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Progressive cardiac arrhythmias and ECG abnormalities in the Huntington's disease BACHD mouse model

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Abstract

Huntington's disease (HD) is a dominantly inherited neurodegenerative disease. There is accumulating evidence that HD patients have increased prevalence of conduction abnormalities and compromised sinoatrial node function which could lead to increased risk for arrhythmia. We used mutant Huntingtin (mHTT) expressing bacterial artificial chromosome Huntington's disease mice to determine if they exhibit electrocardiogram (ECG) abnormalities involving cardiac conduction that are known to increase risk of sudden arrhythmic death in humans. We obtained surface ECGs and analyzed arrhythmia susceptibility; we observed prolonged QRS duration, increases in PVCs as well as PACs. Abnormal histological and structural changes that could lead to cardiac conduction system dysfunction were seen. Finally, we observed decreases in desmosomal proteins, plakophilin-2 and desmoglein-2, which have been reported to cause cardiac arrhythmias and reduced conduction. Our study indicates that mHTT could cause progressive cardiac conduction system pathology that could increase the susceptibility to arrhythmias and sudden cardiac death in HD patients.

Introduction

Huntington's disease (HD) is a progressive neurodegenerative disease which causes degeneration of the striatal medium spiny neurons with other neurons including cortical pyramidal neurons affected to a lesser extent (1,2). HD is a dominantly inherited disease with age of onset between the late 30s and mid-40s (3–6). Patients in early disease stages exhibit subtle changes in coordination and personality, while later stages are characterized by the increasing severity of involuntary movements (chorea) and

cognitive changes. In advanced stages, patients are no longer able to walk or speak, and the most common cause of death is aspiration pneumonia.

HD is caused by a CAG-repeat expansion in a single gene encoding the protein huntingtin (HTT) (5). The CAG codon encodes the amino acid glutamine (Q), resulting in an expanded polyglutamine (polyQ) repeat in the mutant huntingtin (mHTT) protein that causes HD when expanded beyond 36 repeats (4,5,7). The age of onset is inversely correlated with the length of the CAG repeat (8–10). Huntingtin is expressed in many cell types,

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and the protein is not confined to a specific subcellular location (11–14). However, the cellular function of huntingtin is complex and still not completely understood (15,16). The mHTT protein forms aggregates that can be found in many brain regions within the cytoplasm or nucleus of the cells (17,18). However, like most of the other neurodegenerative diseases caused by the expansion of a polyQ domain, there are abnormalities in peripheral tissues as well, including skeletal muscle atrophy, genital system abnormalities (men) and dysfunction of blood-derived cells (19–22).

There is increasing evidence of cardiac involvement in HD. Epidemiological data suggests that cardiac disease is the second leading cause of death in HD patients and cardiac failure is implicated in ~30% of HD patient deaths (23–27). Studies in the R6/2 mHTT-expressing mouse model have revealed various functional and cardiac abnormalities including cardiomyocyte death, abnormal distribution of contractile proteins and arrhythmias (28–30). Additional studies have shown many of these same phenotypes along with cardiomyocyte structure and circadian rhythm disturbances present in other mouse knock-in and transgenic models expressing mHTT (31–33). A recent study showed that mHTT expression in the heart decreases its ability to respond to stress, which could contribute to heart failure in HD patients (34). Furthermore, mouse studies have suggested that cardiac-specific expression of polyQ-containing protein can cause heart failure, thus implicating cell-autonomous cardiomyocyte mechanisms in cardiac dysfunction (35). While these mouse models express mHTT in a transgenic, knock-in, or a cardiomyocyte-specific manner, many of them exhibit similar cardiac phenotypes.

Importantly, direct evidence for cardiac abnormalities has recently emerged from HD patients. Screening electrocardiograms (ECGs) from 590 early symptomatic HD patients (stage I and II) were retrospectively analyzed for ECG abnormalities (36). The study revealed no ECG evidence for cardiac hypertrophy, but the prevalence of cardiac conduction defects was higher than expected in non-HD populations, with increased rates of bradycardia, prolonged QRS (electrical impulse that indicates ventricular depolarization) and other cardiac conduction system-related findings. These observations indicate that HD patients may be more susceptible to cardiac arrhythmias and may be at increased risk for sudden cardiac death.

To further assess if the HD mutation predisposes to cardiac arrhythmias *in vivo* and to identify potential molecular mechanisms, we utilized the bacterial artificial chromosome Huntington's disease (BACHD) mouse model and examined cardiac changes as CNS phenotypes progressed in this model (37). These mice express full-length human huntingtin with 97 CAA-CAG repeats under control of the endogenous human promoter and recapitulate many aspects of human HD. In the current study, we evaluated progressive changes in overall cardiac structure and function in BACHD mice. Abnormalities in gross cardiac structure in these mice are unremarkable. Despite this, BACHD mice showed histological changes in the penetrating His-bundle and progressive changes in ECG parameters. Upon challenge with sympathetic and parasympathetic agonists, BACHD mice developed more severe and more frequent supraventricular and ventricular arrhythmias than control mice. In addition, BACHD hearts showed a progressive decrease in the expression level of the desmosomal plakophilin-2 (PKP2) protein and desmoglein-2 (DSG2) protein. Recent findings suggest that decreased expression or impairment of PKP2 and DSG2 causes cardiac arrhythmias and decreased conduction (38–41). Our data suggests that the HD gene mutation in the BACHD mice leads to progressive

cardiac conduction system pathology and decreased expression of desmosomal proteins that could contribute to progressive ECG abnormalities and increase the susceptibility to cardiac arrhythmias in HD mice.

Results

Histological analyses of BACHD hearts reveal His-bundle abnormalities

In order to assess whether the HD mutation in mouse models caused increased arrhythmia susceptibility, we chose to use the BACHD mouse model (37). This model expresses the human mHTT protein in all cell types that express normal HTT. BACHD mice express the mHTT protein at about the same level as endogenous mouse HTT protein in the brain. This is a slowly progressing model, where motor coordination is slightly impaired in the mice as early as 2 months of age but becomes progressively worse with age. Significant atrophy of the striatum and cortex, the brain regions most affected in HD, is apparent at around 12 months of age. We have used mice at 8 months at what we define as the mid-stage of disease in this model when motor dysfunction as measured by abnormal motor coordination on the rotarod is readily apparent and at 18 months a more advanced stage after an age where mHTT expressing aggregates are observed in the brain and brain volume decreases (37,42).

We examined the gross histological features of the BACHD mouse hearts at 15 months of age. We used Masson's trichrome staining of sections from these hearts to assess fibrosis, which is associated with many cardiac diseases. We analyzed 7 wild-type (WT) and 10 BACHD mice. The hearts of the BACHD mice were histologically unremarkable (Fig. 1A and B) with the exception of one BACHD mouse that developed subepicardial replacement fibrosis with associated interstitial and perivascular fibrosis. Figure 1A and B shows representative normal histological images of trichrome staining in WT and BACHD, respectively. Thus, fibrotic changes usually indicative of cardiomyocyte changes are largely absent in this group of BACHD mice hearts (in FVB/NJ background). However, further examination of images of trichrome-stained heart tissue from WT (Fig. 1C) and BACHD (Fig. 1D) revealed histological and structural changes in the penetrating His-bundle, which is a target of conduction diseases, in BACHD mice. His-bundle is an important part of the electrical conduction system. In histological cross section of BACHD and WT hearts, we identified changes in the septal architecture. We measured the extent of fibrotic deposition in a portion inferior to the septal crest in sections of BACHD ($n = 4$) and WT ($n = 3$) hearts. We quantified the histological parameters with a threshold method using NIS-Elements software. We noted a significant increase in fibrotic deposition in this region in BACHD sections (Fig. 1E). More specifically, whereas WT hearts had a thin layer of fibrotic deposition in this area of the septal crest, BACHD hearts display invaginations of fibrotic tissues that network deep in the endocardium. Importantly, this structural change could cause cardiac conduction system function to deteriorate.

Echocardiographic measurements in BACHD mice

We used echocardiography to assess heart structure and function in our cohort of BACHD mice at 18 months of age. Changes in the heart structure or morphology could indicate abnormal heart function. The hearts of BACHD mice ($n = 6$) at this advanced age showed no significant structural differences in the parameters measured as compared to WT mice ($n = 6$) (Fig. 2 and Table 1).

Figure 1. Histological analysis of BACHD and WT hearts. (A) Representative images of a WT and (B) BACHD heart section at 15 months of age stained with Masson's trichrome. Gross histological appearance of BACHD hearts is unremarkable. Representative histological images of trichrome-stained penetrating His-bundle in (C) WT and (D) BACHD. The penetrating His-bundle in BACHD mice demonstrated histological and structural changes that could cause cardiac conduction system function to deteriorate. (E) Quantitation of fibrotic deposition inferior to the septal crest in the hearts of BACHD and WT mice at 15 months of age, $n = 3$ WT and $n = 4$ BACHD (* $P < 0.05$).

The end-diastolic dimension (EDD) and end-systolic dimension (ESD) were the same in this cohort of mice. Our analysis of the left ventricular posterior wall thickness (Lv PWT) did not reveal any differences at this age. In addition to these structural correlates, overall cardiac function was not significantly altered in this group of BACHD mice as shown by no significant differences in the ejection fraction (EF) or cardiac output (CO). Thus, the BACHD mice showed normal values for echocardiographic measurements (Fig. 2 and Table 1). While this group of mice was only analyzed at one advanced age, there does not appear to be

Figure 2. Representative echocardiographic images in WT and BACHD. WT (A) and BACHD (B) short-axis recordings. Contractile parameters ((C), fractional shortening (FS)) and cardiac dimensions ((D), EDD, ESD) were similar between groups ($n = 6$ WT, 6 BACHD).

significant abnormalities in baseline heart function at this age. In general, this data is in agreement with some of the previous work using the BACHD mouse although on a different strain background (31).

Abnormal ECGs observed in BACHD mice

The recent work of Stephen *et al.*, (36), observed ECG abnormalities in early symptomatic stage HD patients. We performed ECGs on BACHD mice at mid-stage (8 months) and an advanced stage (18 months). In Figure 3A and B, representative ECG tracings with ECG interval measurements in WT and BACHD mice are shown. We observed significant ECG abnormalities in the BACHD mice as compared to WT mice. Our analysis revealed that PR interval was normal in BACHD mice. R peak time was prolonged in BACHD mice in surface ECG analysis (6.92 ± 0.39 ms in BACHD mice vs. 5.67 ± 0.38 ms in WT mice, $P < 0.05$). QRS complex was prolonged (10.13 ± 0.38 ms in BACHD mice vs. 8.02 ± 0.42 ms in WT mice, $P < 0.01$), and QT interval was longer in BACHD mice (47.40 ± 0.37 ms in BACHD mice vs. 44.54 ± 1.04 ms in WT mice, $P < 0.05$).

There are clinical data to support dysautonomia in HD patients with autonomic nervous system dysfunction present even in pre-symptomatic HD mutation carriers (24,43–45). The dysfunction of the autonomic nervous system has also been observed in various mHTT-expressing mouse models including the BACHD model (32,33,46,47). In order to assess how BACHD mice respond to autonomic stimulation, sympathetic and parasympathetic agonists were injected intraperitoneally. Figure 4 shows representative ECG tracings illustrating various cardiac arrhythmias in response to sympathetic and parasympathetic agonists in BACHD mice. BACHD mice had a higher incidence of arrhythmias in response to sympathetic and parasympathetic agonists including PACs, PVCs and SA exit block as compared with WT mice. The ECG shown

Table 1. Echocardiographic measurements in WT and BACHD mice

Group	HR (bpm)	EDD (mm)	ESD (mm)	FS (%)	LV PWTd (mm)	LV PWTs (mm)	EDV (uL)	ESV (uL)	EF (%)	CO (mL/min)
WT	500 ± 22	3.5 ± 0.1	2.1 ± 0.1	38.6 ± 3.2	1.2 ± 0.1	1.6 ± 0.1	50.4 ± 4.6	15.6 ± 2.6	69.3 ± 4.0	17.4 ± 2.2
BACHD	519 ± 21	3.5 ± 0.2	2.0 ± 0.1	41.5 ± 1.8	1.2 ± 0.1	1.6 ± 0.1	49.8 ± 6.3	13.8 ± 2.4	72.9 ± 2.0	18.6 ± 2.1

Values are means ± SE. HR, heart rate at the time of echocardiography; LV PWTd, left ventricular posterior wall thickness at end diastole; LV PWTs, left ventricular posterior wall thickness at end systole; EDV, end-diastolic volume; ESD, end-systolic volume. $P > 0.05$ between WT and BACHD mice.

Figure 3. Representative surface ECG recordings in WT and BACHD. (A) WT and (B) BACHD at 18 months of age. Detailed analysis of ECG intervals (C): PR interval was similar, but R peak time, QRS and QT interval were longer in BACHD mice ($n = 12$ WT, 11 BACHD) (* $P < 0.05$ in R peak time, * $P < 0.01$ in QRS, * $P < 0.05$ in QT).

in Figure 4C showed an irregular rhythm with intermittent pauses in BACHD mice. There were no premature complexes preceding the pauses. However, during each of the pauses, there were no P waves at the expected time interval. These ECG features are in keeping with sinoatrial (SA) exit block. As for ventricular arrhythmias, bigeminy, ventricular couplets, triplets and ventricular tachycardia only occurred in BACHD mice.

Arrhythmias in response to autonomic stimulation in BACHD mice

Autonomic nervous system dysfunction in HD could cause more cardiac arrhythmias in response to sympathetic and parasympathetic stimulation and/or altered interaction with the intrinsic cardiac nervous system. We examined the response to the various stimulation protocols in BACHD mice at 8 months of age, considered a mid-stage (Mid) of disease progression based on behavioral and neuropathological changes present in the mice, and at 18 months of age, a more advanced (Adv) stage of disease progression in this model. Cardiac

Observation in both groups, but more frequent in BACHD

Figure 4. Representative ECG tracings in response to sympathetic and/or parasympathetic agonists. Traces illustrating various cardiac arrhythmias in response to sympathetic and parasympathetic agonists in BACHD mice at 18 months of age. (A) Premature atrial contraction (asterisks), (B) premature ventricular contraction (asterisks), (C) SAN exit block (arrows), (D) bigeminy, (E) ventricular couplets, (F) ventricular triplets and (G) ventricular tachycardia (all indicated by asterisks) in BACHD mice.

atrial and ventricular arrhythmias increased in BACHD mice in response to sympathetic stimulation (norepinephrine and epinephrine), parasympathetic stimulation (carbachol) and autonomic conflict stimulation (norepinephrine, epinephrine and carbachol). In Figure 5, we show scatter plots for PACs, PVCs, SA exit block, bigeminy, couplets/triplets in response to sympathetic stimulation, parasympathetic stimulation and autonomic conflict stimulation in WT and BACHD mice at an advanced age (18 months), as well as autonomic conflict in

Figure 5. Summary analysis of ECG observations. BACHD mice had a higher incidence of rhythm abnormalities such as premature ventricular contractions (PVCs) (A), bigeminy (B), couplets/triplets (C), premature atrial contraction (PACs) (D) and SAN exit block (E) than WT mice in response to sympathetic and/or parasympathetic stimulation. At mid-stage (8 months), BACHD mice showed few observations, but at a more advanced/adv-stage (18 months) abnormalities increased, indicating progressive effect ($n = 5-6$ WT, $5-8$ BACHD).

mid-stage mice (8 months). In atrial arrhythmias, one of the BACHD mice had 1133 PACs (the numbers are always per 20 min in this paper) in response to autonomic conflict stimulation. As a group, BACHD mice had increased PACs with autonomic conflict stimulation (354 ± 228 in BACHD vs. 3 ± 2 in WT, $P < 0.05$). Sympathetic stimulation also triggered more PACs in BACHD mice (21 ± 13 in BACHD vs. 3 ± 1 in WT, $P < 0.05$). Autonomic conflict stimulation increased PVCs in BACHD mice (12 ± 6 in BACHD vs. 1 ± 0 in WT, $P < 0.05$). Sympathetic stimulation also led to more PVCs in BACHD mice (6 ± 2 in BACHD vs. 1 ± 0 in WT, $P < 0.05$). BACHD mice developed significantly more SA exit block in response to parasympathetic stimulation than WT mice (205 ± 79 in BACHD vs. 13 ± 4 in WT, $P < 0.05$). We observed ventricular arrhythmia, such as ventricular bigeminy, couplets, triplets and ventricular tachycardia in response to autonomic stimulation only in BACHD mice. Cardiac arrhythmias also increased in mid-stage BACHD mice in response to autonomic conflict stimulation compared with WT mice at the same age. However, mid-stage BACHD mice had smaller increases (although not statistically significant) in arrhythmias than the more advanced BACHD mice (18 months) (Fig. 5). All BACHD mice showed notable sinus node dysfunction during parasympathetic stimulation (Fig. 5E).

In order to investigate the relationship between ventricular arrhythmia and ventricular depolarization duration (R peak time and QRS width), the BACHD mice were divided into two groups according to their numbers of PVCs (low PVC had < 10 PVCs, and

high PVC had ≥ 10 PVCs). On average, WT mice had 1 ± 0 PVCs, low PVC BACHD had 2 ± 1 PVCs, and high PVC BACHD had 19 ± 3 PVCs. Frequent PVCs in response to sympathetic and autonomic conflict stimulation were observed in mice with prolongations in R peak time or QRS complex at baseline (Fig. 6A). The R peak time was significantly increased in the BACHD group with high PVCs (6.01 ± 0.29 ms in low PVC BACHD vs. 5.67 ± 0.38 ms in WT and 7.69 ± 0.48 ms in high PVC BACHD). The QRS duration was significantly increased in the high PVC BACHD group (9.14 ± 0.35 ms in BACHD with low PVCs vs. 8.02 ± 0.42 ms in WT, 10.96 ± 0.37 ms in high PVC BACHD). Widening of the R peak time and QRS complex is related to slower spread of ventricular depolarization, and QRS complex prolongation at baseline could be associated with pro-arrhythmic conditions.

In order to assess the progression of QRS phenotype, we examined the same WT and BACHD mice (a subset of the group) from 8 to 18 months of age. Those mice showed QRS width from the same mice at 8 and 18 months in WT and BACHD mice (Fig. 6B). There was a progressive prolongation of QRS duration with age in BACHD mice. QRS in BACHD mice at 18 months were significantly wider than those at 8 months (8.38 ± 0.49 ms at 8 months vs. 10.60 ± 1.74 ms at 18 months in BACHD mice, $P < 0.05$). In WT mice, an age-related QRS prolongation was not observed (7.57 ± 1.14 ms at 8 months vs. 8.03 ± 0.41 ms at 18 months in WT mice, $P > 0.05$). Thus, these deficits progress with age and motor and neuropathological changes in BACHD mice.

Figure 6. ECG abnormalities are progressive in BACHD mice. (A) The frequency of premature ventricular contractions (PVCs) correlated with ECG parameter prolongation at 18 months. (B) QRS width from the same mice shows QRS in BACHD mice at 18 months was longer than those at 8 months ($n = 4$ WT, 6 BACHD). QRS in BACHD mice at 18 months were significantly wider than those at 8 months (8.38 ± 0.49 ms at 8 months vs. 10.60 ± 1.74 ms at 18 months in BACHD mice, $P < 0.05$). In WT mice, an age-related QRS prolongation was not observed (7.57 ± 1.14 ms at 8 months vs. 8.03 ± 0.41 ms at 18 months in WT mice, $P > 0.05$).

Desmosomal protein decreases in BACHD mouse heart

We observed arrhythmias in the BACHD mice which may be due to pathological changes in the cardiac conduction system, especially in light of the observed changes in the penetrating His-bundle. Typically, cardiac conduction system disease manifests in slowing and/or blocking of conduction at various sites, but it is also associated with ventricular arrhythmias. Perhaps the best example is certain mutations in desmin (DES), an intermediate filament protein (48). In order to determine, if there were changes in DES protein levels in the hearts of BACHD mice, we performed western blots at 12 and 21 months of age. We did not observe any significant changes in DES levels at either age (data not shown).

Interestingly, the phenotypes of arrhythmias in BACHD mice are similar to those observed when there are mutations in desmosomal proteins or mutations in proteins that regulate them (41,49,50). Desmosomes provide cell-to-cell adhesion between cells and are involved in mechanical and electrical stability in the heart. The mutations in these proteins are typically found in those with different forms of arrhythmogenic cardiomyopathy (ACM), including arrhythmogenic right ventricular cardiomyopathy (ARVC) (51). These mutations could lead to arrhythmogenic phenotypes and cause sudden cardiac arrest due to ventricular arrhythmias in affected HD patients. The specialized cells in the cardiac conduction system also contain desmosomes with the requisite desmosomal proteins (52). Furthermore, mutations in PKP2, encoding an armadillo protein, likely account for the largest proportion of ACM patients who show arrhythmias and sudden cardiac death (53,54). When *Pkp2* is haploinsufficient in mice, ventricular arrhythmias develop (40), and embryonic knockout in mice shows that it is required for normal desmosome formation (55). We assessed the expression of PKP2 in protein extracted from BACHD and WT hearts at 12 and 21 months of age. We observed a significant decrease of ~20% in PKP2 levels at 12 months (Fig. 7A and B) and ~40% at 21 months of age (Fig. 7D and E). We also observed a significant decrease in the level of another desmosomal protein, DSG2, in the hearts of BACHD mice at 12 months of age

(Fig. 7A and C). The decrease in expression is comparable to the change observed for *Pkp2* at 12 months of age. It is known that when *Pkp2* is knocked out in mice, there is a significant reduction in the levels of DSG2 (55). Thus, decreases in desmosomal proteins in the BACHD mouse hearts could contribute to the arrhythmia phenotype observed.

Discussion

In this study we investigated if the expression of full-length human mHTT induces cardiac pathology with a focus on cardiac electrophysiology. Tissue histology did not reveal overt cardiac pathology in the ventricular myocardium, and cardiac function was similar in BACHD mice compared to control. Despite the absence of overt structural and functional changes, we detected progressive ECG abnormalities, most notably a progressive prolongation of R peak time and QRS duration. More focused analyses of tissue revealed degenerative changes in penetrating His-bundle in BACHD mice. We observed a decrease in the expression of the proteins PKP2 and DSG2. These abnormalities were associated with increased arrhythmia susceptibility upon stimulation with sympathetic and parasympathetic agonists *in vivo*. Observations include arrhythmias originating from the ventricle as well as the atria. All BACHD mice showed notable sinus node dysfunction during parasympathetic stimulation.

The presence of mHTT has previously been implicated in cardiac abnormalities. Those studies in models expressing expanded polyglutamine repeats and N-terminal mHTT-EGFP fragments in cardiac tissue have identified aggregate accumulation with increasing polyQ repeat lengths (56). Increased stress in the cardiac tissue, including oxidative stress, was observed in models expressing mHTT (57,58). Moreover, a recent study has indicated the presence of mHTT leads to an abnormal response to cardiac stress (34). In addition, mitochondrial abnormalities have been observed in some models expressing mHTT (56,58), while other studies did not detect changes in the mitochondria (59). Taken together, along with our studies, the previous findings clearly indicate that the presence of mHTT

Figure 7. Desmosomal proteins are decreased in BACHD hearts. (A) Western blot and (B) chart demonstrating PKP2 and DSG2 reduction in BACHD mice hearts at 12 months of age reveals a 19% reduction in PKP2 levels ($*P < 0.05$) when normalized to GAPDH. (C) Analysis of DSG2 levels at 12 months of age reveals a significant reduction ($P < 0.03$) of about 25%. (D and E) Western blot analysis demonstrating further PKP2 reduction at 21 months of age ($P < 0.009$) when normalized to GAPDH. Data are mean $\pm$ SEM, $n = 4$ WT and $n = 4$ BACHD.

causes abnormalities that could contribute to abnormal cardiac function.

Evidence for pathological changes within the cardiac conduction system

We observed QRS prolongation in BACHD mice that likely indicate dysfunction in the upper ventricular conduction system. A similar QRS prolongation has been described in mice heterozygous for the *Six5* gene, a gene implicated in the molecular mechanisms contributing to the cardiac abnormalities in myotonic dystrophy (60). *In vivo* intracardiac electrophysiological studies revealed that infrahisian conduction delay was significantly delayed in *Six5* heterozygous mice. These findings indicate that QRS prolongation was associated with the specific conduction delay in the His-bundle (infrahisian conduction delay) (60). Trichrome-stained histological images revealed degenerative changes as indicated by increased fibrosis in the penetrating His-bundle that could cause the cardiac conduction system function to deteriorate and conduction delay in the His-bundle. Altogether, our data suggest that the His-bundle and the sinoatrial node (SAN) are functionally impaired in the BACHD mice. Other aspects of the conduction system may be impaired as well, but there appears to be some specificity. The PR interval is normal, and none of the drug injections induced any degree of AV block which indicates that AV nodal function is not compromised.

At times 'desminopathies' manifest predominantly as cardiac conduction disease, and affected patients also exhibit PVCs and other forms of arrhythmias (61,62). The BACHD mice display premature contractions from atrial as well as ventricular origin in the absence of an overt structural substrate. Our data show that mice with higher number of PVCs have a significantly higher degree of R peak prolongation (Fig. 6A). The mechanistic

relationship is uncertain; it could be a direct causal connection, or it may only be indirectly linked.

It is also interesting to note that myocardial hyperinnervation as well as denervation can both contribute to arrhythmias (63). We would like to point out that we also tested the arrhythmia susceptibility using drugs that imitate stimulation by the parasympathetic or sympathetic nervous system. However, when considering that the autonomic nervous system plays an active role, it needs to be considered that the arrhythmias were observed during isoflurane anesthesia, which is known to dampen autonomic nervous system output (64).

Cause of cardiac conduction system dysfunction

It seems widely accepted that altered functioning of the autonomic nervous system occurs already in early stages of HD and that this is probably the result of compromised high-order autonomic control. This is similarly observed in several HD mouse models including the BACHD mice and seems to occur early at an early age (33). The sinus node is continuously regulated by parasympathetic and sympathetic outflow and thus may be very sensitive towards dysfunction of the autonomic nervous system. One indicator for autonomic nervous system influence on the sinus node is the observation of heart rate variability (HRV), which studies the variance of time between individual beats. HRV is altered in BACHD mice, the average HRV is lower and the temporal patterning was diminished (33). Sinus node dysfunction in the BACHD mice may be due to acute autonomic dysregulation and that especially manifests during parasympathetic stimulation. It may also be the result of chronic functional remodeling as a result of persistent altered autonomic control. Either effect may also apply to other functional regions of the cardiac conduction system like the His-bundle.

In addition, through dysregulation by the autonomic nervous system, transgenic expression of a polyQ peptide led to cardiac dysfunction which suggests that proteins like mHTT may also directly damage the heart (31,35). If that occurs in the BACHD mice, then it is not in the context of broad tissue remodeling since the septal wall and the area beyond the His-bundle appear to be structurally unremarkable (Fig. 1). However, it is intriguing that with the exception of the peripheral nerves in the heart, the cells of the cardiac conduction system are considered most neuronal-like. The cardiac conduction system expresses markers that are also expressed in neuronal lineages, for example, cardiac Purkinje fibers have a high activity of acetylcholinesterase (65), share HCN4 with thalamic nuclei in the brain and express neuronal isoforms of intermediate filaments (66) and the neuronal adhesion molecule contactin (67). Since mHTT expression leads to selective neurodegeneration, we can speculate that the most likely cell type in the heart that might become damaged directly is in the cardiac conduction system.

Decreased expression of PKP2 and DSG2 leads to cardiac arrhythmias and reduced conduction

Recent findings suggest that decreased expression or impairment of desmosomes with defective cell adhesion proteins, such as PKP2 and DSG2, causes cardiac arrhythmias and decreased conduction. Most cases of familial ACM are associated with a mutation in a desmosomal gene (68). These desmosomal proteins interact with intracellular calcium handling proteins and other junctional molecules such as other cadherins, catenins, ion channels, e.g. the sodium channel Nav1.5, and gap junction molecules (38,49,68,69). Patients with PKP2 variants are associated with exercise-induced sudden cardiac arrest or sudden cardiac death (39). In addition, in patients with a large deletion of PKP2, exercise-induced ventricular arrhythmias develop (70).

The loss of *Pkp2* can markedly reduce the transcriptional expression of genes critical to intracellular calcium handling and could contribute to isoproterenol-induced arrhythmias (38). In a *Pkp2* conditional knockout mouse model, shortly after loss of PKP2 expression, mice showed a high propensity for ventricular arrhythmias. While *Pkp2* conditional knockout mice did not show structural heart disease, *pkp2* deficiency in adult ventricular myocytes is sufficient to cause ACM (ARVC). However, there are data to support that a loss-of-function *Pkp2* variant is a potential cause of life-threatening ventricular arrhythmias even in the absence of overt structural heart disease in humans (38). Additionally, decreases in PKP2 protein levels in mice lead to reduced amplitude and shift in gating and kinetics of sodium current (I_{Na}) and directly impair the function of the voltage gated sodium channel complex and cardiac electrophysiological behavior, which could facilitate the development of arrhythmias (40,68). Western blot analysis of the conditional mice revealed decreased PKP2 protein levels, and the mice showed decreased conduction velocities (40). Sodium channel deficit and heterogeneous distribution in the conduction system and across the ventricle could contribute to arrhythmogenesis (40). Reduced amplitude could also facilitate the development of arrhythmias, even in the absence of a structural cardiomyopathy syndrome (68).

In a *Pkp2*-deficient mouse model, altered sodium current amplitude and kinetics were crucial to reentry initiation, decrease gap junction coupling, slow conduction and increase

arrhythmogenicity. PKP2 deficiency can set up a substrate for reentry (49). In another study with the loss of PKP2 expression and decreased sodium channel, optical mapping demonstrated increased reentrant activity and significantly decreased conduction velocity (71).

Furthermore, mechanical junction protein abnormalities with reduction in I_{Na} amplitude have been observed in mouse models with *Pkp2* haploinsufficiency or with cardiac overexpression of mutated DSG2 (40,72,73). Similar to PKP2, DSG2 is also a common gene for ACM (73,74). Mutations in DSG2 may be related to fatal arrhythmic events even in patients with a morphologically normal heart (75). Mice with a *Dsg2* mutation display ACM and reduced functional sodium channel availability leading to slowed conduction and increased arrhythmia susceptibility (72,76).

Background mouse strain variability

The cardiac effects of the huntingtin disease gene appear to be variable depending on the background mouse strain. Another study previously reported cardiac dysfunction in BACHD mice that were bred in the C57BL6/J background (31). In contrast to FVB/N, the C57BL6/J BACHD hearts developed a variety of mild to moderate structural and functional abnormalities including hypertrophy and fibrosis, increased ESD and decreased LV EF. These changes started at 3 months of age and mostly persisted. Interestingly, we do not find the same degree of tissue changes observed by Schroeder et al. 2016 (31). However, it must be noted that there is a spectrum of cardiac phenotypes in humans without all of them displaying fibrotic changes including those with mutations in PKP2. Despite the lack of gross observations, the FVB/N BACHD mice exhibit increased arrhythmia susceptibility consistent with selective functional impairment in the cardiac conduction system. The data in the published report using C57BL6/J BACHD does not allow the evaluation of conduction system function, and this may have been missed.

Implications for HD patients

This study reports progressive ECG abnormalities, degenerative His-bundle and decrease in the expression of the protein PKP2 and DSG2 consistent with selective conduction system dysfunction and associated with increased arrhythmia susceptibility in BACHD mice. Our observations in the BACHD mice are largely consistent with the recent study in early symptomatic HD patients reporting higher than the expected numbers of conduction abnormalities including QRS prolongation and intraventricular conduction delay (36). This suggests that HD patients may need to be screened for rhythm abnormalities and that screenings may need to be repeated as the disease progresses. Moreover, severe sinus node dysfunction could contribute to cerebral hypoperfusion that would facilitate cognitive dysfunction, which may be treatable by pacemakers. Other implications are that drugs which prolong QT interval or promote bradycardia may require additional monitoring if administered. It is also important to note that novel HD treatments are being developed that are applied via intrathecal injection and thus will specifically target the brain and spinal canal, not peripheral tissues (77). At this time, it is unclear if that will prevent the effects on peripheral tissues, and additional mechanistic studies in the BACHD mice will be valuable.

Figure 8. Summary model linking mHTT to arrhythmias. In BACHD mice, we observe an increase in cardiac conduction system abnormalities that increase risk of sudden arrhythmic death. Increased fibrosis is observed in His-bundle. Decreases in two desmosomal proteins, PKP2 and DSG2 that are known to cause cardiac arrhythmias and reduced conduction are seen in aged BACHD mice.

Conclusions

Our results suggest that it would be valuable to monitor heart rhythm in HD patients even in the absence of overt heart disease. The BACHD mouse model in the FVB/NJ background appears to reproduce observations reported in a large cohort of human patients (36). We detected progressive ECG abnormalities in BACHD mice, most notably a progressive prolongation of QRS duration. BACHD mice showed increased fibrosis in the penetrating His-bundle. Upon challenge with sympathetic and parasympathetic agonists, BACHD mice developed supraventricular and ventricular arrhythmias. Finally, we observed decreases in desmosomal proteins, PKP2 and DSG2, which have been reported to cause cardiac arrhythmias and reduced conduction (Fig. 8). Our study indicates that mHTT could cause progressive cardiac conduction system pathology that would increase the susceptibility to arrhythmias and sudden cardiac death in HD.

Materials and Methods

Animals

All experiments were conducted in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The protocols were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. All studies were performed in the conditional human HTT bacterial artificial chromosome (BACHD) transgenic mouse model in the FVB/NJ background strain as originally reported (37). Transgenic and littermate controls of either sex were used. The age of the mice was from 8 to 18 months.

Drugs

The following veterinary grade injectable drug formulations were used: norepinephrine (Claris Lifesciences, North Brunswick, NJ, 1 mg/ml) and epinephrine (VetOne, Boise, Idaho,

1 mg/ml). Carbachol USP (Sigma Aldrich, Saint Louis, MO) was dissolved in sterile saline.

Surface ECG and analysis

Mice were anaesthetized with 1–3% isoflurane in oxygen and placed on an infrared heating pad (Kent Scientific). Surgical steel needle electrodes were placed under the skin of each limb and the ECG recorded in lead I and lead II configuration, and ECG continuously recorded using PowerLab data acquisition system (ADInstruments, Colorado, USA) and analyzed using LabChart 8.0 software. Baseline ECGs were recorded for about 10 min until a stable baseline was reached. 'R peak time' was measured from the beginning of the QRS complex to the peak of the R wave. ECG intervals were determined by averaging consecutive beats during sinus rhythm in 15 s.

Arrhythmia induction and analysis

For arrhythmia induction, we used three basic protocols: sympathetic (sym), parasympathetic (para) and both (both). In sympathetic stimulation, baseline ECGs were recorded for 10 min before sympathetic agonists (0.6 mg/kg norepinephrine and 0.15 mg/kg epinephrine, i.p., norepinephrine/epinephrine 4:1 on a weight basis) were injected. Parasympathetic agonists (0.3 mg/kg carbachol, i.p.) were injected in parasympathetic stimulation. In autonomic conflict stimulation, sympathetic agonists (0.28 mg/kg norepinephrine and 0.07 mg/kg epinephrine, i.p.) were injected first and followed by an injection of parasympathetic agonists (0.06 mg/kg carbachol, i.p.) 2 min later. ECG recordings were screened for premature atrial beats (PACs), premature ventricular beats (PVCs or triggered beats), bigeminy (alternating sinus beat/triggered beat), couplets (two triggered beats in a row), triplets (three triggered beats in a row), ventricular tachycardia (four or more triggered beats in a row) and SAN exit block.

Echocardiography and analysis

Mouse fur was removed using Nair up to 24 h before the procedure. The mice were anaesthetized using 1–3% isoflurane in oxygen and stretched out on a heating pad. A high-resolution MX400 transducer was held to the chest, and B- and M-mode images were obtained using a Vevo 3100 system. Left ventricular (LV) dimensions (ESD and EDD) and LV wall thickness (PWT in systole (s) and diastole (d)) were analyzed using M mode, while the strain was analyzed using B-mode (vevo strain). Fractional shortening (FS), EF and CO were calculated using the following formulas:

$$FS (\%) = \frac{EDD - ESD}{EDD} \times 100 \quad (1)$$

$$EF (\%) = \frac{EDV - ESV}{EDV} \times 100 \quad (2)$$

$$CO = \frac{(EDV - ESV) \times HR}{1000} \quad (3)$$

Histological analysis

Mouse hearts were harvested under isoflurane anesthesia, fixed in formalin and embedded in paraffin using standard protocols. 5-µm-thick sections were stained with Masson's trichrome protocol, and images of the heart and septal wall were evaluated in a blinded fashion. Fibrosis was quantified by a fibrosis threshold method using NIS-Elements software (Version 5.20.01) in 80 × 80 µm region inferior to the septal crest. The percentage of fibrosis deposition was compared between WT and BACHD mice.

Protein isolation and western blotting

The hearts were taken from mice at 12 and 21 months of age and frozen immediately after dissection. 1 ml of urea lysis buffer (8 M urea, 63 mM Tris (pH 6.8), 1% SDS, 10% glycerol, 0.5% 2-mercaptoethanol) was added to each heart and disrupted with a tissue homogenizer until complete solubilization. Protein concentration was determined with Pierce BCA Protein Assay Kit (ThermoFisher, cat#23225). Each sample was diluted in 4× LDS sample buffer (Invitrogen, cat NP0008) and heated at 70°C for 10 min. 20 µg of total protein was loaded per lane in a NuPAGE 3–8% tris-acetate gel (ThermoFisher, cat#EA0378BOX) and transferred to Immuno-Blot PVDF membrane (Bio-Rad, cat#1620177). The membrane was blocked for 1 h in 5% non-fat milk-PBST (0.1% Tween) and incubated overnight at 4°C with the appropriate primary antibody. Each membrane was incubated with the appropriate secondary antibody. Pierce enhanced chemiluminescent blotting substrate (ThermoFisher, cat#32106) and imaged on a Bio-Rad ChemiDoc Imaging System. ImageJ software was used to calculate the densities of the bands. We used anti-PKP2/plakophilin 2 antibody (aa125–139) IHC-plus™ (Lifespan Biosciences, cat# LS-B9231) diluted 1:1000. GAPDH loading control monoclonal antibody (GA1R) (ThermoFisher, cat# MA5-15738) was diluted 1:10 000. Goat anti-Mouse IgG (H+L) secondary antibody, HRP (ThermoFisher, cat# 31430) was used for the mouse primary antibodies.

Statistics

Data were reported as means ± SEM. Significance was determined using paired, unpaired t-test, one-way analysis of variance (ANOVA), Mann-Whitney U test and negative binomial

model as appropriate by GraphPad Prism 5 and SAS. Western blot data is expressed as mean ± SEM. The significance between the BACHD and the WT group band intensities was determined using the paired samples t-test. Differences were considered significant at $P < 0.05$.

Conflict of Interest statement

The authors declare no competing interests.

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Abbreviations

HD, Huntington's disease; BAC, bacterial artificial chromosome; ECG, electrocardiogram

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