Cardiomyopathy phenotypes in human-induced pluripotent stem cell-derived cardiomyocytes—a systematic review

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Abstract

Human-induced pluripotent stem cells (hiPSC) can be differentiated to cardiomyocytes at high efficiency and are increasingly used to study cardiac disease in a human context. This review evaluated 38 studies on hypertrophic (HCM) and dilated cardiomyopathy (DCM) of different genetic causes asking to which extent published data allow the definition of an in vitro HCM/DCM hiPSC-CM phenotype. The data are put in context with the prevailing hypotheses on HCM/DCM dysfunction and pathophysiology. Relatively consistent findings in HCM not reported in DCM were larger cell size (156 ± 85%, n = 15), more nuclear localization of nuclear factor of activated T cells (NFAT; 175 ± 65%, n = 3), and higher β-myosin heavy chain gene expression levels (500 ± 547%, n = 8) than respective controls. Conversely, DCM lines showed consistently less force development than controls (47 ± 23%, n = 9), while HCM forces scattered without clear trend. Both HCM and DCM lines often showed sarcomere disorganization, higher NPPA/NPPB expression levels, and arrhythmic beating behaviour. The data have to be taken with the caveat that reporting frequencies of the various parameters (e.g. cell size, NFAT expression) differ widely between HCM and DCM lines, in which data scatter is large and that only 9/38 studies used isogenic controls. Taken together, the current data provide interesting suggestions for disease-specific phenotypes in HCM/DCM hiPSC-CM but indicate that the field is still in its early days. Systematic, quantitative comparisons and robust, high content assays are warranted to advance the field.

Keywords hiPSC · Disease modelling · Cardiomyopathy · Quantitative phenotypes

Introduction

The seminal discovery of means to reprogram human somatic cells into embryonic stem cell-like induced pluripotent stem cells (hiPSC; [89]) opened the possibility to generate patient- and disease-specific hiPSC lines and study disease mechanisms in an individualized and human context. An underlying assumption is that human diseases can be studied in hiPSC-derived differentiated cells cultured in vitro or, in other words, that such cells exhibit disease-specific phenotypes. Indeed, soon after the discovery by Yamanaka and colleagues, the first papers appeared that reported specific abnormalities in the function of patient-derived hiPSC derivatives compared to unrelated genetically normal controls. In the cardiac field, the first examples were longer action potentials in hiPSC-cardiomyocytes (hiPSC-CM) from patients with genetically determined long QT syndrome 1 (LQT1 [68] or LQT2 [40]) and larger cells with a higher degree of sarcomeric organization and preferential localization of NFATc4 in the nucleus in hiPSC-CM from a patient with Leopard syndrome [11]. In the meantime, most genetically determined cardiac diseases have been studied in hiPSC-CM and generally revealed some phenotypic abnormalities that have been described before in native cardiomyocytes from patients with the respective disease. However, it was soon realized that hiPSC-CM are relatively immature cells (for review, see [105]) that exhibit large phenotypic heterogeneity, e.g. in terms of action potential width and shape [68], cell size and sarcomeric organization. Reasons include variability of the original somatic cells used for reprogramming (e.g. skin cells with mosaic mutations or...
variable levels of epigenetic modifications [53], the reprogramming procedure itself [46], differentiation protocols with less than 100% efficiency, a varying level of maturity in hiPSC-CM in culture as well as methodological issues such as the difficulty to measure action potentials in small cells by patch clamping [38]). The recent introduction of transcription activator-like effector nuclease-mediated gene correction (TALEN) or CRIPSR/Cas9-based methods for gene editing has increased the level of trust in the conclusion that the observed phenotypes were indeed the consequence of the suspected gene mutation [4]. The reader is referred to several excellent reviews on this subject (e.g. [8, 70, 107]).

Hypertrophic and dilated cardiomyopathy—clinical phenotype and pathophysiology

This review will concentrate on the question to which extent a specific “cardiomyopathy phenotype” exists, which can be studied in hiPSC-CM in the dish. It restricts itself to hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), because they are the two most common and clinically relevant cardiomyopathies, often have a defined genetic cause, have been most often studied in hiPSC-CM and present with relatively clearly defined and partially opposing clinical phenotypes (Table 1). The key morphological features of HCM are thickened left ventricular (LV) walls in the absence of apparent hemodynamic reason (e.g. aortic stenosis, severe hypertension). Hypertrophy preferentially affects the interventricular septum, whose thickness is commonly used as inclusion criterion for patients in clinical studies. HCM is generally associated with a normal or rather small LV cavity, preserved LV systolic contractile function and early diastolic dysfunction [37, 66, 102]. Most patients develop various degrees of LV obstruction [60]. Histomorphological signs of HCM are myocardial disarray and increased fibrosis. DCM in contrast is characterized by LV systolic dysfunction, dilation of LV cavities and normal wall thickness. While HCM is the prototypic genetic cardiomyopathy (likely disease-causing mutations can be found by cardiac gene panel, exome or whole genome sequencing in approximately 32–70% of cases [2, 16, 58, 78]), DCM is classified as a mixed cardiomyopathy, which is familial in ~20–35% [25, 60], and a recent whole exome sequencing identified mutations in only 12% of cases [58]. The majority of DCM cases are caused by (mainly viral or parasitic) infection, toxins such as alcohol or anti-tumour agents and mitochondrial disorders.

While the partially opposing clinical pictures of HCM and DCM allow a relatively straightforward clinical differentiation, overlaps between the two types of cardiomyopathies exist. Both HCM and DCM exhibit increased serum levels of brain natriuretic peptide and cardiac fibrosis, and HCM patients can develop severe systolic dysfunction requiring heart transplantation. Both can lead to life-threatening ventricular arrhythmias [60] and are accompanied by an increased rate of atrial fibrillation [81, 106] and dilation of the left atrium [36]. Despite the discovery of numerous mutations in genes that underlie HCM and DCM, our understanding of the pathomechanisms leading from the mutation to the phenotype remains incomplete. Reasons are not only the diversity of mutations causing similar clinical pictures, particularly in DCM, the incomplete and highly variable penetrance of both HCM and DCM, but also the fact that mouse models only partially recapitulate the human phenotype. For example, no single mouse model in which a classical HCM mutation in the gene coding for cardiac myosin-binding protein C (MYBPC3, cMyBPC) or β-myosin heavy chain (MYH7, β-MHC) has been introduced in the heterozygous state develop the pathognomonic septal hypertrophy seen in patients (for review, see

| Table 1 | Clinical, morphological and functional characteristics of patients with hypertrophic (HCM) or dilated cardiomyopathy (DCM) |
|-----------------------------|---------------------------------------------------------------|
| **Symptoms and biomarkers** | **HCM** | **DCM** |
| Arrhythmias and sudden cardiac death | Dyspnoea (initially exercise-induced) |
| Atrial fibrillation | Heart failure |
| Exercise-induced dyspnoea | Arrhythmias and sudden cardiac death |
| Heart failure | Atrial fibrillation |
| Increased serum BNP levels | Increased serum BNP levels |
| **Morphology** | **LV hypertrophy ± outflow tract obstruction** | **LV chamber dilatation** |
| Cardiac myocyte hypertrophy (width) | Cardiac myocyte hypertrophy (length) |
| Myofiber/myocardial disarray | |
| Fibrosis | Fibrosis |
| **Function** | **Diastolic dysfunction (pre-hypertrophy stage)** | **Systolic dysfunction** |
| Hypercontractility (inconsistent) | Diastolic dysfunction |
| Systolic dysfunction (late stage) | |
| Energy depletion (early stage) | Energy depletion (early stage) |

Parameters distinguishing between HCM and DCM are marked in italics. Note overlap of many parameters.
Neither has LV obstruction been observed in any such model. Either homozygous knockout or knockin of the respective gene is lethal (as in the case of α-MHC, the rodent pendant on the dominant myosin isoform [28]) or the animals develop severe LV dysfunction (as in the case of cMyBPC [10, 27, 33, 61, 62, 64, 65, 98]). It is also apparent that the mouse work still did not answer a number of fundamental questions: (1) What is the exact physiological role of the sarcomeric proteins most commonly affected in HCM such as cMyBPC and β-MHC? (2) How do they cooperate to ensure proper systolic and diastolic function? (3) What are the specific consequences of even relatively well-studied gene mutations? (4) How do mutations in numerous sarcomeric and non-sarcomeric genes with diverse function lead to the uniform induction of “autonomous” cardiac hypertrophy and disarray in HCM? These questions have been discussed in recent reviews to which the reader is referred [21, 26, 59, 94]. In any case, the experiences with mouse models thus raise the question to which extent they really reflect the human disease and provide an argument to study HCM and DCM in hiPSC-CM.

Another reason for our limited understanding of HCM/DCM pathophysiology is that access to isolated heart tissue and cells from patients with HCM and DCM is sparse, and only very few studies specifically reported on the in vitro phenotype of these diseases (for review, see [22]). The most commonly used sources for human tissues are septum biopsies acquired during surgical correction of LV outflow tract obstruction by myectomy in the case of HCM and LV tissues obtained during implantation of LV assist devices or heart transplantation in case of DCM. Both tissue sources represent a late stage of the disease, raising the question to which extent the abnormalities observed in comparison to (even rarer) non-failing heart tissue comparators reflect primary defects or secondary compensatory changes. The highly fibrotic texture of the terminally diseased tissue imposes a further challenge to such studies as enzymatic isolation of cardiomyocytes requires harsher conditions, introducing a systematic error.

Prevailing in vitro phenotypes of HCM and DCM

Despite the limitations discussed above, some observations prevail and have led to hypotheses that can be tested in hiPSC-CM.

Abnormal myofilament calcium sensitivity The relation between intracellular Ca\(^{2+}\) concentrations and force development of the myofilaments is a highly regulated biological constant with half-maximal force development (of skinned myofibers) at a pCa of \(\sim 5.8 (\sim 1.6 \mu M)\). Numerous studies reported increased Ca\(^{2+}\) sensitivity on HCM [3, 13, 19, 23, 67, 95, 96] and decreased in DCM [19, 20, 56]. The shift in the pCa/force relation leads to more force development at lower Ca\(^{2+}\) concentrations in case of HCM and less force development in DCM. Importantly, the increased Ca\(^{2+}\) sensitivity in HCM also predicts delayed relaxation in the descending part of the intracellular Ca\(^{2+}\) transient. Both consequences are well compatible with the predominant clinical phenotypes of preserved systolic function and diastolic dysfunction in HCM and systolic dysfunction in DCM. Increased Ca\(^{2+}\) sensitivity in HCM would even predict LV hypercontractility at rest, and indeed, a study in 36 mutation carriers found significantly increased LV ejection fraction by echocardiography compared to 36 healthy controls [36]. This observation forms the basis of novel therapeutic concepts to reduce myosin activity by small molecules to treat HCM [30]. However, it is also clear that increased Ca\(^{2+}\) sensitivity in HCM is not a universal finding. Several studies reported HCM mutations to be associated with either no change [33, 101] or decreased Ca\(^{2+}\) sensitivity [88] in various experimental systems, suggesting mutation-specific differences. Of note, even the knockout of a protein such as cMyBPC [13, 33, 48] has been associated with different effects on myofilament Ca\(^{2+}\) sensitivity. The latter emphasizes the importance of the respective experimental context and supports the notion that altered myofilament Ca\(^{2+}\) sensitivity cannot be the sole unifying mechanism underlying HCM or DCM.

Abnormal actin-myosin sliding velocity Another parameter of sarcomere function is the unloaded sliding velocity of thin filaments on immobilized S1-myosin. Several studies indicate that HCM mutations are associated with increased sliding velocity [44, 45, 88] and DCM with decreased sliding velocity [1, 79], and for a review, see [24].

Altered maximal force development Interestingly, both HCM and DCM mutations were found to generally associate with decreased maximal force development [95, 101], but normal or even higher force output and increased force redevelopment have also been reported [48, 92, 102].

Increased Cal\(^{2+}\)-independent cross-bridge cycling in HCM Mutations (or full deletion) of MYBPC3 or cardiac troponin T (TNNT2, TnT) have been associated with a shallow pCa-force relationship (lower Hill coefficient) and residual force development at very low or nominal absence of Ca\(^{2+}\) [3, 56, 75]. In the case of cMyBPC, the effect may be explained by mutations (or its absence) disturbing its normal role in stabilizing the super-relaxed, inactive state (SRX) of myosin heads [63]. The concept implies that one of the abnormalities in HCM is incomplete arrest of crossbridge cycling in diastole, which could participate in diastolic dysfunction and increased energy expenditure.

Decreased energetic efficiency Many HCM mutations lead to decreased energetic efficiency of crossbridge cycling, i.e.
The phenomenon in a general sense indicates less-than-normal functioning of the sarcomeres harbouring mutated sarcoma proteins and may relate to the partial loss of the myosin SRX state in the case of eMyBPc. In any case, it may well contribute to the decreased phosphocreatine/ATP ratio observed in patients with HCM even in the prehypertrophic state [17]. Energy starvation is not specific to HCM. In fact, it has been shown already in 1992 as a common feature of patients with heart failure due to non-ischemic DCM [71]. However, it is possible that the more diverse causes of DCM include both decreased energetic efficiency of the myofilaments with higher energy expenditure and decreased efficiency of mitochondrial energy generation like in Barth syndrome [39].

**Allelic imbalance of β-MHC as a cause of myocardial disarray**

Early work (in skeletal muscle fibres from β-MHC-expressing soleus muscle) showed a high variability of myofilament Ca\(^{2+}\) sensitivity between individual muscle fibres [47]. This observation was later related to marked cell-to-cell differences in the expression of the mutated β-MHC in cardiomyocytes and marked differences in individual Ca\(^{2+}\) sensitivity [49]. The interesting phenomenon could well contribute to another hallmark of HCM, myocardial disarray, by individual cardiomyocyte developing different degrees of contractile force. It is not clear whether allelic imbalance is restricted to β-MHC mutations.

**HCM and DCM phenotypes in hiPSC-cardiomyocytes**

By searching PubMed (keywords: *hiPSC cardiomyocytes* and *hypertrophic cardiomyopathy or dilated cardiomyopathy*), we identified 38 original papers reporting phenotypes of hiPSC-CM either derived from hiPSC lines of patients with HCM/DCM (or related syndromes) or from hiPSC lines in which a HCM or DCM mutation had been genetically introduced (Tables 2 and 3). Initial studies compared the phenotype of disease-related hiPSC-CM to unrelated genetically healthy controls; more recent studies used TALEN or CRISPR/Cas9 gene editing approaches to correct a mutation in a patient-specific line or introduce it into a wild-type line, allowing comparison under isogenic conditions. While most studies validated the absence of off-target effects only at the predicted top-10 sites, one TALEN-based study performed whole exome sequencing and reported in two corrected clones 318 and 1331 de-novo indel mutations, respectively, close to possible off-target sites. The significance of this finding is unclear.

The initial analysis of the papers concentrated on abnormalities in contractile function, based on the hypothesis formulated by Davis and Molkentin that differences between HCM, DCM and wild type (WT) should primarily result in a different tension-time integral of the contraction peak, i.e. the area under the curve of an averaged contraction peak [19]. The hypothesis corroborates the idea that HCM mutations lead to increased, DCM mutations to decreased myofilament Ca\(^{2+}\) sensitivity. As elegantly shown in mouse models with different cardiac troponin C mutations (and in examples of hiPSC-CM), this should lead to higher peak force and prolonged relaxation (T2) in case of HCM and lower peak force and an abbreviated contraction peak (both contraction [T1] and relaxation time [T2]). Unfortunately, contraction kinetics were only studied in a small minority of cases (Tables 2 and 3). Only two studies of a DCM mutation (heterozygous phospholamban (*PLN*) R14del and truncating titin (*TTN*) mutation) showed a representative contraction peak, which indicated prolonged relaxation in one case [43] and lower T1 and T2 in the other [35]. Statistics were not provided. Two papers on HCM mutations (*TNNT2*, *MYH7*) reported statistically evaluated data on T1 and T2, showing no alteration or the expected increase in time of relaxation [69, 100]. Thus, clearly, more work has to be done to decide whether or not HCM/DCM mutations have a systematic effect on contractile kinetics in hiPSC-CM.

Many studies reported peak force, size of intracellular Ca\(^{2+}\) transients, sarcomere structure and gene expression (Tables 2 and 3, Fig. 1). Interestingly, while almost all studies on HCM lines reported cell sizes in 2D culture, only two did in case of DCM lines (Fig. 1). Similar differences in reporting frequency were observed with regard to multinucleation, nuclear NFAT, contraction kinetics (only HCM), ANP/BNP (*NPPA/NPPB*) and rhythmicity (more in HCM) or Ca\(^{2+}\) transient kinetics (only DCM). Reasons are unknown, but a reporting bias appears likely.

Figure 1 summarizes the data from all studies in which functional data were reported in a quantitative manner and presents them compared to the respective controls (log scale; \(n = 16\) HCM, 14 DCM). Three abnormalities appeared to be relatively consistent in both HCM and DCM—sarcomeric disarray (274 ± 81%, \(n = 6\) HCM; 298 ± 146%, \(n = 8\) DCM), increased *NPPA* or *NPPB* gene expression (284 ± 249%, \(n = 11\) HCM; 500%, \(n = 2\)) and arrhythmic behaviour (327 ± 164%, \(n = 12\) HCM; 350%, \(n = 2\) DCM). HCM lines showed an increase in cell size (156 ± 85%, \(n = 15\); DCM +/-), in *MYH7* gene expression (or the ratio of *MYH7/MYH6* (500 ± 547%, \(n = 8\); DCM +/- or reduction) and nuclear accumulation of the transcription factor NFAT (175 ± 65%, \(n = 3\); DCM not determined). The most consistent abnormality in DCM lines was lower peak force development compared to the respective control (47 ± 23%, \(n = 9\); HCM +/- with variability).

Besides the reported disease-associated abnormalities in function, structure or gene expression, it is apparent that absolute values varied largely. For example, reported cell surface area in 2D ranged from 100 \(\mu m^2\) [86] to > 2000 [57, 76], with reported cell volumes from 5.8 [100] to 120 \(\mu m^3\) [69]. Both...
| Mutation       | Disease         | Peak force | T1  | T2  | Cell size | Disarray | Other phenotypes                                                                                     | CRISPR/TALEN Ctr. | Karyotype Ctr. | Reference |
|---------------|-----------------|------------|-----|-----|-----------|----------|-----------------------------------------------------------------------------------------------------|-------------------|----------------|-----------|
| MYH7          | HCM             | n.d.       | n.d.| n.d.| + 60%     | n.d.     | Multinucleation 50 vs. 20%, mRNA of NPPA, NPPB, MYH7 up, higher nuclear NFAT, Ca^{2+}-induced arrhythmias, DAD, higher basal [Ca^{2+}], Iso-induced DAD NPPA, TNNT2 up, MYBPC3–20% in mut, stronger hypertrophic, disarray and NFAT response to ET1, contractile abnormalities linked to disarray | No                | No             | [50]      |
| MYBPC3        | HCM             | n.d.       | n.d.| n.d.| + 20%     | + 50–100%| No further hypertrophic response to stimuli                                                                | No                | Yes            | [90]      |
| MYBPC3        | Pompe           | +/- or -60%| +/- | +/- | n.d.      | n.d.     | Glycogen accumulation, glycan processing abnormality, but normal autophagic flux                        | No                | Yes            | [77]      |
| MYH7          | HCM             | n.d.       | n.d.| n.d.| + 15%     | + 200%   | Higher nuclear NFAT, arrhythmias + 300%, APD prolongation + 60%, resting [Ca^{2+}] up 20%, I_{Ca} and I_{Na} up | No                | No             | [18]      |
| MYBPC3        | Pompe           | n.d.       | n.d.| n.d.| n.d.      | n.d.     | Glycogen accumulation, no functional data                                                                | No                | No             | [82]      |
| BRAF          | HCM Syndrome    | n.d.       | n.d.| n.d.| + 230%    | n.d.     | Irregular Ca^{2+} transients + 400%, MEA FP + 100%                                                     | No                | No             | [73]      |
| BRAF          | HCM Syndromic   | n.d.       | n.d.| n.d.| + 300%    | + 260%   | mRNA of NPPA, NPPB, MYH7 up, PLN down, higher Ca^{2+} transients and store, calcium arrhythmias, "fibroblastic" profibrotic phenotype | No                | Yes            | [41]      |
| FXN           | Friedreich's ataxia | n.d.     | n.d.| n.d.| n.d.      | n.d.     | ROS, unusual iron responses                                                                             | No                | No             | [51]      |
| MYBPC3        | HCM             | n.d.       | n.d.| n.d.| + 200% (M), not clear in T | n.d.     | More multination (40 vs 20%), Ca^{2+} arrhythmias (T, not M), more DAD in M, not T, APD high in T, RMP - 75, NPPA +/-, NPPB, MYH7 and many others up, more in M than T, cMyBPC +/-, TPM up in T | No                | Yes            | [72]      |
| MYH7          | HCM             | - 54%      | n.d.| n.d.| n.d.      | n.d.     | Yes, not quantified                                                                                     | No                | No             | [74]      |
| MYH7          | HCM             | n.d.       | n.d.| n.d.| n.d.      | n.d.     | Skinned myofiber from hiPSC-CM: Fmax 8.2 vs. 18.6 mNmm^-2 (adult 110), KAct + 62%, increased Ca^{2+} sensitivity | N.d.              | Yes            | [80]      |
Table 2 (continued)

| Mutation                      | Disease        | Peak force | T1 | T2 | Cell size | Disarray | Other phenotypes                                      | CRISPR/TALEN Ctr. | Karyotype Ctr. | Reference |
|-------------------------------|----------------|------------|----|----|-----------|----------|------------------------------------------------------|-------------------|----------------|-----------|
| GLA                           | Fabry          | n.d.       | n.d.| n.d.| n.d.      | n.d.     | Galact. down, GB3 accumulation, low beating rate, arrhythmias | No                | Yes            | [15]      |
| c.919+4G>A                    |                |            |    |    |           |          |                                                      |                   |                |           |
| LAMP2                         | Danon          | n.d.       | n.d.| n.d.| n.d.      | n.d.     | Mitochondrial abnormalities, decreased autophagic flux | No                | Yes            | [34]      |
| Het c.129–130insA             |                |            |    |    |           |          |                                                      |                   |                |           |
| Het c.64+1G>A                 |                |            |    |    |           |          |                                                      |                   |                |           |
| MYBPC3                        | HCM            | n.d.       | n.d.| n.d.| +65%      | n.d.     | cMyBPC haploinsufficiency, BNP, MYH7 and others up | Corrected by gene therapy | No             | [76]      |
| Het c.1358_1359insC           |                |            |    |    |           |          |                                                      |                   |                |           |
| PRKAG2                        | HCM + WPW      | n.d.       | n.d.| n.d.| +10–30%  | n.d.     | MDR, APD +/−, If +/−, AP irregularity, RR scatter + 500% | Yes               | Yes            | [5]       |
| Het Arg302Gln                 |                |            |    |    |           |          |                                                      |                   |                |           |
| SCO2                          | HCM syndrome   | +/- (??)   | n.d.| n.d.| n.d.      | n.d.     | Mitochondrial abnormalities, no Iso or Ca2+ response, DAD, arrhythmic response to Iso | No                | Yes            | [31]      |
| Hom c.577G>A                  |                |            |    |    |           |          |                                                      |                   |                |           |
| CpHet c.418G>A/c.17Ins19       | Mitochondrial HCM | n.d.       | n.d.| n.d.| +30%      | n.d.     | NPPA, NPPB, NFA7 up, slightly increased intracellular calcium, SR store, reduced Lc, APD prolonged, arrhythmias, RMP −55, upstroke 5–10 v/s, DAD | No                | Yes            | [52]      |
| MT-RNR2                       |                |            |    |    |           |          |                                                      |                   |                |           |
| m.2336T>C                     |                |            |    |    |           |          |                                                      |                   |                |           |
| MYL3                          | HCM-associated | n.d.       | n.d.| n.d.| +/- (also in mut) | n.d. | No phenotype detected in VUS, mean cell size 1800 μm², NPPA and MYH7 up in the two diseased, contraction and relaxation velocity slightly up, arrhythmias, good stats | Yes               | Yes            | [57]      |
| Het c.170C-A, Exac 0.0001154, |                |            |    |    |           |          |                                                      |                   |                |           |
| introduced 170C-g and MYBPC3   |                |            |    |    |           |          |                                                      |                   |                |           |
| Het p.Val321Met               |                |            |    |    |           |          |                                                      |                   |                |           |
| TNNT2                         |                |            |    |    |           |          |                                                      |                   |                |           |
| Het p.Ile79Asn                |                |            |    |    |           |          |                                                      |                   |                |           |
| MYH7 and MYH6; Het/Hom p.Arg453Cys, |                |            |    |    |           |          |                                                      |                   |                |           |
| frameshift KO, +MYH6 frameshift |                |            |    |    |           |          |                                                      |                   |                |           |
| HCM                           |                |            |    |    |           |          |                                                      |                   |                |           |
| MYH7 and MYH6; Heterozygous   |                |            |    |    |           |          |                                                      |                   |                |           |
| Heterozygous, Hom homozygous, |                |            |    |    |           |          |                                                      |                   |                |           |
| L-type Ca2+ current, L Na+ current, Iso isoprenaline, KATC rate constant reflecting crossbridge turnover rate, LAMP2 lysosome-associated membrane protein 2 gene, MEA Fp multiplexed array field potentials, MT-RNR2 mitochondrial encoded 16S RNA gene, Mut mutation, MYBPC3 cardiac myosin-binding protein C gene/mRNA, MYL3 myosin light chain 3 (MLC1v) gene, MYH6/MYH7 gene or mRNA of α/β-myosin heavy chain, NCX sodium-calcium exchanger, NFA7 nuclear factor of activated T cells, NPPA atrial natriuretic peptide mRNA, NPPB brain natriuretic peptide mRNA, PLN phospholamban gene/mRNA, Pt(s) patient(s), RMP resting membrane potential, ROS reactive oxygen species, RR scatter beat-to-beat irregularity, SERCA2a sarcolemmal reticulum ATPase, SCO2 cytochrome c oxidase assembly protein gene, T1 time to peak force, T2 time from peak to relaxation, TALEN transcription activator-like effector natriuretic peptide-mediated gene correction, TNNT2 cardiac troponin T gene, TnT cardiac troponin T, TPM1 α-tropomyosin, VUS variant of unknown significance | Yes               | Yes            | [100]     |
| Heterozygous                  |                |            |    |    |           |          |                                                      |                   |                |           |
| frameshift KO, +MYH6 frameshift |                |            |    |    |           |          |                                                      |                   |                |           |
| HCM                           |                |            |    |    |           |          |                                                      |                   |                |           |

ANP/BNP atrial/brain natriuretic peptides, AP action potential, APD action potential duration, ALPK3 α-kinase 3, BRAF B-Raf proto-oncogene, serine/threonine kinase, cMyBPC cardiac myosin-binding protein C, CpHet compound heterozygous, CRISPR clustered regularly interspaced short palindromic repeats/Cas9 gene correction, Ctrl control, DAD delayed after depolarizations, Del deletion, Disarray abnormal sarcomeric organization, ET1 endothelin 1, FFR force-frequency relation, Fmax maximal force development, FXX frataxin gene, GAL α-galactosidase A, GB3 glycosphingolipids, Heterozygous, Hom homozygous, IC50 L-type Ca2+ current, IcNa Na+ current, Iso isoprenaline, KATC rate constant reflecting crossbridge turnover rate, LAMP2 lysosome-associated membrane protein 2 gene, MEA Fp multiplexed array field potentials, MT-RNR2 mitochondrial encoded 16S RNA gene, Mut mutation, MYBPC3 cardiac myosin-binding protein C gene/mRNA, MYL3 myosin light chain 3 (MLC1v) gene, MYH6/MYH7 gene or mRNA of α/β-myosin heavy chain, NCX sodium-calcium exchanger, NFA7 nuclear factor of activated T cells, NPPA atrial natriuretic peptide mRNA, NPPB brain natriuretic peptide mRNA, PLN phospholamban gene/mRNA, Pt(s) patient(s), RMP resting membrane potential, ROS reactive oxygen species, RR scatter beat-to-beat irregularity, SERCA2a sarcolemmal reticulum ATPase, SCO2 cytochrome c oxidase assembly protein gene, T1 time to peak force, T2 time from peak to relaxation, TALEN transcription activator-like effector natriuretic peptide-mediated gene correction, TNNT2 cardiac troponin T gene, TnT cardiac troponin T, TPM1 α-tropomyosin, VUS variant of unknown significance
| Mutation          | Disease       | Peak force | T1 | T2 | Cell size | Disarray | Other phenotypes                                                                 | CRISPR/TALEN Ctr. | Karyotype Ctr. | Reference |
|------------------|---------------|------------|----|----|-----------|----------|---------------------------------------------------------------------------------|-------------------|---------------|-----------|
| TNNT2 Het p.Arg173Trp | DCM           | −80% (AFM) | n.d. | n.d. | +/−       | Yes      | Desensitized NE response of rate, RMP −40 mV, APD +/−, smaller Ca²⁺ and caffeine transient (~2 s), TTP and TTD caffeine + 100% (WT 0.5/1.2 s), rescue by SERCA OE, metoprolol rescues disorganization | No                | Yes           | [87]      |
| LMNA Het p.Arg225X (n = 3), another fs mut | DCM           | n.d.       | n.d. | n.d. | n.d.      | n.d.     | Nuclear abnormalities, apoptosis, MEK-inhibition-sensitive, ERK up                | No                | No            | [84]      |
| DES Het p.Ala285Val, +43 stop/gain mut | DCM           | n.d.       | n.d. | n.d. | n.d.      | n.d.     | Morphological abnormalities + 700%, aggregates, peak Ca²⁺ transient +/−, − dF/dt − 40%, abnormal Iso response | No                | No            | [91]      |
| TAZ Het c.517delG Het c.328T>C | Barth | −50–70%  | n.d. | n.d. | n.d.      | Yes      | Decreased mito ox rate, immature cardioliopin, reduced ATP content, excess ROS, tafazzin-sensitive, MitoTempo-sensitive | Yes               | Yes           | [99]      |
| TNN2 p.Arg173Trp | DCM           | −60% (TFM) | n.d. | n.d. | n.d.      | n.d.     | ISO response down, TTP/TTD +/− (~1 s), cAMP response to Iso − 50%, rate − 60%, PDE2,3,5 mRNA several folds up, rescue of cAMP and force by FSK + IBMX | No                | No            | [103]     |
| TTN p.Ser1450fsX4 DMD Dp427m | DCM           | n.d.       | n.d. | n.d. | n.d.      | Yes      | MYH6, MYH7, ACTC1 − 50%, rescue by exon skipping                             | No                | No            | [29]      |
| TTN 3 diff. Truncating mut, CRISPR induction PLN Het p.Arg14del (R14del) RBM20 p.Arg636Ser 2 clones each WT/mut | DCM           | −60–90% (EHT) | n.d. | n.d. | n.d. | n.d. | +400% Stronger phenotype on stiffer posts, rate − 50%, lower SL (1.6 vs. 1.75 μm), lower MYH6/MYH7 ratio, beneficial effects by Poloxamer 188 | No                | No            | [35]      |
| PLN              | DCM           | −60–90%    | n.d. | up | n.d.      | n.d.     | Resting [Ca²⁺] + 30, irregular Ca²⁺ transient + 500%, caffeine transient + 75%, RMP −49 vs. − 58 mV, NPPA/NPPB + 300–900%, MYH7/MYH6 + 60% | Off-target effects | Yes           | [43]      |
| RBM20 p.Arg636Ser 2 clones each WT/mut TNNT2 p.Arg173Trp 2 clones each | DCM           | −40% (EHT) | (+/− (100 μm²)) | n.d. | n.d. | Yes | Increased sarcomere length (WT 1.4 μm), increased Ca²⁺ peak, AUC, decay time at much lower rate | No                | No            | [104]     |
| RBM20 p.Ser635Ala 2 clones each BAG3 KO induced in WT, 2 lines MYBPC3 KO DMD | DCM           | −50% (MTF) | n.d. | n.d. | n.d.       | Yes | Lower rate of cells beat, myosin ATPase − 20%, beneficial effects of omecamtiv | No                | No            | [9]       |
| BAG3 KO induced in WT, 2 lines MYBPC3 KO DMD | DCM           | −50% (MTF) | n.d. | n.d. | n.d.       | Yes | Stronger disarray increased by bortezomib, normal bortezomib-response in MYBPC3-KO | Yes               | Yes           | [42]      |
| DMD              | DCM           | −30–70% (EHT) | n.d. | n.d. | n.d.       | n.d.     | Correction of phenotype by CRISPR                                              | Yes               | No            | [55]      |
Table 3 (continued)

| Mutation | Disease | Peak force | T1 | T2 | Cell size | Disarray | Other phenotypes | CRISPR/TALEN Karyotype | Reference |
|----------|---------|------------|----|----|-----------|----------|------------------|-------------------------|-----------|
| 3 different mut DMPK CTG repeats in, 4 × 6 clones analysed | DM1 Rundown | n.d. n.d. n.d. | n.d. | Nuclear RNA CUG foci, nuclear size +30%, irregularity, MDP −52 vs. −60 mV, APD −20%, AP amplitude −20%, upstroke velocity down, force rundown (AFM); altered splicing of MBNL1, MBNL2, TNNT2, SCN5A; fetal SCN5A isoforms, α-MHC and TnT protein down | No | Yes | [85] |

ACTC1 α-cardiac actin gene, α-MHC α-myosin heavy chain, AFM atomic force microscopy, ANP/BNP atrial/brain natriuretic peptide, AP action potential, APD action potential duration, AUC area under the curve, BAG3 BCL2-associated athanogene 3, cAMP cyclic adenosine monophosphate, CRISPR clustered regularly interspaced short palindromic repeats Cas 9 gene correction, DES desmin gene, Disarray abnormal sarcomeric organization, DM1 myotonic dystrophy type 1, DMD Duchenne muscular dystrophy, DMD dystrophin gene, DMPK dystrophia myotonica protein kinase, EHT engineered heart tissue, ERK extracellular signal-regulated kinase, an important MAPK, F5 frameshift, FSK forskolin, IBMX isobutylmethylxanthine, Ica L-type Ca2+ current, Iso isoprenaline, KO knockout, LMNA lamin A/C gene, MBNL muscle blind-like, MDP maximal diastolic potential, MEK mitogen-activated protein (MAPK) kinase kinase, Mut mutation, MYBPC3 cardiac myosin-binding protein C (cMyBPC) gene, MYH6/MYH7 mRNA of α-β-myosin heavy chain, MYL2 regulatory myosin light chain gene, ventricular isoform (MLC2v), MYL3 essential myosin light chain gene (MLC1v), NPPA atrial natriuretic peptide gene/mRNA, NPPB brain natriuretic peptide gene/mRNA, NE norepinephrine, PDE phosphodiesterase, PLX phospholamban gene, RBA20 RNA binding motif protein 20 gene, RMP resting membrane potential, ROS reactive oxygen species, SCN5A sodium voltage-gated channel alpha subunit 5 (Nav1.5) gene, SERCA OE sarcoplasmic reticulum ATPase overexpression, SL sarcomere length, T1 time to peak force, T2 time from peak to relaxation, TALEN transcription activator-like effector nucleasemediated gene correction, TAZ talazin gene, TFN traction force microscopy, TNNT2 cardiac troponin T gene/mRNA, TPM1 tropomyosin gene, TTD time-to-decay, TTN titin gene, TTP time-to-peak, VEGF vascular endothelial growth factor, WT wild type
Again, it is likely that not only biological differences between hiPSC lines and influences of cell culture conditions and CM maturity but also technical issues explain the large variation. We have shown recently that the sharp microelectrode technique provides more reliable action potential data than patch clamping of single cells [38]. In this study, patch clamp-recorded APD90 in isolated hiPSC-CM amounted only to 119 ± 17 ms (human atrial cardiomyocytes 220 ms, human LV cardiomyocytes 434 ms), while those in intact hiPSC-CM or 3D engineered heart tissue were 271 ms (human right atrial tissue 317 ms, LV tissue 334 ms).

**Conclusion**

The present overview on published reports on the phenotype of HCM/DCM-derived hiPSC-CM allows some preliminary conclusions. (1) The most consistent and to a certain degree differentiating phenotype of hiPSC-CM appears to be decreased force production in DCM, correlating well with the dominant clinical presentation of the disease. (2) HCM lines appear not to exhibit consistent alterations in force development but show increased CM size, nuclear NFAT and increased MYH7 or MYH7/MYH6 ratio. Given the paucity of measurements of these parameters in DCM, it is not possible at present to decide whether these parameters allow a distinction between HCM/DCM phenotypes. (3) Sarcomere disorganization is a common finding in all disease lines and does not appear to allow differentiation between the clinically opposing phenotypes. (4) Overall, the analysis indicates that hiPSC-based disease modelling of cardiomyopathies is still in its early days. Suggestions for a basal set of parameters to be analysed in future studies are given in Table 4. More statistical rigor and robust high content methods are necessary to uncover potentially meaningful but discrete abnormalities of cardiac function in these cells. In this respect, it is interesting to note that only one study evaluated myofilament Ca²⁺ sensitivity in skinned fibres [74], yet myofilament Ca²⁺ sensitivity is one of the most commonly studied parameters in HCM/DCM-related human or animal specimens.

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**Compliance with ethical standards**

**Conflict of interest** T.E. is co-founder of EHT Technologies GmbH, a university spin-off providing equipment for EHT generation and analysis.

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