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Loss of MD1 increases vulnerability to ventricular arrhythmia in diet-induced obesity mice via enhanced activation of the TLR4/MyD88/CaMKII signaling pathway

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KEYWORDS
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Signal transduction

Abstract *Background and aim:* Obesity is an important risk factor for ventricular arrhythmia (VA), and myeloid differentiation protein 1 (MD1) has been reported to decrease in obese hearts. Nevertheless, underlying mechanisms linking MD1 and VA have not been fully studied. This study aims to investigate the regulatory role of MD1 in VA caused by diet-induced obesity.
Methods and results: MD1 knock-out (KO) and wild type (WT) mice from experimental groups were fed with a high-fat diet (HFD) since the age of six weeks for 20 weeks. The body weight gain, fast glucose and serum lipid levels were measured and recorded. In addition, pathological analysis, echocardiography, electrocardiography, langendorff-perfused heart and molecular analysis were performed to detect HFD-induced vulnerability to VA and its underlying mechanisms. After a 20-week HFD feeding, the mice showed an increase in body weight, glycemic, lipid levels, QTc interval, LVEDd, LVEDs and LVFS. HFD feeding also increased vulnerability to VA, as shown by the prolonged action potential duration (APD), enhanced APD alternans threshold and greater incidence of VA. Moreover, HFD feeding caused LV hypertrophy and fibrosis, and decreased the protein expressions of Kv4.2, Kv4.3, Kv1.5, Kv2.1 and Cav1.2 channels. At last, the above-mentioned HFD-induced adverse effects were further exacerbated in KO mice compared with WT mice. Mechanistically, MD1 deletion markedly enhanced the activation of TLR4/MyD88/CaMKII signaling pathway in HFD-fed mice.
Conclusion: MD1 deficiency increased HFD-induced vulnerability to VA. This is mainly caused by the aggravated maladaptive LV hypertrophy, fibrosis and decreased protein expressions of ion channels, which are induced by the enhanced activation of the TLR4/MyD88/CaMKII signaling pathway.

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Introduction

Obesity predisposes to metabolic syndrome, and can also result in cardiac structural and functional disorders in humans and animal models [1]. Previous studies have demonstrated that obesity can significantly increase the risk of ventricular arrhythmia (VA) and sudden cardiac death (SCD) [2,3].

In addition, several mechanisms, which were reported to play vital roles in VA pathophysiology, are related to obesity [4–6]. One of the mechanisms is structural remodeling, which is characterized by ventricular fibrosis and dilatation. It can increase VA vulnerability by promoting multiple reentries in the ventricle. Meanwhile, studies have found that obesity can cause cardiac hypertrophy and fibrosis and impairment of left ventricular systolic and diastolic function [7,8]. Another mechanism is electrical remodeling. According to pervious research, modulation of ion channel expressions following obesity could cause important changes in cellular electrical activity, resulting in prolonged action potential duration (APD) [9]. Both of the above mechanisms demonstrate that obesity can significantly increase the risk of VA, but only limited treatment is currently available for obesity-induced VA. Therefore, finding out the key molecules involved in obesity-induced VA are extremely impendency for the treatment of obesity-induced VA.

The toll-like receptor 4 (TLR4) signaling pathway is acknowledged as one of the main triggers of the obesity-induced inflammatory response [10]. TLR4/MyD88 mediated activation of Ca2+/calmodulin-dependent protein kinase II (CaMKII) has been reported in the heart by previous studies [11–13]. A recent study also found that TLR4 is the upstream signal of cardiac CaMKII which plays an important role in obesity related cardiac remodeling [14,15]. In addition, studies have illustrated that the activation of CaMKII can impair ion channel function, and thus prolong repolarization, revealing the crucial role of CaMKII activation in remodeling ion channels function under obesity conditions [16–18]. Therefore, the TLR4/MyD88/CaMKII signaling pathway plays a crucial regulatory role in obesity-induced ventricular structural and electrical remodeling, which may be as a key signaling pathway involved in VA caused by obesity.

Myeloid differentiation 1 (MD1) is a physiological negative regulator of the TLR4 signaling pathway [19]. Our former study found that MD1 was diffusely expressed in heart, and loss of MD1 deteriorated LV electrical remodeling through heightened activation of the TLR4/MyD88/CaMKII signaling pathway under chronic pressure overload conditions [20]. Moreover, we also found that MD1 deficiency could worsen atrial dilatation, and increase susceptibility to atrial fibrillation in obese mice [21]. However, the underlying mechanisms linking MD1 and VA has not been fully studied. Thus, the current study elaborated the mechanism linking MD1 and high-fat diet (HFD)-induced VA, and first provided evidence of MD1 playing a vital role in development of VA caused by HFD-induced obesity.

Methods

Experimental animals

MD1 knock-out (MD1-KO) mice were generated as described by former research [20]. In brief, male MD1-KO mice were purchased from the Japan RIKEN Bio Resource Centre Mouse (BRC) (B6.129P2-MD-1 <tm1Kmiy>). MD1-KO male mice and their wide type (WT) littermate male mice were housed in a circumstance with controlled light cycles (12 h light/12 h dark), temperature and humidity. All experiments involving animals were confirmed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of health (the 8th Edition, NRC 2011) and approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University.

Mouse model of HFD-induced obesity

The MD1-KO mice were confirmed by Western blot. From the age of 6 weeks, 40 MD1-KO and WT male mice, 20 mice per group, were fed with an HFD (60% kcal from fat) for 20 weeks. Another 40 MD1-KO and WT male mice, 20 mice per group, were fed with a normal diet (ND) (10% kcal from fat). Mice from all four groups were weighed weekly. The levels of fast glucose, total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-c) were tested and recorded when they reached 26 weeks of age.

Surface electrocardiography (ECG) and echocardiography analysis

Surface ECG and echocardiography analysis were performed using previously described methods [21]. After a 20-week HFD feeding, mice were anaesthetized by inhaling gaseous anesthesia (1.5–2% isoflurane). After anesthesia, the electrodes were positioned subcutaneously to the approximate ECG surface-lead II. The heart rate (RR interval) and PR, QRS, and QT interval (in ms) were recorded for the four mice groups. QT interval was corrected with the modified Bazett's formula [22]. All the above collected data was analyzed off-line using Lab-Chart 7 Pro (AD Instruments). In addition, echocardiography was performed to evaluate the LV function under anesthesia (1.5–2% isoflurane) by a Mylab30CV(ESAOTE) ultrasound system with 15Mz probe after the 20-week HFD feeding. Finally, LV end-diastolic dimension (LVEDd), LV end-systolic diameter (LVESd), LV fraction shortening (LVFS), and LV ejection fraction (LVEF) were measured.

Histological analysis

After a 20-week HFD feeding, the heart weight (HW), ratios of HW/body weight (BW) and HW/tibia length (TL) of the sacrificed mice were assessed. Left ventricle (LV) samples were fixed in paraformaldehyde solution for 24 h, embedded in paraffin and cut into 4–5 μm thick sections. Several slices of heart were also prepared for the following procedures: LV morphometric and myocardial fibrosis

Table 1 The characteristic of HFD-induced obese mice model.

	WT-ND	KO-ND	WT-HFD	KO-HFD
BW, g	29.48 ± 0.32	31.18 ± 0.24	35 ± 0.33*	45.36 ± 0.47*#
Glucose, mmol/l	6.31 ± 0.13	6.34 ± 0.11	7.41 ± 0.15*	9.73 ± 0.26*#
TC, mmol/L	1.96 ± 0.16	1.93 ± 0.06	3.8 ± 0.19*	5.13 ± 0.21*#
TG, mmol/L	0.65 ± 0.05	0.73 ± 0.05	1.42 ± 0.1*	1.93 ± 0.25*#
LDL-c, mmol/L	0.84 ± 0.07	0.8 ± 0.03	1.7 ± 0.08*	1.92 ± 0.07*#
RR interval, ms	132.39 ± 9.09	133.54 ± 6.49	141.02 ± 8.8	152.84 ± 7
PR interval, ms	39.78 ± 0.9	39.68 ± 0.71	39.07 ± 0.74	40.56 ± 1.86
QRS duration, ms	9.97 ± 0.25	10.5 ± 0.48	10.34 ± 0.36	10.68 ± 0.26
QTc interval, ms	43.36 ± 0.39	44 ± 1.3	51.12 ± 1.03*	56.91 ± 2.15*#
LVEDd, mm	3.69 ± 0.25	3.76 ± 0.21	4.11 ± 0.15*	4.6 ± 0.15*#
LVEDs, mm	1.84 ± 0.18	1.93 ± 0.13	2.34 ± 0.11*	2.99 ± 0.26*#
LVFS, %	50.24 ± 0.64	48.45 ± 1.97	43.05 ± 0.55*	35.12 ± 1.62*#
LVEF, %	78.25 ± 3.96	76.14 ± 2.61	79.29 ± 2.29	75.5 ± 4.69

N = 8 for each group. Data are presented as mean ± SEM. BW: body weight; TC, total cholesterol; TG, triglyceride; LDL-c: low density lipoprotein cholesterin; HFD, high-fat diet; HR, heart rate; LVEDd, left ventricular end-diastolic diameter; LVEDs, left ventricular end-systolic diameter; LVFS, left ventricular fraction shortening; LVEF, left ventricular ejection fraction. *P < 0.05 vs. WT-ND group, #P < 0.05 vs. WT-HFD group.

were dyed by Hematoxylin and eosin (H&E) and picrosirius red (PSR) stain, respectively.

Monophasic action potential (MAP) recording and electrical stimulated protocol

MAP recording protocols, electrical stimulated protocols, data acquisition and analysis are described in Supplementary methods.

Western blotting analysis

Western blotting was according to a published method [21]. Membrane proteins were extracted from the frozen LV tissues. Protein concentrations were determined and normalized using the Bicinchoninic Acid (BCA) Protein Assay Kit (AS1086, ASPEN). After that, proteins (40 μg) were firstly separated by sodium dodecylsulphate (SDS)-polyacrylamide gel electrophoresis (PAGE). Secondly, they were transferred onto a polyvinylidene difluoride (PVDF) membrane, and finally incubated with primary antibodies (Supplementary Table S1) overnight at 4 °C. In addition, secondary antibodies were incubated with the membranes for 30 min at room temperature. Enhanced chemiluminescence was to visualize the signals.

Statistical analysis

The data analysis was performed by SPSS 24 and GraphPad Prism software. Continuous variables are expressed as mean ± SEM. Comparisons of two groups were conducted with the Student's 2-tailed unpaired t test. Categorical data were presented as percentages and used the Fisher exact test. A $p < 0.05$ was considered statistically significant.

Results

Characteristics of HFD-induced obese mice model

After the 20-week HFD feeding, we first assessed the characteristics of obese mice. As shown in Table 1, HFD-fed mice showed an increase in body weight, glycemic and lipid levels. These metabolic disorders caused by HFD-induced obesity were further exaggerated in MD1-KO mice compared with WT mice. Apart from the above metabolic disorders, the ECG results demonstrated that the QTc interval was more markedly extended in KO-HFD mice compared with WT-HFD mice, with the QTc interval of WT-ND and KO-ND mice being comparable. However, no statistically significant differences were found among the RR interval, PR interval and QRS duration of four groups. In terms of the echocardiographic measurement, the LVEDs, LVEDd and LVFS increased more in HFD-fed mice than that of ND-fed mice, and these indexes were further exaggerated in KO-HFD mice. However, the LVEF was comparable in four groups.

Loss of MD1 prolonged APD and increased vulnerability to VA in HFD-fed heart

To clarify the underlying role of MD1 in regulating obesity-induced LV electrophysiology and arrhythmogenesis, we further used the langendroff-perfused heart system to characterize APD and ventricular repolarization in mice of four groups. After the 20-week HFD feeding, APD_{20}, APD_{50}, APD_{90} were significantly prolonged in mice of both WT-HFD and KO-HFD groups, and the prolongation was considerably greater in the KO-HFD mice than that in the WT-HFD mice (Fig. 1A and B). In addition, the threshold of APD alternans increased markedly after HFD feeding, and the enlargement was greater in the MD1-KO mice (Fig. 1C and D). Finally, burst stimuli did not induce VA in either the WT-ND group or KO-ND groups (0/10). On the

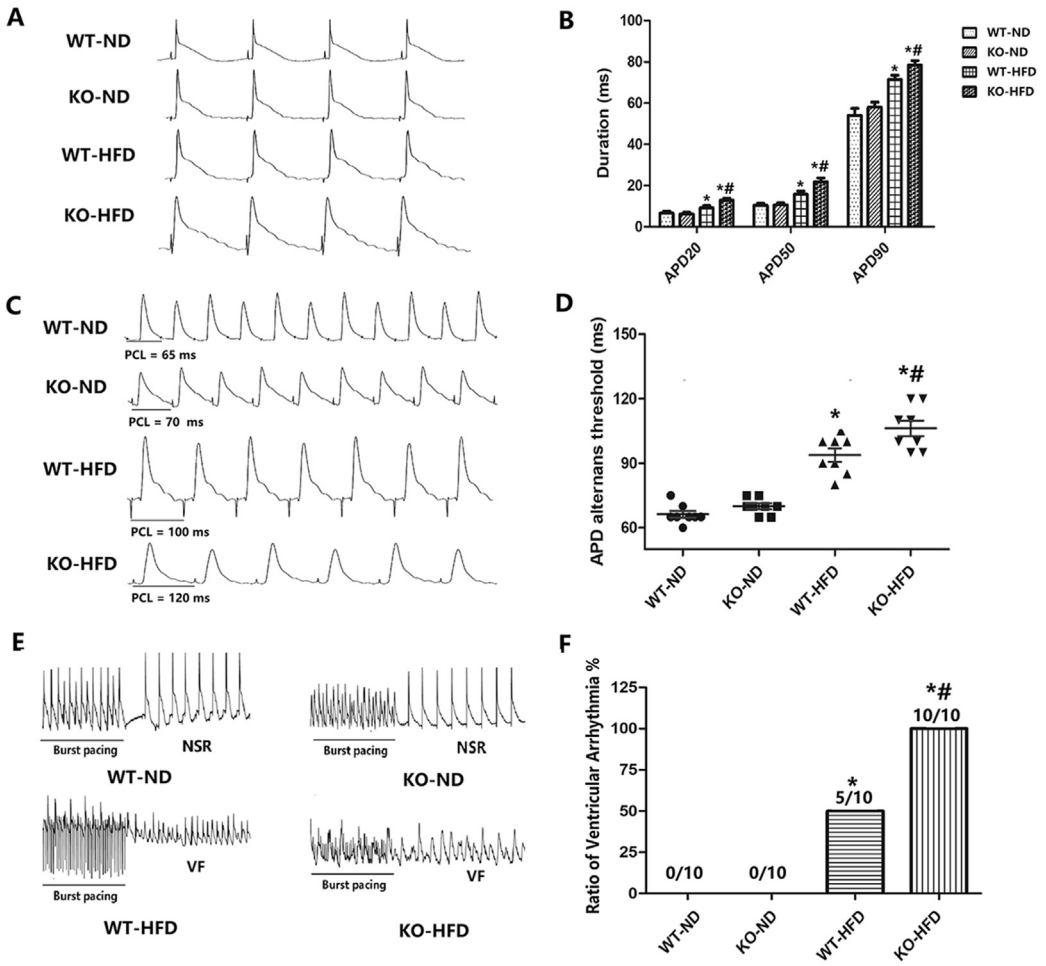

Figure 1 Loss of MD1 prolonged APD and increased susceptibility to arrhythmia in HFD-fed heart. (A, B) Representative action potential figures and statistical analysis of the APD_{20}, APD_{50}, APD_{90} in WT and KO mouse hearts after 20 weeks ND or HFD feeding (n = 8). (C, D) Representative electric alternans figures and statistical analysis of the ALT thresholds in WT and KO mouse hearts after 20 weeks ND or HFD feeding (n = 8). (E, F) Representative arrhythmia induced by burst-pacing stimulations and statistical analysis of WT and KO mouse hearts after 20 weeks ND or HFD feeding (n = 10–11). Data are expressed as mean ± SEM. $^*p < 0.05$ vs. WT-ND group, $\#p < 0.05$ vs. WT-HFD group.

contrary, the VA induction rate was remarkably increased in the HFD-fed group, with the KO-HFD group (10/10, 100%) being significantly higher than the WT-HFD group (5/10, 50%) ($p < 0.05$) (Fig. 1E and F). These results suggested an increased arrhythmic vulnerability in HFD-fed heart, and this increased vulnerability can be exacerbated by MD1 deletion.

MD1 deletion aggravated HFD-fed induced maladaptive LV hypertrophy and fibrosis

Evidence indicated that ventricular dilatation and fibrosis increase the VA vulnerability by promoting multiple re-entries [2,8]. In this study, we further explored the role of MD1 in obesity-related LV hypertrophy and fibrosis. First of all, the myocardium of MD1-KO mice was verified by the data that showed the robust decease in the protein level of MD1 (Fig. 2A). After the 20-week HFD feeding, the myocardium of KO-HFD mice hypertrophied more than that of WT-HFD mice, as confirmed by the higher HW, increased the ratio of HW/TL, and lager cardiomyocyte cross-sectional area (CSA) (Fig. 2B, D–F). Consistently, the protein levels of hypertrophy (ANP, BNP, and β-MHC) markers were also more markedly increased in the LV tissues of MD1-KO mice than in those of WT mice (Fig. 2G and H). No significantly differences in these parameters were observed between the WT-ND and KO-ND mice.

However, the ratio of HW/BW was decreased more in the KO-HFD mice compared with WT-HFD and WT-ND mice (Fig. 2C). This is probably because BW gaining was more pronounced than HW gaining in the HFD-fed mice. In addition, cardiac fibrosis, a major feature of maladaptive cardiac remodeling, was more prominent in KO-HFD mice than in WT-HFD mice (Fig. 2I–L). In principle, all the data suggests that MD1 deletion aggravated HFD-induced maladaptive LV hypertrophy and fibrosis.

Loss of MD1 down-regulated the protein expressions of ion channels in HFD-fed mice

Electrical remodeling is proposed as another mechanism involved in VA pathophysiology. To clarify whether altered

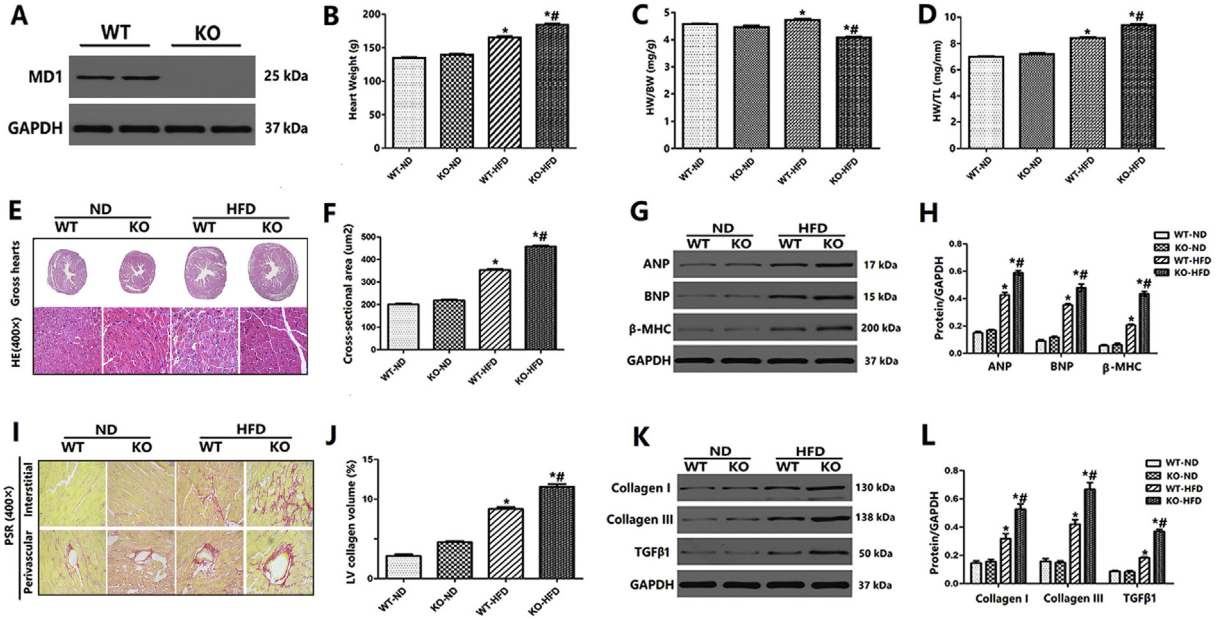

Figure 2 Deletion of MD1 aggravated HFD-induced maladaptive LV hypertrophy and fibrosis. (A) Representative western blots of MD1 expression in LV tissues from WT and MD1-KO mice (n = 6). (B–D) HW, HW/BW, HW/TL values of the indicated groups (n = 8). (E) Gross hearts and H&E staining performed in WT and KO mouse hearts 20 weeks after the ND or HFD feeding (n = 6). (F) Statistical analysis of the cardiomyocyte cross-sectional area (CSA) from H&E-stained slices of LV from WT and KO mouse after 20 weeks ND or HFD feeding (n = 100 + cardiomyocytes in four samples). (G, H) Representative western blots and statistical analysis of the hypertrophy markers ANP, BNP and β-MHC in WT and KO mouse hearts after 20 weeks ND or HFD feeding (n = 4). (I) PSR staining of histological sections prepared from LV samples of WT and KO mouse hearts after 20 weeks ND or HFD feeding (n = 6). (J) Statistical analysis of the LV collagen volume (%) in PSR-stained slices of WT and KO mouse after 20 weeks ND or HFD feeding (n = 25 + fields in four samples). (K, L) Representative western blots and statistical analysis of the fibrosis markers collagen I, collagen III and TGFβ1 in WT and KO mouse hearts after 20 weeks ND or HFD feeding (n = 4). Data are expressed as mean ± SEM, $^{*}p < 0.05$ vs. WT-ND group, $\#p < 0.05$ vs. WT-HFD group.

ion channel expression underlies the prolongation of APD, we assessed the protein expression levels of some potassium and calcium channels in mouse heart.

The protein expressions of Kv4.2 and Kv4.3, which both encode $I_{to,f}$ [23,24], were significantly reduced in the HFD-fed mice. The protein levels of Kv1.5 and Kv2.1, which are used to form $I_{K,slow}$ in mice [25], were also significantly reduced in HFD-fed mice. The protein expression of Cav1.2, the pore-forming subunit of $I_{Ca\text{-}L}$ [26], was markedly reduced in the HFD-fed heart, compared with the ND-fed groups. Reduction in the protein expressions of above channels was far greater in the KO-HFD group (Fig. 3A and B). Above results indicate that MD1 deficiency could prominently down-regulate the expressions of ion channel proteins in HFD-fed mice.

MD1 regulated TLR4/MyD88/CaMKII signaling pathway in the LV after HFD feeding

The foregoing results indicate that MD1-KO may have facilitated HFD-induced LV structural and electrical

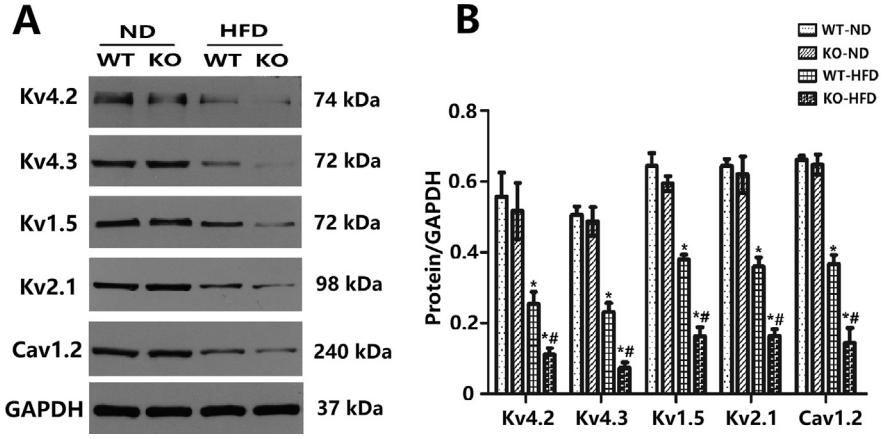

Figure 3 Loss of MD1 decreased the protein expression of ion channels in HFD-fed heart. (A, B) Representative western blots and statistical analysis of Kv4.2, Kv4.3, Kv1.5, Kv2.1 and Cav 2.1 in WT and KO mouse hearts after 20 weeks ND or HFD feeding. Data are expressed as mean ± SEM, n = 4 mice per group. $^{*}p < 0.05$ vs. WT-ND group, $\#p < 0.05$ vs. WT-HFD group.

remodeling. However, the underlying mechanism by which MD1-KO exerts is unclear. The TLR4/MyD88/CaMKII signaling pathway had been reported to be imperative in regulating LV remodeling [11–16]. In addition, our previous studies showed MD1 could affect CaMKII expression [20]. Thus, we investigated the TLR4/MyD88/CaMKII signaling pathway to identify the effect of MD1 in LV remodeling. As shown in Fig. 4A and B, the expressions of TLR4, MyD88, CaMKII and CaMKII phosphorylation (p-CaMKII) were obviously enhanced in KO-HFD mice, indicating MD1 deletion could strongly active the TLR4/MyD88/CaMKII signaling pathway. In consequence, MD1 could interfere HFD-induce LV remodeling to a certain degree by modulating TLR4/MyD88/CaMKII signaling pathway.

Discussion

The major novel findings of this study are as follows: 1) HFD-induced metabolic disorder, LV dilation, prolonged QTc interval were deteriorated in MD1-KO mice compared with WT mice; 2) Loss of MD1 increased HFD-induced vulnerability to VA, as shown by the prolonged APD, enhanced APD alternans thresholds and greater incidence of VA; 3) Loss of MD1 further exacerbated LV hypertrophy and fibrosis and down-regulated the protein expressions of ion channels (Kv4.2, Kv4.3, Kv1.5, Kv2.1 and Cav1.2) in KO-HFD mice compared with WT-HFD mice; 4) In the setting of HFD feeding, MD1 deletion increases the vulnerability to VA through the enhanced activation of the TLR4/MyD88/CaMKII signaling pathway. These findings demonstrated that MD1 may significantly affect vulnerability to VA post HFD feeding.

Nowadays, obesity has become an epidemic, and represents one of the most prevalent disorders. The preponderance of evidence reveals that obesity is associated with ventricular repolarization, particularly lengthening of the QTc interval [27]. Prolongation of the QT interval, which is a hallmark of the abnormally altered electrophysiology or adverse electrical remodeling, increases the risk of arrhythmias and SCD [28,29]. In a recent study, HUNAG and his coworkers found that QTc intervals were increased in HFD mice compared with ND mice [6]. The above-mentioned finding is also detected in this study as the QTc interval was increased in HFD mice compared with ND mice. In addition, this study also detected that the HFD group showed a prolonged APD and elevated incidence of VA in langendroff-perfused hearts in ex vivo. Similarly, a previous study had also indicated that HFD feeding increased arrhythmia inducibility, prolonged APD, and elevated APD alternans thresholds [15]. At last, our previous study found MD1 deficiency could worsen increased vulnerability to atrial fibrillation in obesity mice [21]. Similar with our previous findings, in this study, we found that compared with WT-HFD mice, KO-HFD mice showed a more prolonged APD, enhanced APD alternans thresholds and greater incidence of VA. Thus, we propose that MD1 deficiency could worsen the already increased vulnerability to VA following HFD feeding.

Clinical research showed obesity-related cardiomegaly, LV dilation, and myocyte hypertrophy are all important pathological manifestations of SCD [30]. A recent study also suggested that LV interstitial fibrosis could markedly increase VA inducibility in HFD-fed mice [15]. In the present study, HFD feeding induced LV hypertrophy, fibrosis, LV dilation, and these effects were worsened by MD1 deletion. Thus, loss of MD1 may have exacerbated the LV hypertrophy and fibrosis, and therefore facilitated ventricular reentry forming and increased the VA vulnerability in HFD-induced obese mice.

Apart from structural remodeling, electrical remodeling is proposed as another mechanism involved in VA pathophysiology. A study conducted by Zhong has reported expressions of several ion channels have been associated with LQTS development by regulating APD in the ventricle [15]. In this experiment, HFD-fed mice heart showed decreased protein levels of Kv4.2, Kv4.3, Kv1.5 and Kv2.1, which may be decreased the outward current. Several studies have shown that expression reduction of the potassium channel proteins would induce a prolonged APD [31,32].

Furthermore, decreased expression of Cav1.2, which is used to encode $I_{Ca\text{-}L,}$ was observed in HFD-fed mice. This

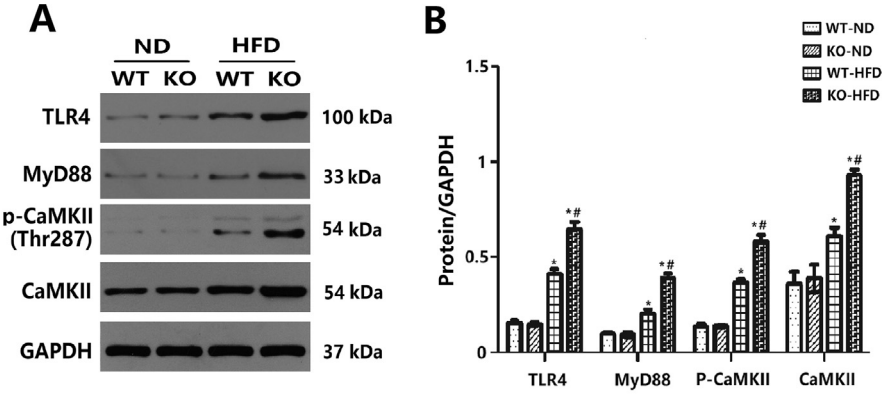

Figure 4 MD1 regulated the activation of TLR4/MyD88/CaMKII signaling pathway in HFD-fed heart. (A, B) Representative western blots and statistical analysis of TLR4, MyD88, CaMKII and p-CaMKII in WT and KO mouse LV tissues after 20 weeks ND or HFD feeding. Data are expressed as mean ± SEM, n = 4 mice per group. $^{*}p < 0.05$ vs. WT-ND group, $\#p < 0.05$ vs. WT-HFD group.

result is in agreement with previously published works, which showed a decrease in calcium channel protein levels and current density of $I_{Ca\text{-}L}$ in an obese animal model [33–35]. Interestingly, $I_{Ca\text{-}L}$, an inward current, was decreased, while the APD in our results was extended. There are two possible reasons for this. Firstly, the expressions of potassium channel proteins in the HFD-fed heart were down-regulated, resulting in the decrease of outward current. And compared with the decrease of L-type calcium channels, the decrease of outward currents may have a stronger effect on prolonging the APD. Secondly, the study conducted by Lin has reported a similar situation, in which the peak current of $I_{Ca\text{-}L}$ decreased, but the APD prolonged due to continuous inward current caused by defective calcium inactivation [33].

All of above HFD-induced ion channel changes, which could prolong APD and enhance automaticity, were further exacerbated in MD1-KO mice compare with WT mice. As the above prolong APD and enhance automaticity can increase the VA vulnerability in HFD-induced obese mice, we suggest that loss of MD1 would significantly increase vulnerability to VA in the setting of HFD-fed obesity.

The underlying mechanisms by which MD1 regulates LV remodeling may involve the TLR4/MyD88/CaMKII signaling pathway. Previous studies showed that TLR4 activation can sustain activation of CaMKII signaling in stressed myocardial tissues [11,13]. TLR4 is the upstream signal of cardiac CaMKII under hyperlipidemia conditions, and CaMKII is crucial for obesity-induced cardiac remodeling [14,15]. Activation of CaMKII can impact the expression levels and function of these downstream target proteins, which may eventually lead to cardiac dysfunction and arrhythmia [36,37]. Our previous study showed that MD1 deletion led to a more pronounced activation of TLR4/MyD88/CaMKII signaling pathway, which may further exacerbate LV remodeling in response to chronic pressure overload [20]. Consistent with previous findings, this study revealed that the expression and activity of CaMKII were markedly increased after HFD feeding, and these effects were exacerbated in KO-HFD mice. Therefore, we propose that MD1 deletion enhances the activation of TLR4/MyD88/CaMKII signaling pathway, facilitating the LV remodeling developed in the setting of HFD feeding to some extent.

Conclusion

In summary, the present study indicates that MD1 deficiency increased HFD-induced vulnerability to VA. This is mainly caused by the aggravated maladaptive LV hypertrophy, fibrosis and decreased protein expressions of ion channels, which are all induced by the enhanced activation of the TLR4/MyD88/CaMKII signaling pathway.

Conflicts of interest

The authors declare that they have no conflict interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2019.06.004.

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