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RESEARCH

# Opposite effects of $G\alpha_{i2}$ or $G\alpha_{i3}$ deficiency on reduced basal density and attenuated β-adrenergic response of ventricular $Ca^{2+}$ currents in myocytes of mice overexpressing the cardiac $\beta_1$-adrenoceptor

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## Abstract

$Ca^{2+}$ currents ($I_{CaL}$) carried by ventricular L-type $Ca^{2+}$ channels (LTCC) are altered in failing hearts, and increased LTCC activity is discussed as a cause of cardiomyopathy. We have shown that lack of the inhibitory G-protein isoform $G\alpha_{i3}$ improves cardiac outcome and survival in a murine heart-failure model of cardiac $\beta_1$-adrenoceptor ($\beta_1$-AR) overexpression ($\beta_1$-tg), while lack of the $G\alpha_{i2}$ isoform was detrimental in the same heart-failure model. Given the potential role of LTCC and their modulation by β-adrenergic signalling, we now analysed ventricular $I_{CaL}$ in $\beta_1$-tg mice and in $\beta_1$-tg mice lacking either $G\alpha_{i2}$ or $G\alpha_{i3}$. Using the patch-clamp technique, we recorded whole-cell $I_{CaL}$ in ventricular myocytes freshly isolated from adult mice. Compared to age-matched wild-type littermates, basal $I_{CaL}$ was reduced in myocytes from $\beta_1$-tg mice both under basal conditions (− 8.1 ± 1.6 vs. − 5.5 ± 1.5 pA/pF) and upon β-adrenergic stimulation with 1 µM isoproterenol (− 14.3 ± 5.6 vs. − 7.4 ± 1.9 pA/pF). Lack of $G\alpha_{i3}$ normalised basal $I_{CaL}$ to nearly wild-type levels (− 7.5 ± 1.6 pA/pF), while β-adrenergic response remained attenuated (− 9.5 ± 3.6 pA/pF). In contrast, the absence of $G\alpha_{i2}$ did not restore basal $I_{CaL}$ (− 5.7 ± 1.8 pA/pF), but restored the β-adrenergic response of $I_{CaL}$, with the difference from basal current even exceeding that in wild-type mice (− 12.2 ± 2.9 pA/pF).We propose that by restoring basal $I_{CaL}$, $G\alpha_{i3}$ deficiency might contribute to the restoration of contractility in $\beta_1$-tg mice, while maintaining attenuation of the $I_{CaL}$ response upon β-adrenergic stimulation protects against deleterious effects mediated by enhanced β-AR signalling. In contrast, restored and even enhanced $I_{CaL}$ response to β-adrenergic stimulation might contribute to detrimental effects of $G\alpha_{i2}$ deficiency observed in $\beta_1$-tg mice previously.

**Keywords** Calcium channel · Adrenergic receptor · Inhibitory g protein GI · Heart failure · Cardiomyopathy · Transgenic mouse

## Introduction

Alterations of ventricular L-type $Ca^{2+}$ currents ($I_{CaL}$) have been associated with cardiomyopathy and heart failure in animal models and humans (Mukherjee and Spinale 1998; Richard et al. 1998; Schröder et al. 1998; Chen et al. 2002, 2008; Nakayama et al. 2007; Beetz et al. 2009). β-adrenoceptor (β-AR) overexpression and lack of $G\alpha_i$ isoforms play a role both in the modulation of ventricular $I_{CaL}$ and the development of cardiomyopathy (Engelhardt et al. 1999; Liggett et al. 2000; Foerster et al. 2003, 2004; Keller et al. 2015; Schröper et al. 2024). In the murine heart-failure model of $\beta_1$-AR overexpression ($\beta_1$-tg) (Engelhardt et al. 1999), an additional lack of $G\alpha_{i2}$ ($G\alpha_{i2}^{-/-}$) led to early-onset heart failure in mice with cardiac overexpression of $\beta_1$-AR (Keller et al. 2015). In contrast, we recently found that the heart-failure phenotype of $\beta_1$-tg mice is prevented or at least delayed by additional $G\alpha_{i3}$ deficiency (Schröper et al. 2024). Given the link between ventricular $I_{CaL}$ and cardiac (dys-)function, we performed whole-cell $I_{CaL}$ recordings using ventricular myocytes isolated from $\beta_1$-tg mice and $\beta_1$-tg mice lacking either $G\alpha_{i2}$ or $G\alpha_{i3}$ with an explorative intention. Our results revealed differences in ventricular $I_{CaL}$ under basal conditions and upon β-adrenergic stimulation, which hint towards mechanisms underlying isoform-specific roles of $G\alpha_i$ proteins.

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## Material and methods

For details, please refer to supplementary data.

On a C57BL/6J background, mice with cardiac overexpression of the human $\beta_1$-AR (Keller et al. 2015) were crossbred with mice globally lacking either $G\alpha_{i2}$ (Dizayee et al. 2011) or $G\alpha_{i3}$ (Gohla et al. 2007). Male mice at an age of 4–5 ($\beta_1$-tg/$G\alpha_{i2}^{-/-}$) or 10–11 months ($\beta_1$-tg/$G\alpha_{i3}^{-/-}$) were investigated. Age-matched wild-type and $\beta_1$-tg mice were used for comparison. The federal state authority approved animal breeding, maintenance and experiments (references: 84-02.04.2016.A422 and 81-02.04.2022.A141). All animal experiments complied with the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

Ventricular myocytes were isolated by retrograde perfusion of freshly excised hearts with collagenase-containing solutions, kept at room temperature and subjected to patch-clamp experiments within 2–8 h.

By patch-clamp technique, we recorded ventricular whole-cell $I_{CaL}$. Pipette solution (mM): 120 CsCl, 10 EGTA, 4 Mg-ATP, 5 HEPES, 1 $MgCl_2$; pH 7.2. Bath solution (mM): 137 NaCl, 10 HEPES, 10 glucose, 5.4 CsCl, 2 $CaCl_2$, 1 $MgCl_2$; pH 7.4. I-V curves were obtained at room temperature using a double-pulse protocol. To correct for different cell size, $I_{CaL}$ density was analysed, i.e. peak $I_{CaL}$ divided by membrane capacitance (that was similar in all groups). For analysing voltage dependence of activation, data were fitted by combined Ohm and Boltzmann relation using $I(V) = (V - VR) \times \frac{G_{max}}{(1+\exp\frac{(V_{0.5}-V)}{dV})}$ (Dizayee et al. 2011; Despang et al. 2022). We estimated the half-maximum potential of inactivation from steady-state inactivation curves by fitting with a sigmoidal Boltzmann equation, too (Poomvanicha et al. 2011). In addition to basal conditions, patch-clamp recordings were separately performed using cells incubated with 1 µM isoproterenol for 8–10 min.

Throughout, we present mean values ± standard deviation. More than two groups were compared using one-way ANOVA followed by Bonferroni-corrected post-tests. Two groups were compared using unpaired Student's *t* test or the Mann-Whitney *U* test as appropriate. We considered *p* values $< 0.05$ statistically significant.

## Results

### Impaired ventricular $I_{CaL}$ in $\beta_1$-tg compared to WT cardiomyocytes

In $\beta_1$-tg mice (10–11 months of age), ventricular peak $I_{CaL}$ density was significantly reduced compared to age-matched WT, and $I_{CaL}$ activation was shifted to more positive potentials (Fig. 1; Table S1). $I_{CaL}$ response (peak $I_{CaL}$ density and activation potential) to 1 µM isoproterenol was significantly reduced compared to WT. Thus, $\beta_1$-tg mice showed altered ventricular $I_{CaL}$ both under basal conditions and upon β-adrenergic stimulation at an age, when neither cardiac hypertrophy nor cardiac dysfunction were found in previous studies (Keller et al. 2015; Schröper et al. 2024).

### Additional $G\alpha_{i3}$ deficiency normalizes basal $I_{CaL}$ but not the response to isoproterenol in $\beta_1$-tg cardiomyocytes

$I_{CaL}$ density in $\beta_1$-tg/$G\alpha_{i3}^{-/-}$ mice was higher than in $\beta_1$-tg mice, and not significantly different from WT (Fig. 1; Table S1). There was no right-shift of the activation potential as in $\beta_1$-tg mice. Overlap of $I_{CaL}$ activation and inactivation curves suggests only slight differences with respect to window currents, i.e. currents flowing in a voltage range where inactivation is not yet complete while activation already occurs (Fig. S1). The blunted response to β-adrenergic stimulation mainly persisted in $\beta_1$-tg/$G\alpha_{i3}^{-/-}$ mice. In contrast to $\beta_1$-tg mice, however, isoproterenol shifted $I_{CaL}$ inactivation significantly to more negative potentials. Taken together, $G\alpha_{i3}$ deficiency led to normalization of ventricular $I_{CaL}$ density and activation potential in $\beta_1$-tg mice under basal conditions. Response to β-adrenergic stimulation, however, remained disturbed.

### $G\alpha_{i2}$ deficiency does not restore basal $I_{CaL}$ but response to isoproterenol in $\beta_1$-tg cardiomyocytes

Our current findings on $\beta_1$-tg mice aged 10–11 months suggest that changes in ventricular $I_{CaL}$ precede contractile dysfunction previously observed at about 18 months (Schröper et al. 2024), while in another study, $G\alpha_{i2}$ deficiency in $\beta_1$-tg mice led to heart failure already at 10–11 months of age (Keller et al. 2015). Thus, we investigated $I_{CaL}$ here at an even younger age of 4–5 months. Already at this younger age, peak $I_{CaL}$ density was significantly reduced in $\beta_1$-tg mice under basal conditions, and activation appeared to occur at more positive potentials (Fig. 2; Table S2). Furthermore, $I_{CaL}$ response to isoproterenol incubation was attenuated. Basal $I_{CaL}$ density and activation potentials were not normalized by $G\alpha_{i2}$ deficiency, while inactivation was significantly shifted towards more positive potentials (Fig. S2). Overlapping curves of $I_{CaL}$ activation and inactivation indicated an increased window current in case of $\beta_1$-tg/$G\alpha_{i2}^{-/-}$ compared to both wild-type and $\beta_1$-tg mice, respectively. $I_{CaL}$ response to isoproterenol was at least restored in $\beta_1$-tg/$G\alpha_{i2}^{-/-}$ mice. Isoproterenol caused a statistically significant leftward shift of the $I_{CaL}$ activation potential in all three genotypes, but

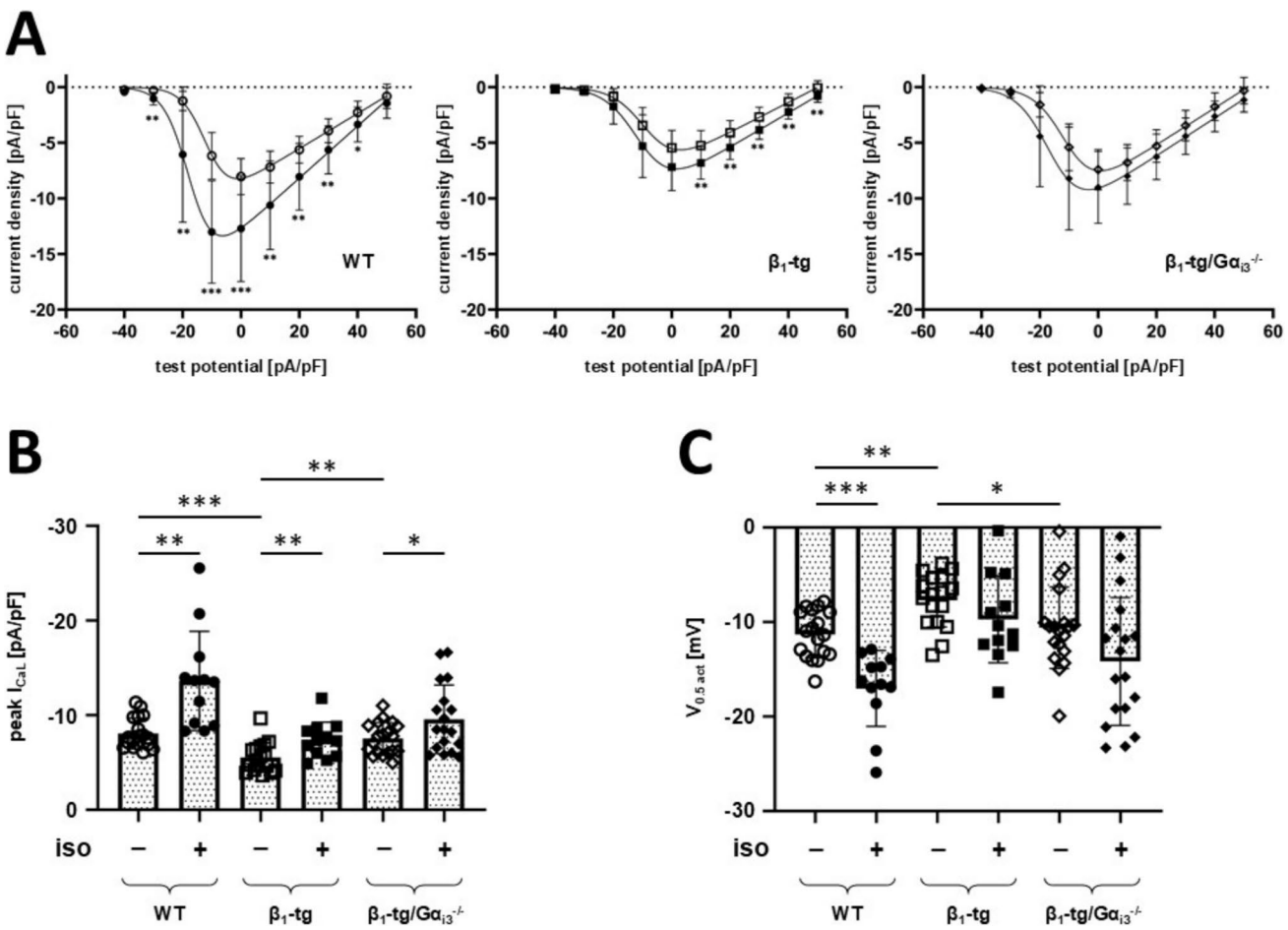


**Fig. 1** In $\beta_1$-tg mice, lack of $G\alpha_{i3}$ reverses the reduction in basal ventricular $I_{CaL}$ but does not restore the response to β-adrenergic stimulation. Current-voltage relationships (**A**) show that both the increase of $I_{CaL}$ density and the shift of activation upon β-adrenergic stimulation (closed symbols) are impaired in mice overexpressing the cardiac $\beta_1$-adrenoceptor ($\beta_1$-tg). Lack of $G\alpha_{i3}$ ($G\alpha_{i3}^{-/-}$) does not reverse this effect. **B** Effect of isoproterenol (iso) on peak $I_{CaL}$ density and **C** on the half-maximum potential of $I_{CaL}$ activation. Data are presented as mean ± SD. Symbols in **B** and **C** represent values derived from individual recordings. $^*p < 0.05$; $^{**}p < 0.01$ and $^{***}p < 0.001$ in multiple unpaired *t* tests (**A**), unpaired *t* tests comparing effects of isoproterenol (**B**, **C**), or Bonferroni-corrected post-tests following one-way ANOVA from comparison of genotypes under basal conditions (**B**, **C**). To test β-adrenergic stimulation, cells were incubated with 1 µM isoproterenol for 8 ± 2 min. Data were obtained in $n = 12$–19 recordings with cells from at least three animals per genotype, aged 10–11 months

compared to WT, this shift was reduced in $\beta_1$-tg, while more pronounced in $\beta_1$-tg/$G\alpha_{i2}^{-/-}$. In contrast to $G\alpha_{i3}$, $G\alpha_{i2}$ deficiency was associated with significant effects on the inactivation rates of $I_{CaL}$ in $\beta_1$-tg myocytes, as reflected by delayed inactivation over almost the entire voltage range (Fig. S3). Even at 4–5 months of age, the absence of $G\alpha_{i3}$ ($\beta_1$-tg/$G\alpha_{i3}^{-/-}$) in contrast to $G\alpha_{i2}$ ($\beta_1$-tg/$G\alpha_{i2}^{-/-}$) appeared to shift basal $I_{CaL}$ properties towards WT levels, while as in $\beta_1$-tg mice, the response to β-adrenergic stimulation was blunted. However, here, data are limited to five recordings under each condition with myocytes from a single animal.

In summary, we found that in contrast to $G\alpha_{i3}$, the lack of $G\alpha_{i2}$ does not normalize basal $I_{CaL}$ in $\beta_1$-tg mice, while it restores or even enhances the response to β-adrenergic stimulation.

## Discussion

Cardiac $\beta_1$-AR overexpression leads to heart failure in mice (Engelhardt et al. 1999; Lee et al. 2015; Schröper et al. 2024). Additional $G\alpha_{i3}$ deficiency was protective (Schröper et al. 2024), while $G\alpha_{i2}$ deficiency exacerbated cardiomyopathy in both $\beta_1$- and $\beta_2$-AR overexpressing mice (Foerster et al. 2003; Keller et al. 2015). In $\beta_2$-tg mice, ventricular $I_{CaL}$ was reduced (Heubach et al. 2001; Foerster et al. 2003, 2004), perhaps due to increased ventricular $G\alpha_{i3}$ expression (Foerster et al. 2003; Dizayee et al. 2011), suggested by enhanced LTCC activity when lacking $G\alpha_{i3}$ (Klein 2009). Consistently, we find $G\alpha_{i3}$ deficiency to revert the reduction in basal $I_{CaL}$ density in $\beta_1$-tg mice. This was not the case in $G\alpha_{i2}$-deficient $\beta_1$-tg mice. Non-selective $G_i$-protein

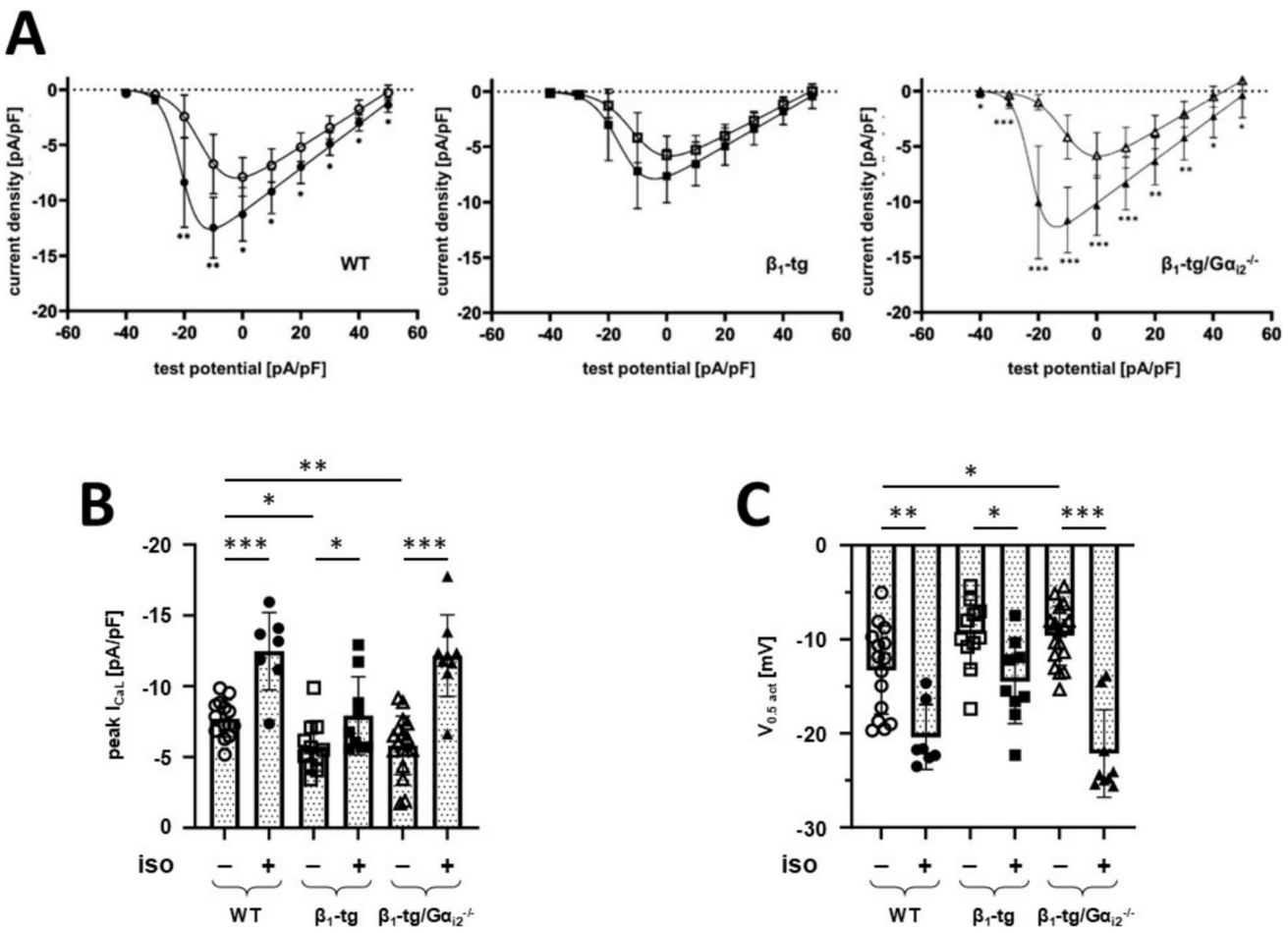


**Fig. 2** In $\beta_1$-tg mice, lack of $G\alpha_{i2}$ restores the β-adrenergic response without influencing the reduced basal $I_{CaL}$. Current-voltage relationships (**A**) indicate a blunted increase of the $I_{CaL}$ density upon β-adrenergic stimulation (closed symbols) by isoproterenol (iso) in mice overexpressing the cardiac $\beta_1$-adrenoceptor ($\beta_1$-tg), which is reversed in $\beta_1$-tg mice lacking $G\alpha_{i2}$ ($\beta_1$-tg/$G\alpha_{i2}^{-/-}$). **B** Respective effects on peak $I_{CaL}$ density and **C** on the half-maximum potential of $I_{CaL}$ activation. Data are presented as mean ± SD. Symbols in **B** and **C** represent values derived from individual recordings. *$p <$ 0.05; **$p < 0.01$ and ***$p < 0.001$ in multiple unpaired $t$ tests (**A**), unpaired $t$ tests comparing effects of isoproterenol (**B**, **C**), or Bonferroni-corrected post-tests following one-way ANOVA from comparison of genotypes under basal conditions (**B**, **C**). To test β-adrenergic stimulation, cells were incubated with 1 µM isoproterenol for 8 ± 2 min. Data were obtained in $n$ = 7–17 recordings with cells from at least three animals per genotype, aged 4–5 months. WT, wild-type littermates

inhibition restored the reduced contractile response to β-adrenergic stimulation in failing cardiomyocytes (Brown and Harding 1992). Thus, the blunted LTCC response to β-adrenergic stimulation in $\beta_1$- and $\beta_2$-tg mice (this study and Foerster et al. 2004) might be explained by $G_i$-protein activity. In heart failure, the (PKA-mediated) response to enhanced β-AR stimulation is detrimental in the long term, and β-AR antagonists can decrease patients' mortality (El-Armouche and Eschenhagen 2009; Baker 2014; Kotecha et al. 2017). Thus, it seems reasonable to consider suppressed β-adrenergic response as protective. We find reduced $I_{CaL}$ response on isoproterenol in $\beta_1$-tg mice with and without $G\alpha_{i3}$ expression. The latter suggests that the above-mentioned restoration of contractile response to β-AR stimulation was not mediated by inhibiting the $G\alpha_{i3}$ isoform. In contrast, our data on $G\alpha_{i2}$ deficiency suggest that preventing effects mediated by this isoform may restore or even enhance the response to β-AR stimulation, which could be detrimental in the long term. The observed effects might be explained not only by the lack of a respective $G\alpha_i$ isoform, but upregulation of the other (Dizayee et al. 2011; Köhler et al. 2014; but: Gohla et al. 2007; Hippe et al. 2013). Our recent studies do not support this reactive change of expression, but we might have missed rather slight alterations (Keller et al. 2015; Schröper et al. 2024). Similarly, different relative expression levels of $\beta_1$- and $\beta_2$-AR might play a role, although a previous study suggests the amount of $\beta_2$-AR negligible in $\beta_1$-tg mice (Keller et al. 2015).

In summary, basal $I_{CaL}$ and its response to β-adrenergic stimulation is altered in ventricular myocytes from mice

overexpressing the cardiac $\beta_1$-AR. Lack of either $G\alpha_{i2}$ or $G\alpha_{i3}$ shows differential effects on these alterations.

## The role of ventricular $I_{CaL}$ in the development and prevention of cardiomyopathy

We used ventricular myocytes of mice at an age apparently preceding the onset of ventricular dysfunction or even an effect on survival for two reasons (Keller et al. 2015; Schröper et al. 2024): first, alterations in ventricular LTCC expression and/or function are already found in compensated hypertrophy (Mukherjee and Spinale 1998; Richard et al. 1998). Second, genetic alterations of ventricular $I_{CaL}$ can lead to cardiac dysfunction, suggesting a causal role of LTCC (Muth et al. 1999; Nakayama et al. 2007; Beetz et al. 2009; Goonasekera et al. 2012).

Increased ventricular $I_{CaL}$ was deleterious in some mouse models (Muth et al. 1999; Nakayama et al. 2007; Beetz et al. 2009). Interestingly, heterozygous knockout of cardiac LTCC expression resulted in reduced $I_{CaL}$ density but also hypertrophy and heart failure (Goonasekera et al. 2012). Compared to wild-type littermates, ventricular $I_{CaL}$ density was reduced in $G\alpha_{i2}$-deficient mice but increased in $G\alpha_{i3}$-deficient mice (Dizayee 2011), but no contractile dysfunction was present in either group (Jain et al. 2001; Keller et al. 2015; Schröper et al. 2024). In $\beta_1$-tg mice, $G\alpha_{i3}$ deficiency was cardioprotective (Schröper et al. 2024) and largely normalized, i.e. increased, ventricular $I_{CaL}$ density, whereas $G\alpha_{i2}$ deficiency, which was detrimental to contractility and survival in $\beta_1$-tg mice (Keller et al. 2015), did not restore basal $I_{CaL}$ density. Regarding an impact on contractility, these results suggest that the mechanism underlying LTCC modulation may play a role.

Since mice in the previous studies died without prior signs (Keller et al. 2015; Schröper et al. 2024) and arrhythmias are the most common cause of death in humans, it is tempting to speculate that the risk of arrhythmias is increased in $\beta_1$-tg/$G\alpha_{i2}^{-/-}$ given the increased window current, which has been associated with rhythm disturbances such as early after depolarizations (Benitah et al. 2010).

We cannot exclude altered LTCC expression, although voltage dependence and response to isoproterenol indicate effects independent of this, and previous studies using $\beta_1$-tg, $G\alpha_{i2}$- and $G\alpha_{i3}$-deficient mice did not indicate such changes (Foerster et al. 2004; Dizayee et al. 2011).

## The possible relevance of a reduced response of ventricular $I_{CaL}$ to β-adrenergic stimulation

Sustained stimulation or overexpression of β-AR leads to ventricular hypertrophy and eventually to heart failure in rodent models (Gomes et al. 2013). Acute β-AR stimulation leads to increased LTCC activity like that observed in human heart failure (Tsien et al. 1986; Yue et al. 1990; Schröder et al. 1998), and increased LTCC activity can lead to cardiomyopathy and heart failure in mice (Nakayama et al. 2007; Beetz et al. 2009). Given the life-prolonging effect of heart failure treatment with β-AR antagonists, one might speculate that the reduced $I_{CaL}$ response to β-adrenergic stimulation observed in human and murine heart failure (Schröder et al. 1998; Muth et al. 1999; Groner et al. 2004; Foerster et al. 2004; Chen et al. 2008; Beetz et al. 2009) is a protective mechanism, albeit insufficient or decompensating in the long term. Thus, protective effects of $G\alpha_{i3}$ deficiency in $\beta_1$-tg mice might be due to maintained attenuation of $I_{CaL}$ response to β-AR stimulation, whereas in contrast, the deleterious effects of $G\alpha_{i2}$ deficiency might be linked to restoration of the $I_{CaL}$ response to β-adrenergic stimulation, i.e. lack of protection against or increased susceptibility to β-adrenergic stimulation. In addition to the increased window currents under basal conditions, $I_{CaL}$ inactivation was delayed upon β-AR stimulation in $G\alpha_{i2}$-deficient $\beta_1$-tg mice compared to WT or mice solely overexpressing the cardiac $\beta_1$-AR. This might as well contribute to arrhythmia as seen for example with mice expressing a mutated LTCC pore (Cheng et al. 2011; Drum et al. 2014). Studies on the modulation of $I_{CaL}$ by muscarinic acetylcholine receptors or β-AR suggest an isoform-specific role of $G\alpha_{i2}$ or $G\alpha_{i3}$ (Nagata et al. 2000; Foerster et al. 2003; Klein 2009). This might involve differential regulation of PKA activity that is known to regulate $I_{CaL}$ activity (Papa et al. 2022). Of note, we recently found $G_i$-isoform-specific differences in phosphorylation of the PKA target phospholamban (Schröper et al. 2024).

## Limitations

Our study is subject to certain limitations. The age of mice used in our experiments was chosen with respect to *in vivo* findings from earlier studies (Keller et al. 2015; Schröper et al. 2024). We cannot exclude that cardiac function is affected already at the age of mice we used now. Again considering previous findings, we used mice at different ages for experiments on either $G\alpha_{i2}$ or $G\alpha_{i3}$ deficiency. Of note, $I_{CaL}$ alterations in $\beta_1$-tg mice were similar at either age, and differences between $\beta_1$-tg mice lacking either $G\alpha_{i2}$ or $G\alpha_{i3}$ were similar at 4–5 months of age, though indicated by experiments with myocytes from only one $\beta_1$-tg/$G\alpha_{i3}^{-/-}$ animal. Experiments with animals of the respective other age are necessary to confirm the hints we found. Since we used exclusively male mice, experiments should be repeated with females. We discuss previous findings obtained with the same mouse lines. Properties of mouse models can change over time. However, not least for animal welfare reasons, it is difficult to repeat experiments. On the other hand, repeating some experiments with animals of the same genotype but a different age seems reasonable. We do not provide sufficient data on molecular mechanisms

underlying our findings. Thus, future studies are needed to address the issues discussed and furthermore analyse interaction partners involved in, e.g., adrenergic signalling.

## Summary and conclusion

Given the limitations of your study, we conclude with caution. We assume that $G\alpha_{i3}$ deficiency contributes to the restoration of contractility in $\beta_1$-tg mice by restoring basal ventricular $I_{CaL}$, whereas maintained attenuation of $I_{CaL}$ response to β-adrenergic stimulation protects against deleterious effects of enhanced β-AR signalling. In contrast, restored or even enhanced $I_{CaL}$ response to β-AR stimulation might explain detrimental effects of $G\alpha_{i2}$ deficiency observed in $\beta_1$-tg mice previously (Keller et al. 2015). Of course, other factors besides $I_{CaL}$ may be relevant for the effects of $G\alpha_{i2}$ or $G\alpha_{i3}$ deficiency. Overall, our current and previous data suggest that isoform-specific effects of inhibitory G proteins should be further explored regarding new options for treatment or prevention of heart failure.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00210-025-03999-y.

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**Author contributions** JM and NK designed the research. NK conducted the experiments and analysed the data, supervised by JM. JM wrote a first draft of the manuscript, NK and JM finalised and approved it. The authors declare that all data were generated in-house and that no paper mill was used.

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**Data availability** All source data for this work (or generated in this study) are available upon reasonable request.

## Declarations

**Ethics approval** The responsible federal state authority approved animal breeding, maintenance and experiments (Landesamt fuer Natur-, Umwelt- und Verbraucherschutz Nordrhein-Westfalen; references: 84-02.04.2016.A422 and 81-02.04.2022.A141). All animal experiments complied with the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

**Competing interests** The authors declare no competing interests.



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