpf R, Mazzanti A *et al*: Long-term follow-up of patients with short QT syndrome. *J Am Coll Cardiol* 2011; **58**: 587–595.
37 Mazzanti A, Kanthan A, Monteforte N *et al*: Novel insight into the natural history of short QT syndrome. *J Am Coll Cardiol* 2014; **63**: 1300–1308.

Supplementary Information accompanies this paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)

Journal of Human Genetics (2016) 61, 3–4

www.nature.com/jhg

COMMENTARY

Atrial fibrillation: an inherited cardiovascular disease—a commentary on genetics of atrial fibrillation: from families to genomes

Hayato Tada, Masa-aki Kawashiri, Masakazu Yamagishi and Kenshi Hayashi

Journal of Human Genetics (2016) **61**, 3–4; doi:10.1038/jhg.2015.63; published online 11 June 2015

ATRIAL FIBRILLATION AS AN INHERITED CARDIOVASCULAR DISEASE

The fraction of the risk for atrial fibrillation (AF) attributable to established factors (hypertension, smoking, obesity, diabetes mellitus, age, male sex and heart disease) is roughly 50% with hypertension being the most prominent modifiable risk factor.[1] On the other hand, it has been shown that at least 5% of all patients with AF and 15% of those with only AF but without established clinical risk factors for AF had a positive history of AF.[2] These facts have motivated researchers to investigate the risk of AF among those with documented familial AF, as well as genome-wide association studies (GWASs) that have screened for common single-nucleotide polymorphisms (SNPs) associated with AF.[3] In this issue of the journal, Christophersen *et al.*[4] have nicely summarized the current understanding of genetics in AF from rare variants to common variants. We acknowledge that AF is heritable; however, most cardiologists do not routinely collect information on family history of AF, nor do they use this information for decision-making in clinical practice due to the lack of evidence. Thus, future clinical trials are needed to verify the clinical utility of family history information of AF beyond established risk factors in the first place. The next step could be genotype–phenotype association studies regardless of allele frequency. Finally, one of the possible tentative goals of genetics in AF could be to develop a risk calculator for AF based on human variations. Such approaches have already been applied in AF. For example, an AF genetic risk score comprising 12 common genetic variations was as powerful as hypertension to estimate AF events.[5] However, further studies with larger samples, and with multiple ethical perspectives, are needed to be accepted as a common clinical practice to investigate such genetic risk scores. Another possible goal could be to find novel molecules as therapeutic targets, as has been shown with lipids. In this regard, whole-exome sequencing (WES) has emerged as a promising tool for gene discovery in families with suspected monogenic (or polygenic) disorders, with a success rate exceeding 20%.[6] In general, hundreds or thousands of variants are found in an individual through the WES approach.[7] Then, typically, the variants predicted as benign, common and unmatched assuming co-segregation should be excluded. In this process, great advances have been made in the fields of *in silico* variant annotation prediction and in the information of allele frequency of a certain variant based on huge collaborative efforts in exome-sequenced data sets publicly available.

AF AS AN ION CHANNEL DISEASE

Since the first gene (*KCNQ1*) responsible for familial AF was identified in 2003, many variants in genes encoding ion channel subunits, cardiac gap junctions and signaling molecules have been identified in monogenic AF families. These genetic variants predispose individuals to AF by enhanced or delayed atrial action potential repolarization, conduction velocity heterogeneity, cellular hyperexcitability and hormonal modulation of atrial electrophysiology.[8] Once a susceptibility variant is identified, it is important to identify the mechanistic links between the variant and disease expression. The

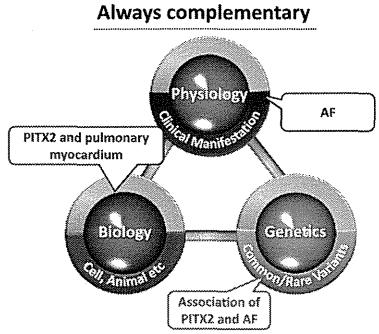

Figure 1 Schema of physiology, biology and genetics. Investigations of physiology, biology and genetics are always complementary, and have contributed to our understanding of AF. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

H Tada, M Kawashiri, M Yamagishi and K Hayashi are at Division of Cardiovascular Medicine, Kanazawa University Graduate School of Medicine, Kanazawa, Japan
E-mail: ht240z@sa3.so-net.ne.jp

functional role of ion channel gene variants in AF can be usually assessed by a cellular electrophysiological study using a heterologous expression system. In addition, elucidating the molecular mechanisms of AF may allow a mechanism-based approach for treatment to be developed. For example, patients with gain-of-function ion channel variants are likely to benefit from a drug that selectively blocks mutant channel complexes. WES applied to familial AF kindreds or lone AF patients will identify many rare variants of different types of genes, and the true causal allele should be identified from the large number of insignificant alleles. To link these variants to a clinical phenotype, a family study, as well as functional evaluation, is required using a model system including zebrafish, mice or patient-specific induced pluripotent stem cells.

PHYSIOLOGY, BIOLOGY AND GENETICS OF AF

In 1998, Häissagurre *et al.*[9] demonstrated that pulmonary veins are an important source of ectopic beats, initiating frequent paroxysms of AF. After this report, myocardial sleeve, in which paired-like homeodomain transcription factor 2 (*PITX2*) has a key role in its formation, has been shown to initiate AF. In 2009, a GWAS identified a strong association between SNPs in the *PITX2* gene and AF.[10] Each of these investigations contributed complementarily to our understanding of AF (Figure 1).

CONCLUSION

Given the development of novel genotyping and sequencing technologies, as well as extensive catalogs of human genetic variation together with functional analyses, cardiologists are now realizing that AF is highly heritable. Rigorous efforts are currently underway, and it is highly likely that these efforts could reveal the contribution of rare and common variants to the overall genetic architecture of AF in the next few years.

1 Magnani, J. W., Rienstra, M., Lin, H., Sinner, M. F., Lubitz, S. A., McManus, D. D. *et al.* Atrial fibrillation: current knowledge and future directions in epidemiology and genomics. *Circulation* **124**, 1982–1993 (2011).
2 Darbar, D., Herron, K. J., Ballew, J. D., Jahangir, A., Gersh, B. J., Shen, W. K. *et al.* Familial atrial fibrillation is a genetically heterogeneous disorder. *J. Am. Coll. Cardiol.* **41**, 2185–2192 (2003).
3 Ellinor, P. T., Lunetta, K. L., Albert, C. M., Glazer, N. L., Ritchie, M. D., Smith, A. V. *et al.* Meta-analysis identifies six new susceptibility loci for atrial fibrillation. *Nat. Genet.* **44**, 670–675 (2012).
4 Christophersen, I. E. & Ellinor, P. T. Genetics of atrial fibrillation: from families to genomes. *J Hum Genet.* **61**, 61–70 (2016).
5 Tada, H., Shiffman, D., Smith, J. G., Sjögren, M., Lubitz, S. A., Ellinor, P. T. *et al.* Twelve-single nucleotide polymorphism genetic risk score identifies individuals at increased risk for future atrial fibrillation and stroke. *Stroke* **45**, 2856–2862 (2014).
6 Stitziel, N. O., Peloso, G. M., Abifadel, M., Cefalù, A. B., Fouchier, S., Motazacker, M. M. *et al.* Exome sequencing in suspected monogenic dyslipidemias. *Circ. Cardiovasc. Genet.* **8**, 343–350 (2015).
7 Tada, H., Kawashiri, M. A., Nohara, A., Saito, R., Tanaka, Y., Nomura, A. *et al.* Whole exome sequencing combined with integrated variant annotation prediction identifies asymptomatic Tangier disease with compound heterozygous mutations in ABCA1 gene. *Atherosclerosis* **240**, 324–329 (2015).
8 Roberts, J. D. & Gollob, M. H. A contemporary review on the genetic basis of atrial fibrillation. *Methodist DeBakey Cardiovasc. J.* **10**, 18–24 (2014).
9 Haïssaguerre, M., Jaïs, P., Shah, D. C., Takahashi, A., Hocini, M., Quiniou, G. *et al.* Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. *N. Engl. J. Med.* **339**, 659–666 (1998).
10 Kääb, S., Darbar, D., van Noord, C., Dupuis, J., Pfeufer, A., Newton-Cheh, C. *et al.* Large scale replication and meta-analysis of variants on chromosome 4q25 associated with atrial fibrillation. *Eur. Heart J.* **30**, 813–819 (2009).

Cardiac Innervation and Sudden Cardiac Death

Keiichi Fukuda, Hideaki Kanazawa, Yoshiyasu Aizawa, Jeffrey L. Ardell, Kalyanam Shivkumar

Abstract: **Afferent and efferent cardiac neurotransmission via the cardiac nerves intricately modulates nearly all physiological functions of the heart (chronotropy, dromotropy, lusitropy, and inotropy). Afferent information from the heart is transmitted to higher levels of the nervous system for processing (intrinsic cardiac nervous system, extracardiac-intrathoracic ganglia, spinal cord, brain stem, and higher centers), which ultimately results in efferent cardiomotor neural impulses (via the sympathetic and parasympathetic nerves). This system forms interacting feedback loops that provide physiological stability for maintaining normal rhythm and life-sustaining circulation. This system also ensures that there is fine-tuned regulation of sympathetic–parasympathetic balance in the heart under normal and stressed states in the short (beat to beat), intermediate (minutes to hours), and long term (days to years). This important neurovisceral/autonomic nervous system also plays a major role in the pathophysiology and progression of heart disease, including heart failure and arrhythmias leading to sudden cardiac death. Transdifferentiation of neurons in heart failure, functional denervation, cardiac and extracardiac neural remodeling has also been identified and characterized during the progression of disease. Recent advances in understanding the cellular and molecular processes governing innervation and the functional control of the myocardium in health and disease provide a rational mechanistic basis for the development of neuraxial therapies for preventing sudden cardiac death and other arrhythmias. Advances in cellular, molecular, and bioengineering realms have underscored the emergence of this area as an important avenue of scientific inquiry and therapeutic intervention. (*Circ Res*. 2015;116:2005-2019. DOI: 10.1161/CIRCRESAHA.116.304679.)**

Key Words: arrhythmias, cardiac ■ autonomic nervous system ■ death, sudden cardiac ■ physiopathology

Cardiac autonomic dysregulation is central to the development and progression of most cardiovascular diseases (hypertension, heart failure [HF], arrhythmias, and myocardial infarction). Impaired cardiac parasympathetic responsiveness and enhanced sympathetic activity are negative prognostic indicators for both morbidity and mortality associated with

Original received October 7, 2014; revision received December 1, 2014; accepted December 11, 2014. In April 2015, the average time from submission to first decision for all original research papers submitted to *Circulation Research* was 13.84 days.

From the Department of Cardiology, Keio University School of Medicine, Tokyo, Japan (K.F., H.K., Y.A.); and UCLA Cardiac Arrhythmia Center, Neurocardiology Research Center of Excellence (J.L.A., K.S.).

Correspondence to Keiichi Fukuda, MD, PhD, Department of Cardiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail kfukuda@a2.keio.jp; or Kalyanam Shivkumar, MD, PhD, UCLA Cardiac Arrhythmia Center, David Geffen School of Medicine at UCLA, 100 UCLA Medical Plaza, Suite 660, Los Angeles, CA. E-mail kshivkumar@mednet.ucla.edu

Circulation Research **is available at http://circres.ahajournals.org** **DOI: 10.1161/CIRCRESAHA.116.304679**

Nonstandard Abbreviations and Acronyms	
AF	atrial fibrillation
ANS	autonomic nervous system
BBs	β-blockers
BRS	baroreflex sensitivity
BS	Brugada syndrome
CHF	congestive heart failure
DM	diabetes mellitus
HF	heart failure
IVF	idiopathic ventricular fibrillation
LQT	long QT
NGF	nerve growth factor
RDN	renal denervation
RyR2	ryanodine receptor 2
SCD	sudden cardiac death
Sema3A	semaphorin 3A
SemaTG	transgenic mouse expressing semaphorin
TWA	T wave alternans
VT	ventricular tachycardia
VF	ventricular fibrillation

arrhythmias and sudden death.[1,2] The autonomic nervous system (ANS) plays a major role in the pathophysiology of arrhythmias leading to sudden cardiac death (SCD), and neuraxial modulation is emerging as an important avenue of scientific inquiry and therapeutic intervention.[3,4] Mechanism-based autonomic regulation therapy holds promise to treat both arrhythmias and HF. Improved basic scientific understanding can result in innovative low-cost therapeutic options, with a global impact, that can not only prevent death but also favorably alter the course of the underlying disease. The ANS intricately regulates cardiac excitability and contractile function. Cardiac afferents provide beat-to-beat sensory information of cardiac muscle activity to the neuraxis, additional information is conveyed by extracardiac circulatory receptors (Figures 1 and 2). The processing of this afferent information at several levels (intrinsic cardiac nervous system, extracardiac-intrathoracic ganglia, spinal cord, brain stem, and higher centers) provides an elegant mechanism for interacting feedback loops to provide physiological stability for maintaining normal rhythm and life-sustaining circulation. These nested feedback loops ensure that there is fine-tuned regulation of efferent (sympathetic and parasympathetic cardiomotor) neural signals to the heart in normal and stressed states. Concepts on cardiac neural control have been revised in recent years based on new physiological data from multiple studies that together provide an elegant framework for understanding regulatory control of the mammalian heart (Figure 2). Direct single neuron and neural network recordings from intrinsic cardiac and extracardiac ganglia provide the methods to study organ level physiology[5–7] and a proper framework of interpretation of the neural control-myocyte interface.

Pathophysiology

Cardiac injury (eg, infarction, focal inflammation) results in the formation of a scar at the level of the organ and likewise alters the integrative regulation of the heart.[4,8] The changes at the level of the organ result in slowed and altered paths of myocardial electric propagation, which together creates the substrate for reentrant arrhythmias. The systemic effects of this scar are characterized by afferent-mediated activation of the neuroendocrine system, primarily sympathoexcitation in conjunction with withdrawal of central parasympathetic tone, which provides short-term benefits to maintain cardiac output, but at a cost.[9] The recovery from acute injury is characterized by a state wherein there is continued abnormal cardiac afferent signaling (cardiocentric afferents).[10] Mechanistically, such dysregulation reflects reactive and adaptive responses of the cardiac neural hierarchy leading to excessive neuronal interactive excitability and network interconnectivity from the intrinsic cardiac nervous system up to and including the insular cortex.[7] This reorganization ultimately leads to conflict between central and peripheral aspects of the hierarchy. This

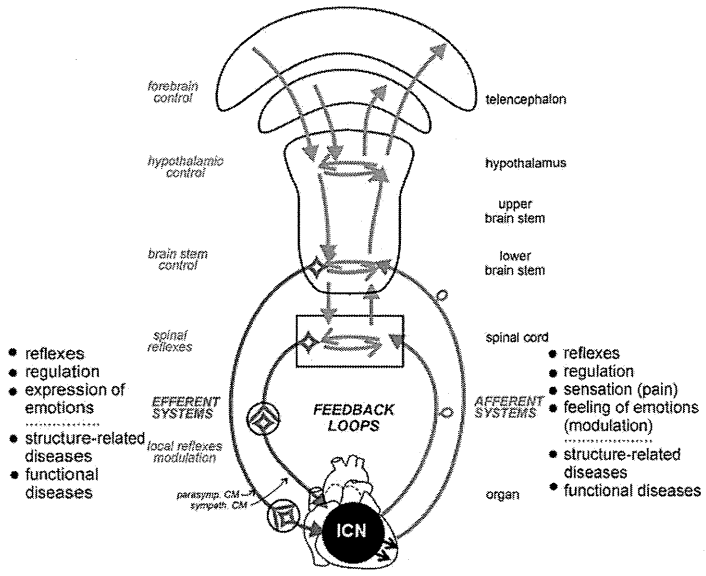

Figure 1 . Cardiac neurotransmission. Adapted from Jänig[143] with permission of the publisher. Copyright ©2014, Elsevier. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation. CM indicates cardiomotor; and ICN, intrinsic nervous system of the heart.

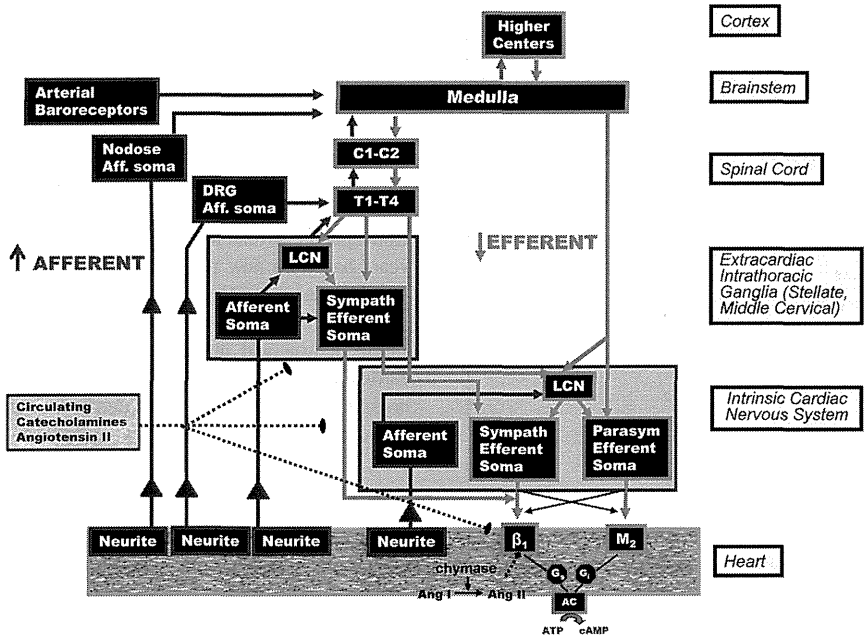

Figure 2. Neurohumoral control and anatomic organization of cardiac innervation. AC indicates adenylate cyclase; Ang, angiotensin; Aff, afferent; C, cervical; DRG, dorsal root ganglion; LCN, local circuit neuron; Parasym, parasympathetic; Sympath, sympathetic; and T, thoracic.

leads to a maladaptive response of excessive sympathoexcitation contributing to the evolution of cardiac disease and fatal arrhythmias.

Cortical and subcortical control of the heart can be demonstrated experimentally in animal models[11] and in humans[12,13] and has been implicated in arrhythmias.[14,15] The efficacy of autonomic regulation therapy such as cardiac afferent denervation after myocardial infarction[16] and sympathectomy to treat ventricular tachycardia (VT) storm could be because of a reduction of these conflicts between levels of the cardiac nervous system.[17,18] In these settings, it is likely that the intrinsic nervous system of the heart is able to provide the neural coordination and ensure electric stability without the interference of central input. The electric stability of the transplanted heart is a clear manifestation of this principle.[19]

Determination of Cardiac Innervation Patterning by Both Neural Chemoattractants and Chemorepellents in the Heart

The heart is abundantly innervated and its performance is tightly controlled by both sympathetic and parasympathetic efferent nerves (Figure 2). Cardiac innervation density including sensory nerves is altered in diseased hearts, which can lead to unbalanced neural activation and lethal arrhythmias. In this section, we focus on the regulatory mechanisms controlling cardiac innervation and the critical roles of these processes on cardiac performance (Figure 3).

Semaphorin 3A Reduces Arrhythmia Potential Through Modulation of Sympathetic Innervation Patterning

Semaphorin 3A (sema3A) is a class 3–secreted semaphorin that has been cloned and identified as a potent neural chemorepellent and a directional guidance molecule for nerve fibers.[20] Initially, we analyzed the kinetics and distribution of cardiac sympathetic innervation in the developing mouse ventricles.[21]

By analyzing sema3A knocked-in lacZ mice (transgenic mouse expressing semaphorin [SemaTG]), we found that sema3A is strongly expressed in the developing heart at embryonic day 12 with sema3A expression in the subendocardium, but not subepicardium, of the atria and ventricles. SemaTG mice have reduced sympathetic innervation and attenuation of the epicardial to endocardial innervation gradient.[21] The expression of sema3A in developing hearts revealed a linear decrease from embryonic day 12 that corresponded with an increase in sympathetic innervation density. Thus, the spatial and temporal expression pattern of sema3A directly mirrors the patterning of sympathetic innervation in developing hearts. These results indicate that sema3A is a negative regulator of cardiac innervation. We also analyzed sema3A-deficient mice and found that the sympathetic nerve density is lower in the subepicardium and higher in the subendocardium. As such, these changes resulted in disruption of the innervation gradient in the ventricles. Overall, these results indicate that cardiomyocyte-derived sema3A plays a critical role in cardiac sympathetic innervation by inhibiting neural growth.

Most sema3A-deficient mice died within the first postnatal week because of sinus bradycardia and abrupt sinus arrest. By comparison, SemaTG mice died suddenly without any symptoms at 10 months of age. Sustained ventricular tachyarrhythmia was induced in SemaTG mice, but not in wild-type mice, after epinephrine administration. Programmed electric stimulation also revealed that SemaTG mice were highly susceptible to ventricular tachyarrhythmias.

Together, these data indicate that the highly organized innervation patterning mediated by sema3A is critical for the

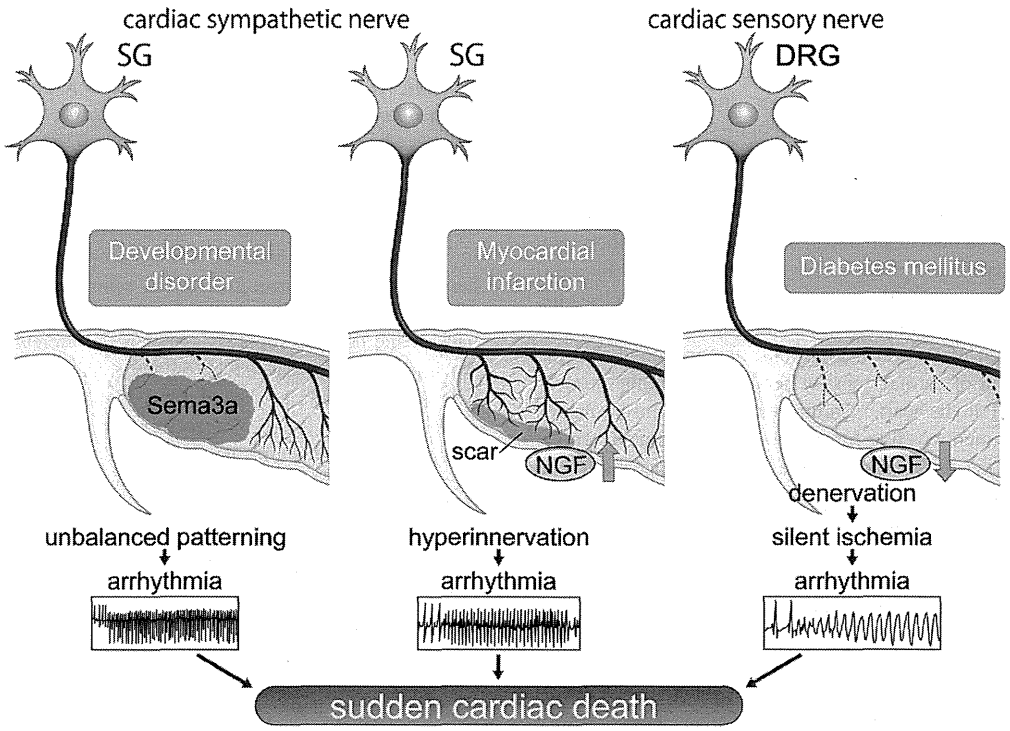

Figure 3. Regulation of cardiac innervation patterning and sudden cardiac death (SCD). Left, Overexpression or lack of semaphorin 3A (sema3A) in endocardium causes unbalanced patterning of sympathetic nerves, which alters the potential for lethal arrhythmia. Appropriate sema3A-mediated sympathetic innervation is crucial for maintenance of arrhythmia-free heart. **Middle**, Upregulation of secreted nerve growth factor (NGF) from cardiomyocytes in diseased heart may cause lethal arrhythmia and SCD. **Right**, Downregulation of NGF in diabetic heart induces denervation of cardiac sensory nerve, which leads to silent ischemia and lethal arrhythmia. SG indicates stellate ganglia. Details of these pathways are referenced in the text.[21,36,41]

maintenance of arrhythmia-resistant hearts. From the clinical perspective, consistent with our data, Stramba-Badiale et al[22] report that developmental abnormalities in cardiac innervation may play a role in the genesis of some cases of sudden infant death syndrome. In a recent study of unexplained cardiac arrest, Nakano et al[23] demonstrated that a polymorphism of SEMA3A(I334V) diminishes the cardiac sympathetic innervation gradient and partially contributes to the pathogenesis of SCD with ventricular fibrillation (VF). These findings are important in elucidating the pathogenesis of cardiac sudden death and indicate the dynamic synergism between neural and cardiac development in control of cardiac electric stability.

Nerve Growth Factor Upregulation Causes Nerve Sprouting and SCD

Sympathetic activation is important in the genesis of SCD in diseased hearts. It has been known for decades that β-blocker (BB) therapy prevents SCD secondary to VT in ischemic heart disease or congestive HF (CHF). It is further recognized that BBs exert this effect by targeting both cardiac myocytes and elements of the cardiac nervous system.[24–26]

Nerve growth factor (NGF) is a prototypic member of the neurotrophin family, the members of which are critical for the differentiation, survival, and synaptic activity of the peripheral sympathetic and sensory nervous systems.[27] The level of NGF expression within innervated tissue corresponds approximately to innervation density. Previous studies show that NGF expression increases during development and is altered in diseased hearts.[28,29] Zhou et al[30] showed that NGF, which is critical for sympathetic nerve sprouting, is upregulated after myocardial infarction in animal models, resulting in the regeneration of cardiac sympathetic nerves and heterogeneous innervation.

It has also been reported that NGF is upregulated in cardiac hypertrophy, leading to sympathetic hyperinnervation.[31] In addition, Cao et al[28] reported that NGF infusion after MI enhances myocardial nerve sprouting and results in a dramatic increase in SCD and a high incidence of ventricular tachyarrhythmias. Chen et al[32] have shown that overexpression of sema3A in the MI border zone could reduce the inducibility of ventricular arrhythmias by reducing sympathetic hyperinnervation after infarction. These results demonstrate that NGF-induced augmentation of sympathetic nerve sprouting in diseased hearts can lead to lethal arrhythmias and SCD.

NGF Downregulation Is Critical for Diabetic Neuropathy and Silent Myocardial Ischemia

Cardiac autonomic neuropathy is a frequent complication of diabetes mellitus (DM), and diabetic patients are at high risk for developing arrhythmias, silent myocardial ischemia, and SCD.[33]

The cardiac ANS is composed of efferent and afferent nerves. In contrast to sympathetic innervation, little is known about sensory innervation and how it is altered in diseased hearts. A subset of the cardiac sensory innervation is responsible for pain perception. Activation of these nociceptive afferents results in multiple somatic and visceral responses during myocardial ischemia.[34] Cardiac sensory nerve impairment causes silent myocardial ischemia and this is a likely a major cause of sudden death in patients with DM.[35] Furthermore, there are data that indicate nerve sprouting induced by a potent stimulator of NGF after myocardial injury increases the incidence of ventricular tachyarrhythmias.[36]

A screen of several neurotrophic factors found that the development of cardiac sensory nerves parallels the production of NGF in the heart.[37] Cardiac nociceptive sensory nerves that are immunopositive for calcitonin gene–related peptide (including the dorsal root ganglia and the dorsal horn) are markedly retarded in NGF-deficient mice and rescued in mice overexpressing NGF specifically in the heart. Thus, NGF synthesis in the heart is critical for the development of the cardiac sensory innervation.[38]

To investigate whether NGF is involved in diabetic neuropathy, type I DM was induced with streptozotocin in wild-type and transgenic mice overexpressing NGF in the heart.[39] DM-induced wild-type mice show downregulation of NGF, calcitonin gene–related peptide–immunopositive cardiac sensory denervation and atrophic changes in the dorsal root ganglia. These defects are prevented in DM-induced NGF-transgenic mice. Cardiac sensory function, as measured by myocardial ischemia–induced c-Fos expression in the dorsal root ganglia, is also downregulated by DM in wild-type mice, but not by DM in NGF-transgenic mice. Direct gene transfer of NGF into diabetic rat hearts improves the impaired cardiac sensory innervation and function, as determined by the electrophysiological activity of cardiac afferent nerves during myocardial ischemia. These findings demonstrate that the development of the cardiac sensory nervous system depends on the synthesis of NGF in the heart, and that DM-induced suppression of NGF expression may lead to cardiac sensory neuropathy.[39] In human clinical trials of recombinant human NGF administered to diabetic patients with polyneuropathy, none of adverse events such as ventricular arrhythmias were reported.[40] However, a better understanding of the regulation of these pathways and precise studies on reliable and efficient methods of gene therapy and optimal dosage or route of administration are required for further clinical trials.

Abnormalities and Alteration of Cardiac Sympathetic Nerve Profile in HF

Recently, crosstalk, through various humoral factors, between cardiomyocyte and cardiac sympathetic nerves has been demonstrated. Axon growth, denervation, and functional alternation of sympathetic nerves have been noted in HF. Using molecular biological approaches, a new concept about the adaptation mechanism of the ANS in HF has been developed. With this understanding, new interventional therapies targeted for the ANS and based on a concept with multiple organ linkage have emerged. In this section, we focus on a framework to understand cardiac sympathetic nerve abnormalities in HF and implications for therapy of HF and SCD prevention strategies that target autonomic nerves (Figure 4).

Systemic Autonomic Nerve Dysfunction as Related to Central and Peripheral Neural Interactions

There is strong evidence that sympathetic efferent neuronal activity is increased in CHF.[42] Such sympathetic activation in HF can also trigger malignant arrhythmias. One of the mechanisms proposed to explain sympathetic activation in HF involves abnormalities in baroreceptors. Signals from baroreceptors are transmitted to the central nervous system via afferent nerves, and after central processing is transduced back to the heart to suppress sympathetic efferent activity.[43] An impairment of carotid baroreflex sensitivity (BRS) has been shown to be a marker of the risk of mortality or a cardiovascular event in HF.[44,45] Baroreceptor activation has been thought to confer benefits in prevention of SCD which is prevalent in patients with HF.[46] The HOPE4HF (Health Outcomes Prospective Evaluation for Heart Failure With EF ≥ 40%) trial (ClinicalTrials.gov Identifier: NCT00957073) is a prospective randomized trial, where patients will be randomized in a 2:1 ratio to receive baroreceptor activation therapy (device arm) or optimal medical therapy alone (medical arm).[45]

Mechanisms mediated by the chemoreceptor reflex that sense hypoxic and hypercapnic conditions could also be involved in sympathetic activation.[47] Recently, Del Rio et al[48] showed that carotid chemoreceptor ablation reduces cardiorespiratory dysfunction and improves survival during HF in rats. In addition, Niewiński et al[49] showed that surgical removal of the carotid body from a patient with systolic HF significantly decreased sympathetic tone.

Brain stem and suprabulbar regions of the central nervous system are critical elements for integrated cardiovascular control.[50] It is well established that the paraventricular nucleus of the hypothalamus and the rostral ventrolateral medulla are involved in the enhanced central sympathetic outflow in HF.[51] Reduced nitric oxide, increased oxidative stress, and activation of angiotensin II type 1 receptors in the rostral ventrolateral medulla all contribute to sympathetic drive.[52] Further oxidative stress can alter cardiac cholinergic control.[53] It is important to note that cardiac afferents are activated after cardiac injury and play a major role in cardiac dysfunction and remodeling. Wang et al,[16] using resiniferatoxin a potent analog of capsaicin to delete transient receptor potential vanilloid 1 receptor–expressing cardiac afferent nerves, have demonstrated attenuation of remodeling and fibrosis in a rat HF model.

Animal and human studies suggest that activation of both efferent and afferent renal nerves play a role in the pathogenesis and progression of disease states such as hypertension and CHF. Renal denervation (RDN), which is a novel catheter-based ablation therapy, interrupts efferent sympathetic and afferent renal sensory nerves. It is being studied as an option for patients with resistant hypertension and HF.[54] Physiological cardiovascular control involves afferent signals from the kidneys which are processed in the hypothalamus[55,56] as well as in the nucleus of the solitary tract, insular cortex, anterior cingulate cortex, and based on functional MRI studies in the infralimbic cortex.[57] Alterations in afferent input would

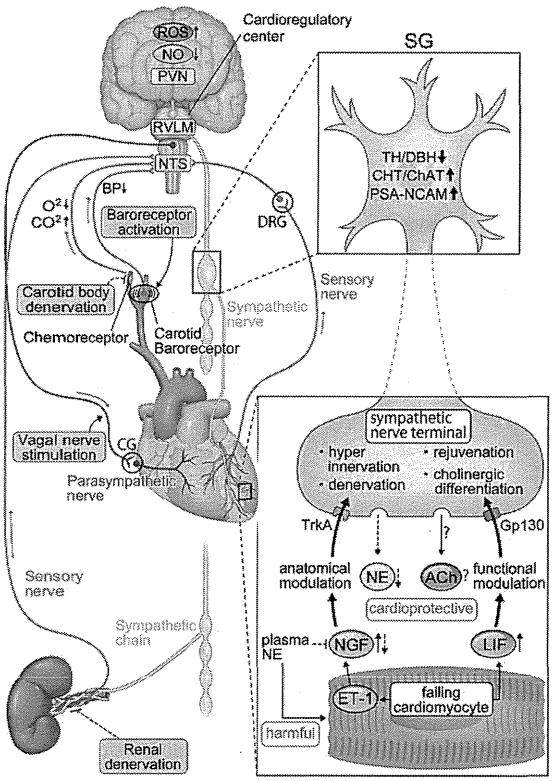

Figure 4. Systemic autonomic interactions and crosstalk between cardiomyocyte and sympathetic nerve terminal via humoral factors in diseased heart. This figure shows that central and peripheral mechanism of the heart and brain interaction including the cardiac autonomic efferent (sympathetic and parasympathetic) and afferent (sensory) nerves. Representative promising interventional therapies are also described in the figure. In addition, alteration of cardiac sympathetic nerves occurs in postganglionic fiber. Failing cardiomyocytes induces nerve growth factor (NGF) via endothelin-1 (ET-1)–mediated pathway and leukemia inhibitory factor (LIF). NGF and LIF lead to hyperinnervation (anatomic modulation) and rejuvenation/cholinergic differentiation (functional modulation), respectively. This phenomenon shows the expression of catecholaminergic markers such as tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) reduced and of cholinergic (choline transporter [CHT], choline acetyltransferase [ChAT]) and juvenile (polysialylated neural cell adhesion molecule [PSA-NCAM]) increased. Ach indicates acetylcholine; BP, blood pressure, CG, intrinsic cardiac ganglia; DRG, dorsal root ganglia; NE, norepinephrine; NTS, solitary tract; PVN, paraventricular nucleus; ROS, reactive oxygen species; RVLM, rostral ventrolateral medulla; SG, stellate ganglia; and TrkA, tropomyosin-related kinase A.

be expected to alter set-points and sensitivities for reflex control of blood pressure.

Treatment of drug-resistant hypertension was the initial therapeutic use of RDN. Both preclinical[58] and clinical trials[59–62] demonstrated decrease ambulatory blood pressure in medication refractory hypertension. However, the recently reported prospective Symplicity HTN-3 trial did not meet the expected antihypertensive end points[63] and was terminated early. Currently, RDN is being evaluated as a potential adjunctive therapy in a spectrum of sympathetically modulated cardiovascular diseases, including left ventricular hypertrophy and diastolic dysfunction,[64] CHF,[65] obstructive sleep apnea,[66] and atrial fibrillation (AF).[67] RDN has also been proposed as a possible treatment strategy in patients with recurrent ventricular arrhythmias.[68–70] Consequently, there are several ongoing clinical trials (Symplicity-HF and REACH [Renal Artery Denervation in Chronic Heart Failure Study]) investigating the safety and efficacy of RDN in patients with CHF and VT (Reset VT).[71]

Finally, considering its systemic relationship with the peripheral sympathetic nerves, the involvement of the central nervous system in sympathetic dysfunction is of considerable interest and further studies in this area are anticipated in the future.[52]

Crosstalk Between Cardiomyocyte and Cardiac Sympathetic Nerves Mediated by Humoral Factors

The pathology of HF involves various abnormalities in sympathetic nerve terminals. During the transition to overt HF, sympathetic neural tone is upregulated, and NGF expression is elevated contributing to hyperinnervation of cardiac sympathetic nerves.[31] However, there is a paradoxical reduction in norepinephrine synthesis concomitant with downregulation of tyrosine hydroxylase (TH), the rate-limiting enzyme in innervated neurons, norepinephrine reuptake into the sympathetic nerve terminals, and depression of norepinephrine levels in the myocardium.[31,72–74] This discrepancy between anatomic or functional integrity and the catecholaminergic properties of the cardiac sympathetic nervous system in HF is longstanding. However, the molecular mechanisms underlying the reduction in catecholaminergic characteristics of cardiac sympathetic nerve system in HF remain poorly understood (Figure 5).

CHF leads to upregulation of a range of growth factors and cytokines in the heart. Leukemia inhibitory factor and other members of the interleukin-6 family, which can induce fetal gene expression (so-called rejuvenation) in adult cardiomyocytes, are upregulated during CHF.[31] In the cardiac sympathetic nervous system in CHF, strong expression of growth-associated protein 43 and highly polysialylated neural cell adhesion molecule has been noted.[31] We also found that this neural rejuvenation can be induced by leukemia inhibitory factor expressed in hypertrophied hearts (unpublished data). These observations are supported by several other studies, which showed that leukemia inhibitory factor changes the neuropeptide phenotype[75,76] or increases the degradation of tyrosine hydroxylase through the ubiquitin-proteasome system.[77] Taken together, these results suggests that cardiac sympathetic nervous system dysfunction is accompanied by neuronal rejuvenation, the so-called functional denervation because of rejuvenation mechanism.[31]

Cardiac sympathetic properties are also altered in CHF as mediated by changes in cardiac-derived humoral factors. In a HF model using Dahl salt-sensitive rats and in autopsy specimens from patients with HF, decreased density of tyrosine hydroxylase–positive neurons was seen.[78] Furthermore, many neurons in the stellate ganglia and left ventricle also expressed parasympathetic markers such as choline transporter

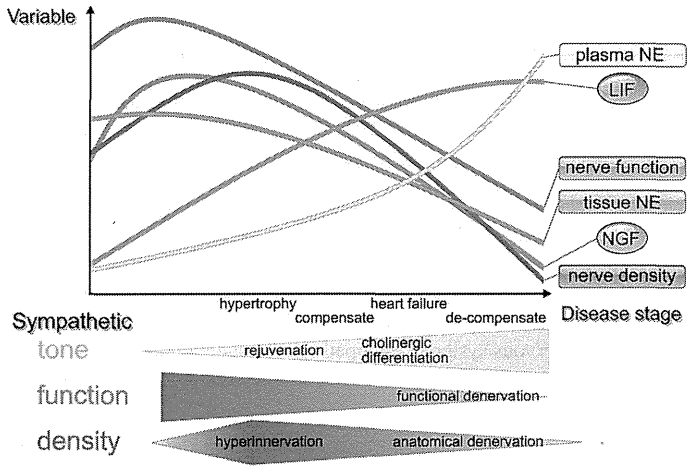

Figure 5. Temporal changes in cardiac innervation with disease progression. NE indicates norepinephrine; NGF, nerve growth factor; and LIF, leukemia inhibitory factor.

and choline acetyltransferase. This was thought to represent cholinergic transdifferentiation of cardiac adrenergic neurons into cholinergic neurons, induced by leukemia inhibitory factor via a gp130 signaling pathway.[78] The diverse potential of sympathetic neurons in terms of plasticity (adaptability to changes in the environment) is implied by the functional changes to cardiac sympathetic neurons in HF.

It remains controversial whether cardiac sympathetic differentiation induced by gp130-mediated cytokines in CHF is a favorable or unfavorable event for cardiac performance and prognosis. We found significantly improved survival rate and ventricular function in reference mice when compared with sympathetic nerve specific, gp130-deficient mice, suggesting a protective role for the transdifferentiation seen in the model of hypoxia-induced HF mice.[78] Together, these results indicate that interleukin-6 family cytokines secreted from the failing myocardium act as negative modulators of sympathetic function by rejuvenation and cholinergic differentiation via a gp130 signaling pathway, possibly affecting cardiac performance and prognosis.[78]

Modulation of parasympathetic function can exert profound effects on sympathetic function in the heart. Previous reports have demonstrated that the vagal nerve stimulation suppresses arrhythmia and prevents sudden death in CHF after myocardial infarction with dogs or rats.[79,80] Indeed, in recent reported clinical trial, vagal nerve stimulation improves cardiac function and quality of life with tolerable safety profile in patients with CHF.[81] However the recent results of another multicenter trial of vagal stimulation failed to demonstrate benefits with regards to cardiac remodeling and functional capacity despite improvement in quality-of-life measures.[82]

Long-term exposure of high plasma norepinephrine concentration caused a reduction in myocardial NGF and associated sympathetic fiber loss in severe decompensated HF animals, the so-called anatomic denervation because of depletion of NGF.[74] Recently, Rana et al[83] showed that mechanical stretch and α-1 adrenergic stimulation attenuated NGF expression via the calcineurin-nuclear factor of activated T-cell signaling pathway in cultured neonatal cardiomyocytes. The spatial and temporal innervation pattern and activity of sympathetic nerves directly affect the pathogenesis in HF (Figure 3). We think that better understanding of the mechanisms of cardiac sympathetic anatomic and functional innervation patterning represents an important approach for future development of therapies to avoid SCD.

Translational Relevance of Cardiac and Extracardiac Neural Remodeling

The electrophysiological effects of neural remodeling have been the subject of recent human and animal studies. Data from epicardial and endocardial recordings in patients referred for interventional cardiac electrophysiology procedures demonstrate that there is global cardiac remodeling in humans (analysis of infarcted regions, peri-infarct regions, and remote/normal parts of the ventricle).[84] This study showed that in humans, sympathetic stimulation increased regional differences in repolarization. The myocardium remote from the infract demonstrated abnormal neural control consistent with denervation (lack of action potential shortening with neural stimulation). This functional denervation is also seen in experimental infarction replicating the human condition.[84,85] Dispersion of action potential duration in response to sympathetic stimulation (heterogeneity in response) is significantly increased in cardiomyopathic hearts which explains the proclivity to lethal arrhythmias. To mechanistically evaluate this disease-induced remodeling, a porcine model of myocardial infarction was developed that reproduces all key aspects of disease observed in humans.[85] From these animal models, we found that remote (noninfarcted) myocardium in these hearts shows abnormal regulation and the stellate ganglia show neuronal remodeling and adrenergic transdifferentiation (greater tyrosine hydroxylase–positive cells in stellate ganglia).[86] We have recently extended this work to human and have found that in addition to cardiac changes, extracardiac neural structures undergo significant neural remodeling in the presence of myocardial dysfunction.[87] Evaluating the stellate ganglia removed from patients with refractory arrhythmias, we found morphological changes (enlargement of neurons), as well as changes in growth-associated protein 43 and synaptophysin consistent with increased activity.[87] Such changes likely reflect the pathophysiological changes in response to neural

transduction in the stressed heart and the removal of these structures is beneficial by interrupting efferent and afferent pathways.[17,88] It is of interest that vagal stimulation, which is being evaluated as a treatment for HF to prevent sudden death, leads to cholinergic transdifferentiation of stellate ganglion in dogs (Figure 6).[89]

Clinical Correlates

Several studies have highlighted the value of autonomic indices to identify patients at risk for sudden death. These typically have related to measurable indices of sympathetic and parasympathetic function. Although several tests are valuable, they have not surpassed simpler measures of risk such as ventricular function assessment. However, the physiological basis of these tests will be alluded to briefly.

Identifying High-Risk Patients for SCD in Diseased Heart by Evaluation of the ANS

Higher sympathetic tone and lower parasympathetic tone promote fatal arrhythmias by multiple mechanisms including reducing ventricular refractory period and VF threshold, promoting triggered activity and automaticity. To identify patients at high risk for SCD, evaluation of the ANS has received attention during the years primarily because of the limitations of only using left ventricular ejection fraction. Multifaceted evaluation using different risk markers is expected to increase the accuracy for detecting cardiac risk and also provides opportunities to initiate protective therapy and continues to be a matter of clinical debate. In this section, we summarize available cardiac autonomic testing strategies including heart rate variability, BRS, heart rate turbulence, heart rate deceleration capacity, and T wave alternans (TWA) to place them in the context of cardiac interventions (Table 1).

Heart Rate Variability

Sinus node automaticity is modulated by both sympathetic and parasympathetic nervous systems. Modulation of heart rate by respiration is well-known phenomenon mediated by cardiopulmonary afferent inputs and central interactions between cardiovascular and respiratory networks.[90,91] Alterations of the heart rate is easily measured clinically from ECG recordings and is used to quantify cardiac autonomic modulations as heart rate variability. Heart rate variability is measured by multiple different methods. The most popular methods are time domain or frequency domain analysis.

Baroreflex Sensitivity

BRS is an index of autonomic input to the sinus node and measured by the reflex changes in R-R interval in response to induced changes in blood pressure. It is usually measured by characterizing the magnitude of induced bradycardia in response to a pressor (phenylephrine) challenge. BRS decreases with advancing age and is reduced in patients with hypertension or HF.[44,92] The Autonomic Tone and Reflexes After Myocardial Infarction (ATRAMI) study showed that, after myocardial infarction, the SD of the average of normal sinus to normal sinus intervals <70 ms or BRS <3.0 ms/mm Hg with left ventricular ejection fraction <35% carried a significant risk of cardiac mortality.[44] Daily exercise prevents VF induced by acute myocardial infarction by decreasing sympathetic and increasing parasympathetic tone.[93]

Heart Rate Turbulence

Heart rate turbulence is an index of changes in sinus rate after a premature ventricular complex followed by a compensatory pause. Normally, the sinus rate initially accelerates and slows thereafter but this phenomenon is disturbed in various heart diseases. Abnormal heart rate turbulence is associated with increased total mortality and sudden death in patients with coronary artery disease and dilated cardiomyopathy.[94] From the substudy of ATRAMI study, relative risk for abnormal values of heart rate turbulence was a strong predictor.[95]

Heart Rate Deceleration Capacity

Heart rate deceleration capacity is based on a signal processing algorithm to separately characterize deceleration and acceleration of heart rate, which in turn distinguish between vagal and sympathetic factors. Heart rate deceleration capacity is reported to be a better predictor of mortality after myocardial infarction than left ventricular ejection fraction and SD of normal sinus to normal sinus.[96]

T Wave Alternans

TWA is beat-to-beat variability in the amplitude or morphology of T waves. TWA reflects temporal heterogeneity or dispersion in ventricular repolarization. TWA is primary used as a tool for the risk stratification for SCD in patients with ischemic and nonischemic heart diseases. Negative predictive value of this test is high and a negative test strongly predicts freedom from VT and VF.[97]

Disease-Specific Treatment by Sympathetic or Parasympathetic Modulation for Patients at High Risk for SCD

Atrial Fibrillation

Several mechanisms have been proposed to explain the pathogenesis of AF suggesting a strong link to the ANS.[98] The clinical correlation of an autonomic influence was noted by Coumel et al[99] and has since then been the subject of several studies. Electric stimulation of autonomic nerves during the atrial refractory period has been shown to produce rapid ectopic beats from the pulmonary veins and superior vena cava, which in turn can initiate AF.[100–102] It is now generally accepted that the ANS has an important contribution to the pathogenesis of AF.[103,104] However, AF still remains poorly understood and the specific mechanisms underlying the relationship between the ANS and AF have yet to be fully elucidated. Imbalances in the intrinsic nervous system of the heart (Figure 2) are thought to be involved in the pathogenesis of AF and therefore strategies have been developed to modify the synaptic efficacy of these structures by spinal cord stimulation,[105] ablate ganglionated plexi,[106] or the vein of Marshall.[107] Recent studies have used an alternative neuromodulation-based strategy for control of the atrial arrythmogenic substrate, spinal cord stimulation.[105] High thoracic spinal cord stimulation stabilizes neural processing within the intrinsic cardiac nervous system, reducing the potential for neurally induced AF. Moreover, the efficacy of such therapy increases with time and is related to induced changes in intrinsic cardiac neural network function.[7,102,105]

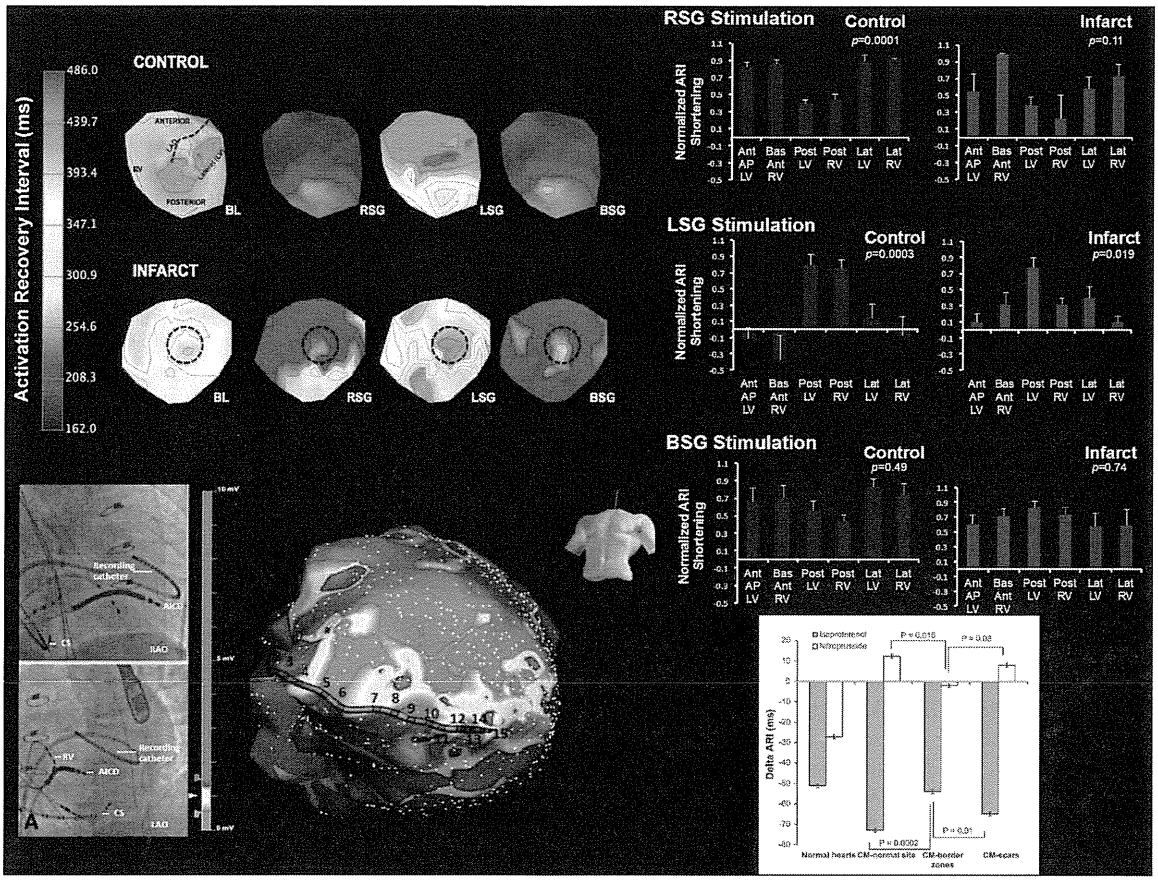

Figure 6. Functional remodeling of cardiac innervation in an experimental infarct model and humans with postinfarct cardiomyopathy. Innervation patterns of the mammalian heart are altered after myocardial infarction. **Left upper**, Polar maps of global epicardial activation recovery intervals (ARIs) recorded from a control and an infarcted porcine heart at baseline (BL), and during stimulation of the right, left, and bilateral stellate ganglion (RSG, LSG, and BSG, respectively). The focal region of myocardial infarction in the anteroapical left ventricle is indicated by the dashed circle in bottom row. The altered pattern of ARI distribution in the infarcted heart extends beyond the region of focal myocardial infarction. **Right upper**, Graphical representation of the regional responses of the porcine heart to stimulation of RSG, LSG, and BSG in control and infarcted hearts, respectively. The anterior and posterior predominance of RSG and LSG stimulations, respectively, are completely lost after infarction. **Left lower**, ARIs recorded from a patient with ischemic cardiomopathy and a large anteroapical scar. The location of the recording multielectrode catheter on fluoroscopy in the right and left anterior oblique (RAO and LAO, respectively) projections; and the corresponding electroanatomic map is shown. On the electroanatomic map, the purple regions indicate tissue with normal voltage (nonscar tissue), whereas the dense gray regions represent dense scar. All other colors represent border zones (tissue with voltage ≥0.5 mV but ≤1.5 mV). **Right lower**, The degree of change in ARI from baseline in response to direct (isoproterenol) and indirect reflex-mediated (nitroprusside) sympathetic stimulation in cardiomyopathic and normal hearts is shown. With isoproterenol, ARI shortening is exaggerated in normal voltage regions in cardiomyopathic hearts (CM-NL) and scarred tissue regions of the cardiomyopathic heart (CM-scar). Border zone regions are slightly less responsive to isopreterenol. With nitroprusside, CM-NL and CM-scar zones paradoxically demonstrate ARI increase when compared with the border zone regions. These observations, when compared with normal hearts, indicate the severe degree of adrenergic nerve dysfunction in human hearts with ischemic cardiomyopathy. AICD indicates automatic internal cardioverter defibrillator lead; AP, apex; CS, coronary sinus electrode; LV, left ventricle; and RV, right ventricular lead.

Ganglionated plexus ablation has been proposed as a strategy for management of AF based on experimental models and human studies. These treatments have the potential to impact ventricular electrophysiology and arrhythmogenesis. Studies have shown significantly increased risk of ventricular arrhythmias in the setting of acute myocardial ischemia heart compared with normal hearts,[108] and there is some evidence of this having relevance to humans post ablation suggesting the need for careful follow-up (Table 2).[109]

Table 1. Cardiac Autonomic Testing

Heart rate variability
Baroreflex sensitivity
Heart rate turbulence
Heart rate deceleration capacity
T wave alternans

Ventricular Tachyarrhythmias Related to Ischemia and Infarction

In patients with myocardial infarction, ventricular arrhythmias develop during the acute and chronic phase. In the acute

phase of coronary occlusion, re-entry caused by heterogeneity of the ischemic myocardium is considered as major mechanism. Reperfusion arrhythmias are caused by washout of various ions such as lactate, potassium, and toxic metabolic substances from the ischemic zone[110] and also oxidative stress alters autonomic function.[53] Reflex activation of the cardiac nervous system, leading to heterogeneous sympathetic activation, contributes to the arrhythmogenic substrate.

VT is often encountered in patients with a healed myocardial infarction.[111] These VTs are mostly monomorphic and caused by re-entry involving a region of infarcted scar. Myocardial scars are most commonly caused by an old myocardial infarction but can also be seen in arrhythmogenic right ventricular cardiomyopathy, sarcoidosis, and other nonischemic cardiomyopathies.[112] Fibrotic scar creates areas of slow conduction or block between surviving myocytes and promotes re-entry. Schwartz et al[113] reported that the presence of a reduced BRS is associated with a greater susceptibility to VF in a canine model of healed myocardial infarction. These data indicate that there are inherent and acquired differences in the neural substrate for cardiac control that contribute to the potential for SCD in the setting of acute and chronic ischemic heart disease.

BBs are essential pharmacological treatment in patients with coronary artery disease and HF. BBs reduce O_2 requirements in myocardium by decreasing heart rate and exercise induced increases in blood pressure. Because BBs block arrhythmogenic sympathetic myocardial stimulation, antiarrhythmic effects also contribute to a favorable outcome. BBs exert this cardioprotective effect by targeting elements of the cardiac nervous system as well as the end-effectors of the heart.[24–26]

Table 2. Disease-Specific Treatment by Sympathetic or Parasympathetic Modulation to Prevent Sudden Cardiac Death

Disease	Trigger	Treatment
Ventricular tachyarrhythmias		
Ischemia and myocardial infarction	Sympathetic	BB, ARB, ACE-I, aspirin, RFCA, ICD, BCSD
Cardiomyopathy		
DCM	Sympathetic	BB, ARB, ACE-I, III, ICD, BCSD
HCM	Sympathetic	BB, Ca-B, III, ICD, BCSD
ARVC	Sympathetic	BB, ARB, ACE-I, III, ICD, BCSD
Arrhythmia		
Long-QT syndrome		
LQT1	Sympathetic	BB, LCSD
LQT2	Sympathetic	BB, LCSD?
LQT3	Parasympathetic	Mexiletine, pacemaker, ICD
Brugada syndrome	Parasympathetic	Quinidine, isoproterenol, ICD
Idiopathic VF	Sympathetic/ parasympathetic	BB?, ICD Quinidine, isoproterenol, ICD
CPVT	Sympathetic	BB, flecainide, LCSD

I and III indicates class I and III antiarrhythmics; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BB, β-blocker; BCSD, bilateral cardiac sympathectomy; Ca-B, calcium channel blocker; CPVT, catecholaminergic polymorphic ventricular tachycardia; ICD, implantable cardioverter defibrillator; LCSD, left cardiac sympathectomy; ?, the efficacy was not established; RFCA, radiofrequency catheter ablation; and VF, ventricular fibrillation.

Cardiomyopathy

Cardiomyopathies including dilated cardiomyopathy, hypertrophic cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy can be associated with VTs. The mechanism of VTs in these patients is re-entry, involving the fibrotic scar with slow conduction. Although TWA may be a useful marker of risk stratification in patients with dilated cardiomyopathy,[114,115] it is difficult to predict patients at high risk of sudden death. The effect of antiarrhythmic drugs is uncertain and implantable cardioverter defibrillator is indicated for primary and secondary prevention of SCD in these patients. In addition to BBs, neuraxial therapy has been valuable in the management of arrhythmias in these patients.[88]

Heart Failure

Increased sympathetic tone in the failing heart causes diastolic Ca^{2+} leak through ryanodine receptor 2 (RyR2) resulting in localized and transient increases in Ca^{2+} in cardiomyocytes. Focally increased Ca^{2+} initiates more Ca^{2+} release and propagates as Ca^{2+} waves. These Ca^{2+} waves can cause delayed after depolarizations resulting in a ventricular premature beats and sustained VT. The effect of β-adrenergic receptor blockers on the survival in patients with HF was proven by multiple placebo-controlled multicenter trials.[116–118] BBs combined with angiotensin-converting enzyme-I produce reverse remodeling of LV, improve patient symptoms, lower hospitalization and prolong survival.[119]

Inherited Arrhythmia

The ANS plays an important role in the development of various inherited arrhythmias.

Long-QT Syndrome

Long-QT (LQT) syndromes are characterized by a prolonged QT interval on the ECG and an increased risk of sudden death by polymorphic VT/torsades de pointes. In congenital LQT syndrome, several clinical phenotypes have been well described including the autosomal dominant Romano–Ward syndrome and the autosomal recessive Jervell and Lange–Nielsen syndrome with or without associated deafness. To date, >17 genotypes have identified but great majority (90%) of cases are LQT1–3. Phenotype–genotype relationships are well studied and the onset of syncope or torsade de pointes (TdP) is initiated by exercise in LQT1 and by noise, sudden wakening from sleep by an alarm clock or telephone rings in LQT2; there are also cardiac changes associated with sleep stages.[120–122] Patients with LQT3 develop events when at rest or asleep. In LQT1 and LQT2, β-adrenergic stimulation enhances transmural dispersion of repolarization and induced TdP. BBs are effective especially in LQT1 but indicated in all LQT patients including genotyped patients with normal QTc. Therapeutic importance of cardiac innervation is evidenced by the fact that left cardiac sympathetic denervation is valuable in high-risk patients who are intolerant or refractory to BBs alone.[123]

Brugada Syndrome

Brugada syndrome (BS) is characterized by ST elevation in the precordial leads and associated with syncope or sudden

death because of VF.[124] VF in BS patients is known to develop more frequently at night than during the remainder of the day. Enhanced vagal tone including a full stomach provokes ST elevation. A decreased nocturnal SD of the average of normal sinus to normal sinus measured in Holter recordings is one of the markers of risk stratification of BS. Sympathetic stimulation such as exercise, isoproterenol infusion improves ST elevation and suppresses syncopal or fibrillatory events.

Idiopathic VF

Diagnosis of idiopathic VF (IVF) is made if patients survive cardiac arrest and the pathogenesis cannot be determined by all available testing. Clinical evaluation should be performed to exclude coronary artery disease, cardiomyopathy, or primary electric disease including BS, LQT, or catecholaminergic polymorphic VT. Although IVF patients are heterogeneous, among the non–Brugada IVF patient, some patients demonstrate similar phenotype with BS. Such patients have higher incidence of J waves. IVF patients with J waves were highlighted as early repolarization syndrome and isoproterenol and quinidine is effective in suppressing VF episodes. There exists a circadian pattern of VF in IVF patients and the presence of J waves was associated with nocturnal occurrence.[125]

Catecholaminergic Polymorphic VT

Catecholaminergic polymorphic VT is characterized by adrenergically induced polymorphic VT which can be reproducibly induced by physical or emotional stress. Mutations in the cardiac *RyR2* gene underlie autosomal dominant catecholaminergic polymorphic VT,[126] whereas cardiac calsequestrin mutations underlie autosomal recessive catecholaminergic polymorphic VT.[127] Intracellular calcium overload triggered by adrenergic stimulation is the disease mechanism. Discontinuation of exercise is required and β-blocking agents are the first line of therapy. Flecainide is alternative pharmacological therapy for patients when cardiac events are not controlled with BBs alone.[128] Left cardiac sympathetic denervation has been reported to be effective in patients with drug refractory ventricular arrhythmias.[129]

VT and Fibrillation Storm in Patients With Structural Heart Disease

Patients with a wide variety of cardiac structural disease present with VT and sometimes this occurs in a cluster (storm) which is associated with a high mortality.[130] Typically these patients are managed with supportive measures, antiarrhythmic drugs, and catheter ablation. The presence of a scar in the heart provides the substrate for VT, but it is not always seen and the pathophysiological role is unclear in patients with dilated cardiomyopathies suggesting a role for functional factors that govern impulse propagation.[112] However, even scar-based reentrant arrhythmias require obligate areas of functional block/conduction changes that allow impulse propagation in preferential directions.[85,131,132] Thus, clinical occurrence of VT reflects the balance between macro structure and functional control. The importance of understanding why only some VTs are clinically encountered when a scar can have multiple circuits is highlighted by the clinical data showing that targeting of the clinical VT is crucial for improved outcomes (not just an arbitrary circuit modification achieved by catheter ablation).[133] In instances when the cardiac substrate is not amenable to catheter modification or refractory to such approaches, neuraxial strategies such as thoracic epidural anesthesia and bilateral cardiac sympathetic denervation have been beneficial.[17,88,134] Patients who undergo such procedures can show changes in cardiac interoception and objective measures of reduced sympathetic outflow to the heart.[18] This again highlights another aspect of the brain heart connection.[12,13]

Perspective on Neuromodulation to Prevent SCD Based on Improved Understanding of Cardiac Innervation

Cardiac disease results in adaptations of afferent and efferent input to various levels of the neuraxis.[2,10] Such adaptations result in changes to the integrated neural function within central and peripheral aspects of the cardiac nervous system. For stress-induced changes in cardiac electric stability, there are interdependent interactions within the nervous system and at the neural–myocyte interface. The following points summarize the current state of the field for neurocardiology with respect to the evolving potential for neuromodulation-based antiarrhythmic therapy based on a better understanding of cardiac innervation.

- Afferent sensory transduction of the pathologically stressed heart results in a reflex-driven adrenergic efferent postganglionic neuronal output to the heart.
- The reflex response of the higher centers to the sensory inputs from stressed heart, especially from ischemic myocardium, is inherently proarrhythmic resulting in augmented norepinephrine release.
- Chronic heart disease adversely remodels multiple levels of the cardiac neuraxis with a resultant shift toward discordant cardiocardiac reflexes, an adaptation by itself that can be proarrhythmic.
- Cardiac neuromodulation/autonomic regulation therapy at different levels of the cardiac neuraxis has the potential to exert antiarrhythmic effects while still preserving basic integrated reflex control the heart.

Recent work has demonstrated that targeting select elements within the cardiac nervous system by electric stimulation or transection and pharmacological manipulation is effective in select cardiac disease states including myocardial ischemia/infarction,[135–137] atrial arrhythmias,[102,105,138,139] and ventricular arrhythmias.[3,88,135] With appropriate neuromodulation therapy, myocytes are rendered stress resistant, autonomic responsiveness for control of the heart is preserved, and the potential for fatal arrhythmias is reduced.[135–137,140–142] Current autonomic regulation therapy therapies are delivered in the open-loop configuration (no feedback) and with the cardiac nervous system considered a black box. To rectify this critical deficit in knowledge, future studies should evaluate reactive and adaptive changes in network function from successive levels of the cardiac neuraxis. This is likely to help develop approaches for mechanism-based targeted neuromodulation for effective cardiac therapeutics.