Precision Medicine and cardiac channelopathies: when dreams meet reality

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Precision Medicine (PM) is an innovative approach that, by relying on large populations’ datasets, patients’ genetics and characteristics, and advanced technologies, aims at improving risk stratification and at identifying patient-specific management through targeted diagnostic and therapeutic strategies. Cardiac channelopathies are being progressively involved in the evolution brought by PM and some of them are benefiting from these novel approaches, especially the long QT syndrome. Here, we have explored the main layers that should be considered when developing a PM approach for cardiac channelopathies, with a focus on modern in vitro strategies based on patient-specific human-induced pluripotent stem cells and on in silico models. PM is where scientists and clinicians must meet and integrate their expertise to improve medical care in an innovative way but without losing common sense. We have indeed tried to provide the cardiologist’s point of view by comparing state-of-the-art techniques and approaches, including revolutionary discoveries, to current practice. This point matters because the new approaches may, or may not, exceed the efficacy and safety of established therapies. Thus, our own eagerness to implement the most recent translational strategies for cardiac channelopathies must be tempered by an objective assessment to verify whether the PM approaches are indeed making a difference for the patients. We believe that PM may shape the diagnosis and treatment of cardiac channelopathies for years to come. Nonetheless, its potential superiority over standard therapies should be constantly monitored and assessed before translating intellectually rewarding new discoveries into clinical practice.

Keywords

Precision Medicine • Cardiac arrhythmias • Cardiac channelopathies • Genetics • Long QT syndrome • Pluripotent stem cells

Introduction

Medicine evolves steadily but sometimes new ideas or discoveries lead to either sudden turns or abrupt jumps forward. It happened with the discovery of blood typing and with the realization that invisible forms of life identifiable by a microscope could cause fatal infections. What followed were the introduction of safe blood transfusions and of specific antibiotics against different types of bacteria. Progressively, these highly selective approaches favoured the development of the term ‘Precision Medicine’ (PM) (still often used interchangeably with the older term ‘Personalized Medicine’), which gained favour because it is objectively attractive and also conveys the reassuring feeling that doctors have therapies that are just ‘right for us’.

Since a few years, this concept is impacting not only the research on inherited cardiac diseases but also their clinical management, and here, we discuss the current role and potential future development of PM for the management of arrhythmogenic disorders of genetic origin. While considering in principle all of them, our focus will be on the long QT syndrome (LQTS) because it is the best characterized
inherited arrhythmia and the one on which we have long-term experience.3,4

As defined by the Precision Medicine Initiative,5,6 ‘Precision Medicine is an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person’. The main purpose of this strategy is to predict treatment and preventive actions, which could be more effective for the individual patient or, more realistically, for groups of patients with common characteristics. This approach is in contrast to the current one-size-fits-all, in which disease treatment and prevention strategies are developed based on the results of randomized trials, without consideration for individual differences. To put it more simply and less elegantly, PM is the opposite of an implantable cardioverter defibrillator (ICD).

We will discuss not only how PM can help to better understand the mechanisms underlying cardiac channelopathies and to improve risk stratification but also which tools are being developed for the identification of patient-specific therapies, including the repurposing of drugs already approved for other uses, a strategy increasingly pursued. Due to space limits, we will not discuss another potentially important aspect of PM, namely gene therapy, which is under progressive development.

While presenting the available approaches and admitting our fascination for PM, especially for its amazing potential to penetrate uncharted territory and our enthusiasm for its intellectual rewards, we will also attempt to honestly recognize its limits—when present—and to preserve common sense when comparing fancy vs. run-of-the-mill approaches. Indeed, besides admiring conceptual elegance, scientists have the responsibility of assessing, as objectively as possible, the superiority of one approach vs. the other.

**Cardiac channelopathies**

The discovery of the first three genes responsible for LQTS in 1995 and 19967–9 had a major impact on the diagnosis and treatment of cardiac arrhythmias of genetic origin. It paved the way to the awareness that genetic variants can produce significant functional alterations in clinical electrophysiology and that, conversely, they could represent potential targets for novel therapies. Ever since, the identification and study of novel disease-causing genes grew exponentially and opened the genetic era for cardiac arrhythmias.

As most of the genes responsible for several inherited arrhythmic diseases encode for ion channels or accessory proteins involved in the excitation–contraction coupling, these disorders are called cardiac channelopathies. The most relevant are LQTS, catecholaminergic polymorphic ventricular tachycardia, Brugada syndrome, and short QT syndrome.3

Being mostly monogenic diseases that manifest early in life, channelopathies represent an ideal model for PM studies since background genetic heterogeneity and the influence of complex environmental and socio-economic factors have generally a low weight in the phenotypic traits of the disease. There are several layers worth considering for the development of PM, all important and with notable intersections (Figure 1). Some of these are discussed in detail below.
Clinical data

Population data collected from large cohorts of patients are the starting point for PM approaches. As the low prevalence of cardiac channelopathies hinders the availability of large sample sizes, the establishment of international registries has allowed the collection and the analysis of clinical data from centres worldwide resulting in significant progress.10–12

For instance, a study on ~700 patients all with arrhythmic events and known genotype has documented that triggers of arrhythmias in LQTS are gene specific.13 LQT1 patients are at risk especially during sympathetic activation, e.g. during physical activity or emotional stress, since they have an impaired slow delayed-rectifier potassium current (IKS) and therefore their QT interval does not shorten as it should during heart rate increases. By contrast, LQT2 and LQT3 patients, whose IKs levels are normal, are at low risk during physical activity. Indeed, whereas 99% of syncope while swimming occur among LQT1 patients, 80% of those triggered by a sudden noise occur among LQT2 patients13, in LQT3 arrhythmias mostly occur during rest/sleep.14 This population-based observation now allows to suspect the genotype simply on the basis of the medical history, before the results of the genetic test, and to implement gene-specific management14 as outlined further down in this section.

The current understanding of LQTS has already allowed clinical decisions that could very well be regarded as aspects of PM, not fancy but effective and dictated by common sense. Two examples are relevant. When in 1995 it was realized that one form of LQTS (i.e. LQT3) was caused by an excess of inward sodium current, we proposed to test in these patients the sodium channel blocker mexiletine to verify whether or not the QT interval would have shortened.15 Our approach was successful and mexiletine for LQT3 patients is now part of standard therapy.16,17 This was the very first gene-specific therapy for LQTS and an early example of PM.

When in 2001 it became evident that the most dangerous time of the day for LQT2 patients was the awakening,13 we decided to shift nadolol administration from the traditional once a day in the morning to twice a day, morning and evening, to ensure an adequate plasma concentration in early morning hours while still asleep.14 This is a simple form of PM, but it is clinically meaningful and shows that understanding the specific, almost ‘private’, aspects of channelopathies impacts on management.

Conversely, it is fair to recognize that the main therapeutic tools used for LQTS, namely β-blockers, left cardiac sympathetic denervation, and ICDs, while being conceptually rather gross interventions, are actually quite effective and have saved the lives of most LQTS patients. As we explore the growing potential of PM using increasingly refined methodologies, the fact that we already have very effective therapies should not be underestimated. To put things in a proper perspective, it might be useful to compare the management of LQTS before and after 1995–1996 (the big divide being represented by the identification of the first three major genes for LQTS).2,9 Until then, the management for patients affected by LQTS, according to the diagnostic criteria in use at that time,18 was essentially the same: β-blockers for all and for those still at risk either left cardiac sympathetic denervation or an ICD.19,20 That was it. Genetics, more than bringing a true revolution led to a major refinement of our management. It became clear, based on the genotype–phenotype correlation mentioned above,13 that LQT1 patients should limit strenuous exercise and that it would be wise for them to swim only in the presence of an adult (one able to swim).2 Similarly, for LQT2 patients, it was recommended to keep the K+ plasma levels at no less than 4 mEq/L to avoid sleep disruption in the post-partum period and sudden noise while asleep. For LQT3 patients, at risk especially during night-time, we recommended sharing the room with an adult or having an audio surveillance system in the room to alert adults in case of gasping noises.21 For them,13 as mentioned above, and more recently also for some LQT2 patients,22 mexiletine was proven useful to shorten QTc. It has also become evident that LQT1 males who, while off therapy, remain without symptoms until age 25 are at extremely low risk of a first cardiac event and may not need therapy, if they so prefer. As to recommendations for exercise and competitive sports, there is uniformity in regarding LQT2 and LQT3 as at lower risk compared to LQT1, but there are differences between the American (more liberal) and the European (less liberal) views.2,23

These changes in management, which may well be labelled as PM, represent not only an undeniable progress in the way we treat and protect our LQTS patients from life-threatening arrhythmias but also a major reward for the efforts to never stop in the search of novel insights into the mechanisms underlying this disease, which constitutes, better than any other, the link between molecular genetics and clinical medicine.4

Genetics

Genetics have a pivotal role in the discovery of new disease-causing genes or variants associated with a specific disease phenotype. Seventeen genes, responsible for or associated with LQTS, have been so far identified.2 The three main ones, KCNQ1 (LQT1), KCNH2 (LQT2) and SCN5A (LQT3), account for ~75% of the clinically defined cases of LQTS, while the minor genes contribute an additional 5% together. About 20% of LQTS cases still do not find a genetic characterization and represent an important opportunity for in-depth diagnostic use of personalized approaches.24

Next-generation sequencing (NGS) technology provides the genome sequence data in hours. The large datasets generated by the NGS technology led to an exponential increase in the amounts of genes and variants associated with specific clinical phenotype but have also caused the proliferation of functionally uncharacterized variants, which represent a non-negligible portion of all genotype-positive patients for a specific disease. For these ‘variants of uncertain significance’ (VUS), the evidence in support of their pathogenicity is lacking or controversial.25 In our clinical databases, ~19% of the variants found in LQT1 and ~33% of those found in LQT2 patients are classified as VUS (Figure 2).

Improvements in diagnostic technologies and the increase in population data worldwide have contributed significantly to the reappraisal and reclassification of genes and variants previously associated with certain disease phenotypes. By strictly revising the criteria with which genes were considered as associated with an LQTS phenotype, Adler et al.26 recently observed that the genotype–phenotype associations behind many genes that were originally identified as disease causing for LQTS were instead limited or disputed for disease causation. Some genes were reclassified due to intrinsic limitations of the ‘candidate gene’ approach in comparison to more modern and
robust genome-wide methods. Another issue included the underestimation of the frequency of the variant in the general population; this led to the conclusion that certain variants, with unknown frequencies at the time of their identification, were common in the general population and even more common in some ethnicities; thus, the disease phenotype was very unlikely to be caused by an autosomal dominant inheritance. Besides the specific case of LQTS, this study indicates that a PM approach to diagnosis and therapy must include a prompt re-evaluation of the interpretation of genetic findings that parallels progress in knowledge and technology.

In addition to variants in coding regions, for which ancillary data such as transcript sequence, protein structure, or functional analyses may facilitate the inference of disease causality, it is necessary to highlight the importance of regulatory variants in non-coding regions of the genome. While current knowledge of the architecture and functional role of regulatory elements in the human genome is scanty, there is a plethora of evidence suggesting that intergenic regulatory elements and non-coding RNAs may play a significant role in both disease causality and disease expression for monogenic disorders. The advent of state-of-the-art genome sequencing technologies will allow functional genomics to explore the contribution of regulatory genetic elements to ultimately explain the incomplete penetrance and variable expressivity observed in LQTS patients.

On a cautionary side, it is important to avoid over-interpretation of genetic findings and assumptions with limited evidence to avoid misdiagnosis and potential harm to patients. Accordingly, it is crucial to identify potentially disease-causing variants and to validate their pathogenicity with high-fidelity in vitro technologies that can circumvent the need of large populations of patients.

**In vitro characterization of variants**

High-throughput approaches based on heterologous systems are a widespread method to acquire information on the pathogenicity of putative disease-causing variants identified with large yield sequencing platforms. Robotic and automated pipelines may allow the rapid electrophysiological characterization of hundreds of variants, significantly contributing to their functional classification and to a more efficient patient risk stratification. However, albeit these high-throughput pipelines should be routinely implemented, it remains evident that the in vitro electrophysiological effects of a variant do not yet necessarily translate into predictable clinical effects for various reasons. First, a variant may have different functionality in immortalized cell lines (e.g. HEK293) transfected with plasmids than in adult cardiomyocytes; this may be due to cell-specific post-translational modifications of ion channels or to different expression levels of accessory subunits or regulatory proteins. The presence of splicing variants further complicates genotype–phenotype correlations and requires additional in vitro studies to identify and characterize the altered transcript and the altered splicing mechanisms prior to any electrophysiological study. However, this additional complexity does not preclude a proper functional characterization, and the putative function of splicing variants can still be captured with an electrophysiological analysis in heterologous systems. Second, the contribution of genetic modifiers may be concealed while approaching the disease characterization in heterologous systems, potentially leading to wrong assumptions of variant pathogenicity that may have relevant clinical implications. Third, the contribution of patient’s genetic background and external factors still cannot be recapitulated by heterologous systems. For instance, the KCNQ1-A341V variant shows a mild in vitro phenotype when expressed in heterologous systems but its in vivo phenotype is very severe. Conversely, the KCNQ1-Y111C trafficking variant exhibits a severe loss of function in heterologous system while patients have a low risk of cardiac events, a condition that might be potentially explained by the concomitant presence of common variants in genes related to protein (ion channel) turnover. Correlating the clinical effect of each variant with their functional in vitro role is a possible first step to identify all the variants that may have outlier or abnormal genotype–phenotype correlations.

**Figure 2** Variants of Unknown Significance. Percentage of variants classified as VUS in our clinical LQT1 and LQT2 patient database. N = LQT1: 153 variants, 29 VUS, 777 subjects; LQT2: 152 variants, 50 VUS, 563 subjects.
However, more physiological cellular models capable to mimic the genetic background in which the variants exert their effect are required for advanced mechanistic studies.

**Patient-specific induced pluripotent stem cell-derived cardiomyocytes**

Human induced pluripotent stem cells (iPSCs) were first generated in 2007 by transforming dermal fibroblasts using retroviruses encoding reprogramming genes. Shortly thereafter, other groups generated human iPSCs by reprogramming different somatic cell sources including mononuclear cells isolated from peripheral blood, a non-invasive method offering clear advantages for the recruitment of patients, especially children.

Human iPSCs can be differentiated into cardiomyocytes (iPSC-CMs). Differentiation efficiency has profoundly increased over time and current protocols generate highly enriched populations of cardiomyocytes with the minimal characteristics required to study channelopathies. As a result, applications and diseases modelled with the iPSC technology have skyrocketed and have validated these cellular models as important preclinical tools for identifying novel mechanisms of disease and for drug screening purposes.

The first study describing LQT1 modelling showed that patient-derived iPSC-CMs summarize the typical electrophysiological features of the disease such as prolongation of action potential duration, abnormal response to the stimulation of catecholamine prevented by β-blockers, and altered activation and deactivation kinetics of IKs. Besides the three main LQTs types, multiple studies further demonstrated the ability of iPSC-CMs to model minor subtypes, including calmodulinopathies.

Correlation between in vitro parameters recorded with Multi-Electrode Arrays (MEAs) and the ECG seem to confirm the potential for iPSC-CMs to model some clinically relevant features in vitro, although large-scale comparisons of electrophysiological parameters from the same donor have still to be generated.

The iPSC-CMs allow researchers to use cells that share ethnicity, gender, and genetic background with the donor, boosting the capabilities to observe and describe patient-specific responses in a human cellular environment comprehensive of the patient’s genetic background. For this reason, disease lines should be matched for gender and ethnicity to perform proper comparisons. The age of the donor should also be considered as somatic cells from older patients have accumulated a larger number of spontaneous mutations that may become relevant after reprogramming. iPSCs derived from relatives, who partially share the genetic background, may help in comparative studies to minimize confounding factors derived from donor-specific genetic background differences that still represent the largest source of genetic variation. Studying multiple cell lines harbouring the same disease-causing mutations should also contribute to dilute potential confounding factors arising from experimental procedures, clonal variability, random variant accumulation, and in vitro selective pressure. A candidate approach to tackle the functional effect of rare genetic variants would ideally involve multiple laboratories in the generation of different iPSC lines for each single variant to triangulate the VUS effect across different centres, cell platforms, and differentiation methods. This in context, genome editing technologies have become a powerful tool to discriminate the effect of VUSs for monogenic disorders as cardiac channelopathies. These approaches integrate two main strategies: (i) insert the variant in a wild-type line to verify whether the variant triggers a disease phenotype or (ii) correct the variant in patient-specific iPSC lines to rescue the disease phenotype. This is also particularly relevant for tackling the intrinsic phenotypic variability exhibited by iPSC-CMs from different lines or donors, and in both approaches each genome edited line represents the proper control for comparison when validating the pathogenicity of a variant.

Operators should also work blindly when investigating VUS pathogenicity to limit bias due to stochastic variations in the data. A remarkable contribution to minimize such variability may come from high-throughput robotics and automated liquid handling platforms, now capable of performing complex tasks as cell culture, line derivation, differentiation, as well as complex functional characterizations.

Such technological advances have also made possible to use the iPSC technology for drug discovery and screening purposes, where new or already marketed drugs are being used to target specific mechanisms underlying different channelopathies. This strategy has the potential to significantly impact the PM concept and promising results are already available.

How to use iPSC-CMs to improve current Precision Medicine strategies

Having said that iPSC technology will help push forward several aspects linked with personalized choices, we also underline that a robust PM strategy should rely more on extensive population data since putting all hopes and efforts in downstream individual patient-specific approaches cannot represent the magic bullet for several reasons. Specifically, it is technically and financially unsustainable at the present state of technology to generate personalized cell lines for each single patient; feature-specific or cohort-specific approaches are instead more realistic. To this end, it is crucial to gather patients in clusters stratified by major factors as age, gender, ethnicity, variant type, and clinical features. The contribution of cell banks will guarantee methodological consistency and proper quality controls. Nevertheless, a personalized approach (i.e. one iPSC line per patient) should not always be discarded since it might be useful for specific situations, for instance, as a filter to evaluate which subjects will likely respond to specific pharmacological treatments.
Use of iPSC-CMs to identify and characterize genetic modifiers

Personalised approaches to study LQTS can also foster the discovery of subtle regulatory mechanisms that would have hardly been captured with large population studies. The incomplete penetrance of LQTS manifests in individuals affected by identical disease-causing mutations having a variable expressivity of the disease phenotype. Combinations of patient-specific iPSC-CMs, genetics, and genome editing have already allowed the identification of putative modifier genes involved in the phenotype of LQT1 and LQT2. Both studies started from LQTS families in which patients showed a different disease severity despite the presence of an identical disease-causing mutation. Subject-specific iPSC-CMs reproduced the variable phenotype severity of their donors in vitro on action potential duration, field potential duration (a gross in vitro equivalent of the QT interval) and ion current levels. Whole exome sequencing allowed the precise identification of variants putatively regulating genotype–phenotype discordance. The correction of these variants by CRISPR/Cas9 genome editing either rescued or unmasked the LQTS disease phenotype, respectively, confirming the damaging and protective role of these variants. The data were further confirmed in small cohorts of patients harbouring the variants but large population studies will now be required to potentially extend these results to other LQTS variants or subtypes. The identification of factors influencing disease severity contributes to a more precise risk stratification system that could impact clinical management (Figure 3).

In addition to these small-scale and patient-specific approaches, factors capable of shaping disease severity may potentially be discovered through genome-wide association studies (GWASs). Despite the requirement of large populations of patients, GWAS-based approaches can identify loci harbouring genetic variants affecting the
QT interval duration in the general population and may provide a reliable starting point for more patient-specific analyses on specific subpopulations of patients. This was the case for two common variants in the nitric oxide synthase 1 adaptor protein (NOS1AP) gene, previously identified as a modulator of QT interval in the general population through a GWAS,86 which was later associated with an increased risk of life-threatening arrhythmias in an LQT1 founder population87 and whose mechanism of action was characterized using patient-specific iPSC-CMs.88 We foresee that combined improvements in the identification of these modifiers and in their downstream functional characterization might lead to more precise risk stratification and clinical management of cardiac channelopathies (Figure 4).

**Drug toxicity**

Perhaps the most intuitive application of iPSC-CMs is their use for the safety assessment of preclinical drug candidates.72,89 Traditional experimental models to assess cardiac toxicity in the preclinical phase of drug development rely on heterologous systems, costly and often inconclusive animal models or more recently on computational approaches. More specifically, the risk of developing ventricular arrhythmias, in particular Torsades de Pointes (TdP), is tested in vitro by measuring I_{Kr} blockade.90 However, this parameter alone is not a robust predictor of TdP or of ventricular arrhythmias in general and the attrition rate of drugs during development raises questions on the reliability of these approaches.91 Indeed, even though some of these systems can be partially automated and performed at high throughput with excellent yields,92–94 their predictive value is often low, and this has resulted in the withdrawal of drugs not only in advanced phases of development but also after having entered the market.95,96

Thus, an easily accessible human-based cellular model, potentially with a higher translational relevance, has attracted basic scientists, pharmaceutical industries and regulatory agencies. An almost complete set of endogenous cardiac ion channels and the ability to potentially perform high-throughput assays make this platform very attractive for the early stages of drug development where medicinal chemistry require guidance.97,98 The suitability of iPSC-CMs for the prediction of arrhythmic cardiotoxicity is currently being investigated also by large consortia as part of the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative, meant to revise tests for hERG blockade and possibly replace thorough QT studies to more
accurately predict the arrhythmogenic risk of compounds, to reduce the rate of false positives. 99–101

To date, the area in which platforms based on iPSC-CMs have been most tested to study drug toxicity in the field of cardiac arrhythmias is that of drug-induced LQTS (diLQTS). 102 Even though, strictly speaking, diLQTS is not a channelopathy, we have shown that 30% of patients developing diLQTS carry LQTS disease-causing mutations. 102 A remarkable example of diLQTS modelling has been recently reported. 63 The investigators first shortlisted healthy volunteers based on their QT prolonging potential exhibited 3 h after a sotalol challenge in a clinical trial (clinicaltrials.gov, NCT01338441). Then, they selected the most extreme phenotypes of low responders, e.g. individuals with an absent or modest QT prolongation after sotalol, and high responders, who did exhibit important QT prolongation after the challenge. They generated subject-specific iPSC-CMs and measured in vitro their pharmacological response using MEA. Despite some variability in ion channel expression (hERG) or drug response, the majority of cell lines clustered according to their in vivo response to sotalol, demonstrating a remarkable correlation between clinical and in vitro data. A similar approach can also be used to screen drug toxicity of new or repurposed drugs in iPSC-CMs derived from patients with LQTS or other types of channelopathies (see below).

Limitations of approaches based on iPSC-CMs

Despite the great enthusiasm for this cellular model, there are still important limitations that may partly impact their use for clinically relevant approaches. The reprogramming of somatic cells into iPSCs alters some of the epigenetic modifications 103 and the effects of stimuli that have been accumulated during lifetime by the subject. This has the unintended consequence of removing the contribution of environmental factors as physical activity, diet or concurrent pharmacological therapies in the exacerbation of the disease phenotype. This may have a minor importance for inherited channelopathies with premature onset, but the potential contribution of environmental factors on the long-term progression of the disease should be considered when investigating the severity of genetic variants.

Another possible limitation is that iPSC-CMs generally show immature characteristics in morphology, cytoskeletal proteins, and ion channel expression and organization. The iPSC-CMs described so far have characteristics more similar to CMs of foetal origin than to mature adult CMs. 38 Since the majority of LQTS mutations do generate clear cut phenotypes as a result of variants whose phenotypic consequence is often manifested at young age, it would be logical to hypothesize that the maturation level currently reached by the iPSC-CM technology could be sufficient to reproduce most of the LQTS phenotypes in vitro. However, the cell maturation level might be critical when investigating mutations that require a fully organized and functional excitation–contraction coupling apparatus, or when the mutation phenotype is shaped by VUS, modifier genes or for proteins which expression levels or isoforms vary with age. 104 Strategies to tackle and partially overcome this issue include the use of long-term cultures 105 or miRNA 106–108 the development of more effective maturation media, 109,110 the use of electrical stimulation, 111 or stretch 112 during differentiation. Numerically modelled I_K,1 injected into iPSC-CMs by dynamic clamp, an advanced state-of-the-art approach, has been successfully used to overcome issues derived by the low expression of this ion current in isolated iPSC-CMs. 45,62,113,114 Another important limitation of standard iPSC-CMs cultures is that they lack the complexity of cardiac tissue. For this reason, three-dimensional multicellular models such as engineered heart tissues, 115–117 cell sheets, 118 or microtissues constituted of two or three defined cell types 119,120 have been developed, with good results also in terms of cell maturation. The generation of organ-on-a-chip platforms will contribute to further refine the predictability and clinical relevance of this technology. 121

Computational approaches

Computational approaches have introduced important breakthroughs in the way experiments are performed and data are analysed and interpreted. Technology has advanced to the point of becoming a key aspect of medicine, with the potential to improve diagnostic accuracy, risk stratification, and drug safety. These methods integrate data generated by clinical, in vitro and in silico sources and use them for predictions, such as the potentially cardiotoxic effect of a drug treatment. They are widely used in safety pharmacology and are a pillar of the CiPA initiative. 122 Recent examples demonstrated that these approaches may even have a superior accuracy than animal models to detect cardiotoxicity caused by proarrhythmic drugs 123 and future technological advancements will certainly contribute to further refine these models. 124

In the context of PM, one of the most mature concept proposed so far is the digital twin, 126 which aims to acquire and combine data from multiple sources as clinical records, imaging reports, -omics, and in vitro experimental data to build mathematical models capable to extract relevant parameters for clinicians and generate new knowledge. This concept has emerged with some notable examples 126–128 in the field of cardiology and could be further refined to embrace also early-onset diseases as congenital channelopathies, with population data, genomics and functional in vitro evaluations guiding risk stratification and clinical pharmacological treatments. However, it is important to include diverse genetic backgrounds, ethnicities, and genders while training models and classifying variants, since a wrong enrichment of specific groups in the training dataset may skew predictions, and thus clinical decisions, towards unnecessary therapy or inaction, both with potentially negative consequences. 129

In this regard, the interpretation of ECG features with neural networks may allow a substantially improved diagnostic accuracy, with the possibility to use the power of large datasets containing hundreds of thousands of annotated ECG records to train algorithms and detect features specific to the main channelopathies. 130 Since ECG interpretation may be considered a statistical classification problem, thus requiring large ‘training’ data sets, the accuracy and precision of these models will likely increase with their use, and this can provide great benefits to small hospitals with little experience in cardiac channelopathies. Some studies already begun with collaborations between large hospitals and industry (e.g. NCT0441892 or NCT02412709 on clinicaltrials.gov); moreover, these technologies have proven their efficacy in enhancing risk stratification for acute coronary syndrome, 131 to identify the patients who could be at
higher risk of developing the disease,\textsuperscript{127} and for the classification of rhythm disturbances to reduce the rate of misdiagnoses in ECG interpretations.\textsuperscript{128} Caution should still be put in the output generated by neural networks as the criteria used by these algorithms for classification are concealed and the data interpretation in these early phases of development should still be further validated.\textsuperscript{132} Nevertheless, these promising results for PM appear particularly exciting when coupled with the widespread use of wearable technologies allowing ECG monitoring without the need of healthcare specialists.\textsuperscript{133,134}

When dreams meet reality

Despite all these promising and fascinating attempts, it is important to note that so far only a tiny fraction of the progress from the described \textit{in vitro} and \textit{in silico} strategies have truly benefitted the patients. The main question is how to handle patient-specific tests that may take months or years to be implemented after the first diagnostic hypothesis is made (\textbf{Figure 5}). The widespread diffusion of high-throughput genetic testing has been coupled, especially in small centres or in developing economies, with insufficient resources to analyse data, functionally validate the output, and extrapolate meaningful information to improve patient’s care. Even though it is acceptable for patients to receive the results of their genetic testing within weeks, at the present stage of technology, it is clearly unfeasible to introduce long-term strategies based on patient-specific cellular models for the majority of our patients. High-throughput platforms often encounter the bottleneck of a low-throughput functional biology.

As previously mentioned, variant-specific therapeutic strategies may be implemented and the power of high-throughput, robotic functional screenings for the biophysical characterization of variants will significantly shorten the time required to obtain the results, and their implementation may be considered alongside with a thorough genetic screening. iPSC-CMs will not offer useful interpretations of clinical realities to guide clinical practice until shared efforts among groups worldwide will be made to develop and predict, in large heterogeneous cohorts, the functional effects of individual variables on disease traits and drug responses. These efforts will require comprehensive consortia and an extensive protocol automation\textsuperscript{75} and standardization similar to what has been done within the CiPA initiative\textsuperscript{122} and others.\textsuperscript{135}

Despite all these limitations, the progress in the understanding of the mechanisms underlying channelopathies has already led to new tools to identify responders to a specific therapy, to proposals for novel targeted therapies, and to attempts for drug repurposing. However, each novel PM strategy should improve on current therapies and would need to survive a careful cost–benefit analysis before becoming clinically meaningful. What follows is a series of a few recent representative ground-breaking examples of PM that, despite the novelty of their approach and scientific quality, still have to overcome a number of obstacles before becoming truly useful to patients.

\textbf{New tools to identify responders}

The pioneering 1995 report on the efficacy of mexiletine to shorten QTc in LQT3 patients\textsuperscript{15} was endorsed by guidelines\textsuperscript{16,17} and confirmed by widespread clinical experience.\textsuperscript{136} However, it soon became evident that not all LQT3 patients shorten their QTc with mexiletine. It was recently shown that there is a blunted response to mexiletine when mutations affect the Domain III voltage sensing domain of Na\textsubscript{v}1.5 (DIII-VSD) and that this peculiarity can be exploited to predict whether or not mexiletine will shorten QTc.\textsuperscript{137} Since the method uses the voltage dependence of DIII-VSD to properly predict the functional response in patients, this must be a known prerequisite for each specific SCN5A variant. However, as biophysical characterization is available only for a minority of variants, it follows that this

\begin{figure}[h]
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\caption{Mexiletine effect. This LQT3 patient (SCN5A-p.M1320V), already on therapy with nadolol (0.28 mg/kg/die limited by bradycardia), continued to show a major QT interval prolongation with a very abnormal T wave. Following mexiletine (5.6 mg/kg/die), both QTc and the T wave morphology became close to normal. The Holter tracings were recorded while the patient was in chronic therapy; the changes observed within 2h from the acute oral administration were similar and prompted the addition of mexiletine to the therapy. The patient continues to remain asymptomatic.}
\end{figure}
effects because of an over- or inaccurate correction of the LQTS has been suggested that some of them could exert proarrhythmic compounds have a very narrow therapeutic window. In addition, it appears premature and requires caution since many of these molecules able to alter the biophysical properties of KV7.1 or KV11.1 and enhance these two key repolarizing currents. PM approaches have been attempted to correct the disease phenotype on patient-specific iPSC-CMs by using pharmacological activators, i.e. small molecule inhibitors of INaL, on repolarization abnormalities, resulting in arrhythmogenic consequences on cardiac conduction. Importantly, the in vitro results were confirmed in knock-in mice harbouring the homologous mutation, demonstrating that iPSC-CMs can provide reliable disease models. Very recently, a chemical refinement of the antiarrhythmic drug mexiletine was obtained via high-throughput screening on iPSC-CMs derived from LQT3 patients, leading to analogues with increased potency and selectivity for inhibiting hERG across a panel of 7 LQT3 variants. The authors hypothesize that these mexiletine analogues can be exploited as mechanistic probes and for clinical development.

**Drug repurposing**

Drug repurposing represents an alternative approach to tailor drug responses to patient needs. By relying on drugs already approved for clinical use, this strategy has the advantage of reducing time and costs related to safety trials and drug approval processes and, if proved effective, offers results readily applicable to the patients. The same approach is currently used and is being optimized by industry with the objective of integrating preclinical drug testing on iPSC-CMs Large-scale assays are being developed with single candidate healthy iPSC lines (bona fide wild-type genomes), with the aid, when needed, of gene editing technologies.

The majority of LQT2 mutations cause a defect in hERG trafficking. The identification of a drug capable of correcting the trafficking defect could therefore have potential positive effects for these patients. Mehta et al. reported that Lumacaftor, a drug acting on protein trafficking and successfully used for the treatment of cystic fibrosis, can restore the trafficking of hERGs in iPSC-CMs derived from LQT2 patients carrying Class 2 mutations and can shorten the corrected field potential duration. This effect was Class-specific as it was absent in iPSC-CMs from LQT2 patients with Class 1 mutations (hERG protein synthesis). We moved from the laboratory to the clinic and performed a first translational study in two of the patients whose iPSC-CMs responded to Lumacaftor in Mehta’s study, having considered that Lumacaftor is already approved for clinical use with a good safety profile. We documented QTc shortening in both patients, and a substantial increase in the time during which the QTc values were in the lower tertile of the values at baseline. However, the magnitude of QTc shortening (~30 ms) was lower than we had expected based on the in vitro results. Very recently, O’Hare et al. confirmed, in similar experimental conditions, that Lumacaftor rescues Herg trafficking in three LQT2 patient-derived iPSCs. The action potential shortening was observed in the two cell lines characterized by a pure trafficking defect, while it was prolonged in a cell line with co-existing trafficking and gating defects. While Lumacaftor may or may not turn out to be sufficiently useful for LQT2 patients with trafficking defect (a clinical trial—clinicaltrials.gov NCT04581408—is ongoing and should provide definitive answers), our combined experimental and clinical studies have shown that iPSC-CMs represent a valid tool for drug-repurposing strategies.

**Conclusions**

PM is an important evolution of traditional medicine and the rapidity of its progress is impressive. It is exciting and stimulating also because its challenge is intellectually rewarding. It has the potential to significantly impact our societies and healthcare systems, by reducing the social and economic burden associated with inadequate prevention strategies, and patients alike, by offering tailored therapies of improved efficacy. To acquire sufficiently large datasets from in vivo and in vitro patient-specific strategies to train models, validate predictions, and implement on a large scale the various systems just described will require time, but this is the way by which science goes forward.

Nevertheless, PM should not be considered as an entirely new path but an ‘evolution’, which means a progress made without the need to jettison the huge amount of practical experience and knowledge accumulated over the years. In this review, we have tried to preserve the thread linking the more advanced technological approaches to the insights derived by relatively simple medical observations emerged by the time-honoured principle that understanding the mechanisms is, most of the time, the key for an effective therapy. We recognize the almost irresistible attraction of complex novelties,
which we embrace, but at the same time we equally respect what has been learnt by those cardiologists who have fruitfully merged knowledge and clinical experience. This consideration should guide us when we have to critically assess whether new approaches to therapy, besides their novelty and elegance, are indeed of greater benefit to our true stakeholders, the patients.

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