ligh-density lipoprotein）粒子中のコステロールをLDLなどに転送するレステリルエステル転送蛋白holesteryl ester transfer protein；ETP）の欠損症は，頻度が高く，高DL-コレステロール血症の約半数を占める[8]。CETP欠損症が「動脈硬化惹起性なのか全く逆に動脈硬化防御性なのか」については，いまだ明確な結論が出ていないが，CETP欠損症の集積地域における疫学研究の結果，血清HDL-コレステロールと虚血性心電図変化とのあいだにU字型の関係があること[9)10)]（図2），最近，欧米で最初に開発されたCETP阻害剤の臨床治験が失敗に終わったことなどから，必ずしも好ましい変化ではなく，動脈硬化性疾患を合併している可能性を考慮して，日常診療にあたる必要がある（http://j-cetpd.org/index.html）。

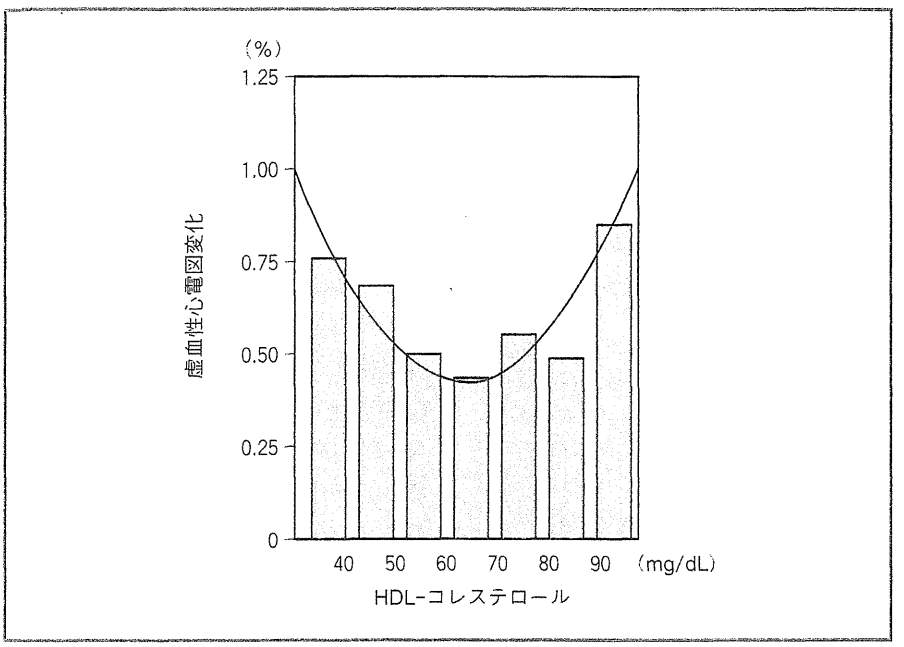

図2　CETP欠損症集積地域における疫学研究―HDL-コレステロール値と虚血性心電図変化の関係

（文献9より改変引用）

5　低HDL-コレステロール血症

HDL粒子産生の必須分子であるABCA1[11]の遺伝的欠損症は，タンジール病として知られている。HDL欠損，オレンジ扁桃，肝脾腫，早発性冠動脈硬化をきたす。また，われわれは，最近，familial intrahepatic cholestasis-1（FIC1/ATP8B1）が，血清HDL-コレステロールレベル調節に重要であることを明らかにしている[12]。FIC1欠損症は，進行性家族性肝内胆汁うっ滞症（progressive familial intrahepatic cholestasis；PFIC）や良性反復性肝内胆汁うっ滞症（benign recurrent intrahepatic cholestasis；BRIC）として知られているが，低HDL血症をきたし，小児期から頸動脈硬化が認めら

表1　FIC1欠損症の血清脂質と頸動脈硬化　脂質プロフィル（単位：mg/dL）

	総コレステロール	中性脂肪	LDL-コレステロール	HDL-コレステロール
FIC1欠損症	121 ± 7	172 ± 9	84 ± 5	20 ± 3
正常者	142 ± 17	90 ± 22	77 ± 15	51 ± 7

頸動脈硬化（超音波検査による評価）

	FIC1欠損症	正常者
IMT（mm）	0.61 ± 0.07*	0.43 ± 0.03
WS	151 ± 22**	109 ± 14

*：$p < 0.05$，**：$p < 0.001$。
IMT：intimal medial thickness，WS：wall stiffness。
（文献13より改変引用）

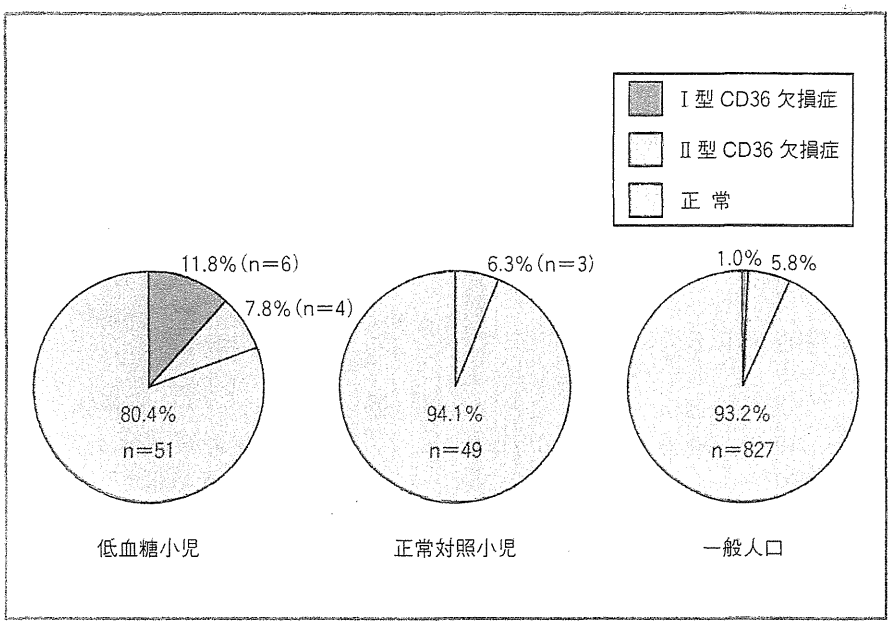

図 3　低血糖小児における CD36 欠損症の頻度

I 型 CD36 欠損症は，小児低血糖のリスクである。　（文献 16 より改変引用）

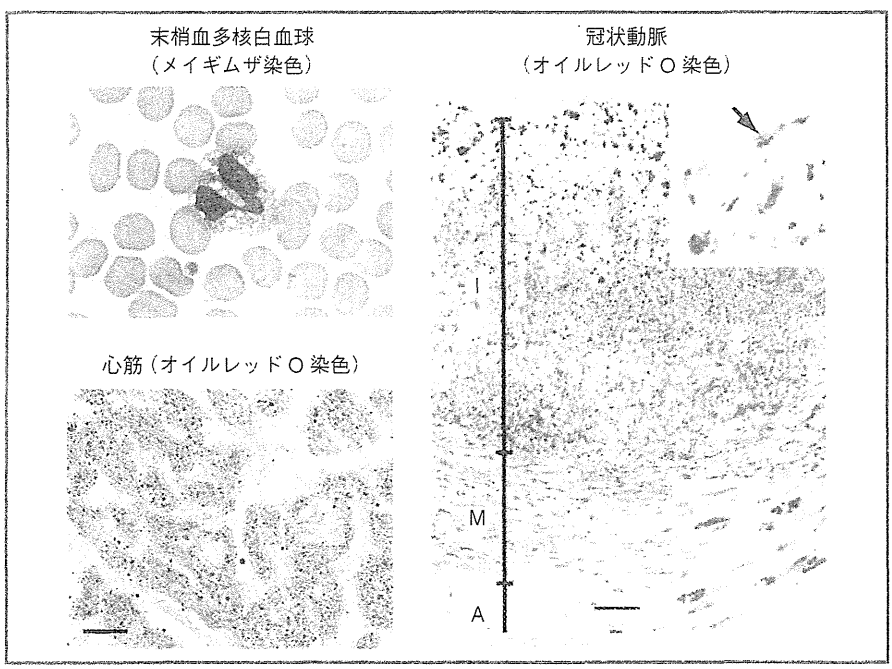

図 4

中性脂肪蓄積心筋血管症における多核白血球の空胞化と心筋および冠動脈における中性脂肪の蓄積。

（文献 20, 22 より改変引用）

（カラーグラビア p2　写真 1 参照）

れる（表 1）[13]。

6　メタボリック症候群

過食や運動不足がメタボリック症候群の後天的原因であることは，よく知られているが，本症候群の遺伝的背景として，日本人の数千人に 1 人程度存在する CD36 欠損症が重要である[14]。CD36 は心筋細胞，骨格筋において脂肪酸を取り込むための重要なトランスポーターで，成人 CD36 欠損症では，インスリン抵抗性[15]，高脂血症（空腹時，食後），高血圧などを呈する。また，小児期では，むしろ低血糖症のリスクになる[16]（図 3）ことも明らかとなり，小児期と壮年期では表現型が大きく異なっており，注意が必要である。CD36 欠損症は，心筋がそのエネルギー源である脂肪酸を利用できないため，心筋症，心筋障害との関連が示唆されている。本症は，BMIPP シンチグラムで心臓に取り込みが欠損していることを契機に見出される場合が多い。血小板，単球における CD36 抗原を検索すること，遺伝子検査することで診断できる[17][18]。

脂質蓄積症
(lipid storage diseases)

1　ファブリー病 (Fabry's disease)

ライソゾーム酵素であるαガラクトシダーゼの遺伝的欠損の結果，セラミドトリヘキソシドが細胞内に蓄積する。Fabry によって記載された古典的ファブリー病では，神経症状，被角血管腫，角膜混濁などが報告されたが，

わが国において，心機能障害，心肥大をきたす心ファブリー病が発見，報告されている[19]。遺伝形式は，X連鎖である。診断は，末梢血の酵素活性測定や遺伝子診断によりなされる。酵素補充療法が開発されている。

2 中性脂肪蓄積心筋血管症

中性脂肪蓄積心筋血管症（triglyceride deposit cardiomyovasculopathy；TGCV）は，2008年，わが国の心臓移植症例より見出された新規疾患単位である。心筋および冠動脈にTGが蓄積する結果，重症心不全をきたす。細胞内の代謝異常の結果，罹患臓器にTGが蓄積するため，必ずしも高TG血症をともなわない[20)21)]（図4）。今のところ明らかな原発性TGCVの原因は，細胞内TG分解の必須酵素であるadipose triglyceride lipaseの遺伝的欠損である。末梢血の多核白血球の空胞化は，発症以前から存在し診断に重要である。厚生労働省難治性疾患克服研究事業として，その病態の解明，診断法，治療法の開発が行われている。

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iPS Cell Modeling of Cardiometabolic Diseases

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Abstract Cardiometabolic diseases encompass simple monogenic enzyme deficiencies with well-established pathogenesis and clinical outcomes to complex polygenic diseases such as the cardiometabolic syndrome. The limited availability of relevant human cell types such as cardiomyocytes has hampered our ability to adequately model and study pathways or drugs relevant to these diseases in the heart. The recent discovery of induced pluripotent stem (iPS) cell technology now offers a powerful opportunity to establish translational platforms for cardiac disease modeling, drug discovery, and pre-clinical testing. In this article, we discuss the excitement and challenges of modeling cardiometabolic diseases using iPS cell and their potential to revolutionize translational research.

Keywords Cardiometabolic disease · Induced pluripotent stem cell · Disease modeling · Storage disease

Introduction

For 30 years, pluripotent stem cells have served as a powerful model system of developmental biology. Beginning with the establishment of murine embryonic stem (ES) cells [1], followed by the first human ES cell line in 1998 [2], the prospect of using pluripotent stem cells for translational research has been a primary goal. The unique properties of immortality and pluripotency, namely the ability to differentiate into all somatic cell types that ES cells possess provide tremendous opportunities for disease modeling, drug discovery, and pre-clinical testing [3]. The breakthrough of somatic cell reprogramming in mouse cells by Takahashi and Yamanaka in 2006 [4] and then in human cells in 2007 [5] was a seminal advance for translational application of pluripotent stem cells. These so called induced pluripotent stem cells (iPS cells) are inherently patient and disease specific, bypassing the technical, ethical, and political limitations of human ES cell research, and a fundamental step towards

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regenerative cell-based therapies. Here we discuss the opportunities to use iPS cell technology for modeling cardiometabolic diseases.

Cardiometabolic Disease

Cardiometabolic diseases are characterized by metabolic disruptions that harm cardiac function. Cardiometabolic syndrome generally refers to the complex interaction of cardiovascular risk factors, anchored by insulin resistance, obesity, dyslipidemia, and hypertension first described in 1988 as "Syndrome X" [6] (Fig. 1). This clinical syndrome is a well-established predictor of premature cardiovascular outcomes with significantly increased morbidity and mortality [7–9]. The confluence of cardiovascular and metabolic pathology captured by this syndrome is perhaps the ultimate goal of in vitro cardiometabolic disease modeling. Although type 1 diabetes was an early interest using iPS cells as a disease model [10, 11] (Table 1), the complexity of recapitulating the full complement of phenotypes expected in this disease is presently prohibitive with the tools currently available for in vitro differentiation and manipulation. The use of well-defined co-culture systems consisting of multiple relevant cell types and factors may be needed to overcome these challenges. Since the field of human disease modeling with iPS cells is at its infancy, we have chosen, in this article, to focus specifically on cardiometabolic diseases with simple Mendelian genetics and well-defined pathophysiology as they illustrated the utility of disease-specific iPS cells in phenotype and pathway discovery. In particular, we describe how monogenic diseases such as glycogen storage diseases and neutral lipid storage diseases may be amenable to in vitro modeling given their cell autonomous cardiac phenotypes.

General Considerations for iPS Cell Cardiac Disease Modeling

Whether a particular cardiometabolic disease is amenable to iPS cell-based modeling depends on the available protocols to derive the cell type of interest and the available assays to assess the disease-relevant phenotype. Thus far, the most significant barrier to finding novel disease pathway through iPS cell disease modeling is the efficiency of generating highly pure and phenotypically mature cells by in vitro differentiation, including the defined subtypes of mature human cardiomyocytes (e.g., atrial, ventricular, or pacemaker cells). To achieve this, the development of more efficient, reproducible, specific, and complete differentiation protocols will be required [12–14]. Some of the known barriers to efficient in vitro differentiation include incomplete reprogramming, epigenetic memory of parental cell type [15, 16], or variability intrinsic to pluripotent cells [17]. These issues must be fully understood before we can fully utilize iPS technology for translational research.

Beyond the efficiency of in vitro differentiation, the disease of interest must be carefully selected based on the known genetic and epigenetic factors that control the clinical characteristic of the disease manifestation. These clinical characteristics dictate whether the disease phenotype would manifest appropriately, particularly since iPS cells generally mimic cells from early embryogenesis and development. In general, monogenic diseases are easier to recapitulate than polygenic diseases, although complex diseases such as familial Parkinson's disease [18] and schizophrenia [19] have recently been described with in vitro phenotypes that mimic their clinical surrogates in animal models.

Since the generation of fully mature cell types from iPS cells has been generally challenging, diseases that present late in life may be particularly difficulty to model with iPS cells. For example, current protocols for deriving cardiomyocytes from iPS cells tend to yield immature cells with

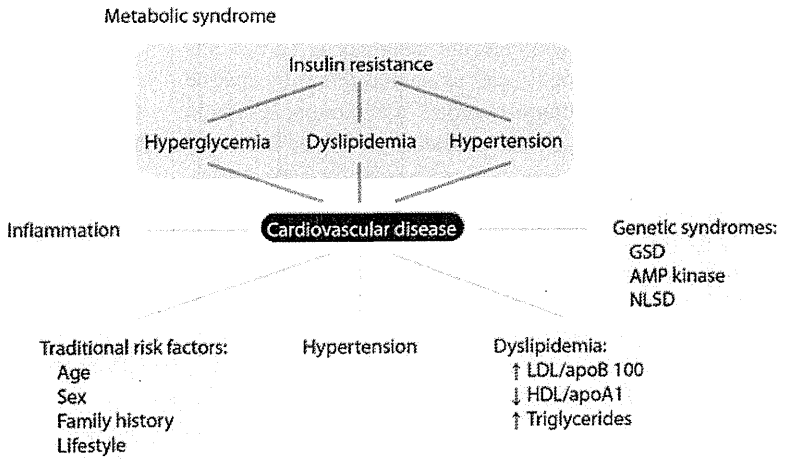

Fig. 1 An overview of common mechanisms in cardiometabolic disease

Table 1 Reported cardiometabolic diseases modeled with iPS cells

Disease	Defect (gene)	Clinical phenotype	iPS-derived differentiated cell type	In vitro phenotype	Notes
Type 1 diabetes [10, 11]	Unknown, multifactorial	Insulin resistance, altered paracrine signaling	Insulin producing β-cell-like cells	Insulin resistance, altered paracrine signaling	In vitro phenotype correlates with clinical phenotype. Cardiovascular phenotypes unknown.
GSD type Ia (von Gierke disease) [48]	Glucose-6-phosphatase (*G6PC*)	Hypertrophic cardiomyopathy, hyperlipidemia	Hepatocyte-like cells	Intracellular glycogen accumulation, increased lactate production, altered paracrine signaling	In vitro phenotype correlates with clinical phenotype. Cardiovascular phenotypes unknown.
GSD type II (Pompe disease) [51]	Lysosomal acid a-1, 4-glucosidase (*GAA*)	Hypertrophic cardiomyopathy, arrhythmia, pre-excitation, hypotonia, muscle weakness, respiratory distress	Skeletal muscle cells	Lysosomal accumulation of glycogen	In vitro phenotype correlates with clinical phenotype. Cardiovascular phenotypes unknown, but likely similar.
Familial hypercholesterolemia [48]	Low-density lipoprotein receptor (*LDLR*)	Hyperlipidemia, accelerated artherosclerosis	Hepatocyte-like cells	Lipid and glycogen accumulation, aggregation of misfolded α_1-antitrypsin	In vitro phenotype correlates with clinical phenotype. Cardiovascular phenotypes unknown.

fetal-like morphology, gene expression profiles [20], ion channel expression [21], and electrophysiological function [22]. While some features such as calcium handling [23] may become progressively more similar to mature adult cells with prolonged culturing in vitro [24] or in vivo [25], the full manifestation of adult phenotypes has not been demonstrated thus far. Until this issue of maturation is addressed, the ideal diseases for iPS cell-based modeling should exhibit clinical phenotypes during fetal or early postnatal stages of development. One possible exception to this may be found in diseases occurring later in life but exhibit phenotypes that can be de-repressed during in vitro culturing. In this case the disease expression may manifest earlier and more robustly than predicted based on clinical information.

While pluripotent stem cells are theoretically able to differentiate into any somatic cell as demonstrated by murine tetraploid complementation studies and human teratoma assays, the currently available protocols are robust for only a subset of specific cell types such as neurons, cardiomyocytes, hematopoietic cells, endothelial cells [26–28], and, to a lesser extent, hepatocytes [29–32]. Fortunately, spontaneously beating cardiomyocytes have been generated from pluripotent stem cell-derived blastocyst-like clusters (the so-called embryoid bodies) for more than 30 years [33, 34]. A wide range of protocols now exist for efficient and cardiac-specific differentiation. Many of these conditions mimic embryonic development by modulating master signaling pathways including WNT [35], BMP/activin [36] and FGF [37] (reviewed in [38]). Small molecules [39] and transgenic selection [40, 41] have also been shown to further increase the efficiency and quality of derived cardiomyocytes.

iPS Cell-Based Modeling of Cardiometabolic Diseases

Cardiometabolic diseases are well suited for iPS cell-based disease modeling when they exhibit readily assayable phenotypes in vitro. The relevant disease phenotype that is expected from clinical presentation, whether molecular or functional, should be sufficiently robust to overcome inherently "noisy" background from the known heterogeneity in the system. Furthermore, the ease of phenotypic assay is also an important consideration given the broad interest from investigators to perform drug screening and validation using iPS cells. A summary of the cardiometabolic diseases that may be amenable to in vitro modeling with iPS cells is provided (Table 1). Here, we also propose in detail two cardiometabolic disease areas—glycogen storage and neutral lipid storage diseases—that exhibit clinical and cellular features particularly amenable to in vitro disease modeling (Table 2).

Glycogen Storage Diseases

Glycogen storage diseases (GSD) are characterized by defective glycogen catabolism or metabolism within many cell

Table 2 Proposed cardiometabolic diseases for iPS cell modeling

Disease	Defect (gene)	Clinical phenotype	Expected in vitro disease phenotype
Fabry disease [42, 43, 45]	X-linked lysosomal hydrolase a-galactosidase A (*GLA*)	Hypertrophic cardiomyopathy, renal failure, rash, neuropathy, arrhythmia	Lysosomal accumulation of glycogen; increased cytoplasmic vacuoles, cardiomyocyte enlargement, high cytoplasmic to nuclear ratio, pleomorphic nuclei; electrophysiological abnormalities (early after depolarizations)
Danon disease [46, 47]	X-linked lysosome-associated membrane protein (*LAMP2*)	Hypertrophic cardiomyopathy, muscle weakness, arrhythmia, pre-excitation	
Cardiac hypertrophy, conduction system disease [52, 53]	AMP-activated protein kinase	Hypertrophic cardiomyopathy, arrhythmia, pre-excitation	Glycogen accumulation; increased cytoplasmic vacuoles, cardiomyocyte enlargement, high cytoplasmic to nuclear ratio, pleomorphic nuclei; electrophysiological abnormalities (early after depolarizations)
Neutral lipid storage disease [55, 66]	Adipose triglyceride lipase (*ATGL*)	Hypertrophic cardiomyopathy, cardiac vasculopathy	Triglyceride accumulation, cardiomyocyte enlargement, suppression of fatty acid oxidation, induction of glycolysis

types, including cardiomyocytes and hepatocytes. Of the 11 distinct GSD described, several have been reported to cause hypertrophic cardiomyopathy and arrhythmia in patients without sarcomere-protein mutations [42], including Pompe disease (a recessively inherited lysosomal acid a-1, 4-glucosidase [GAA] deficiency) [43, 44], Fabry disease (an X-linked lysosomal hydrolase a-galactosidase A [GLA] deficiency) [42, 43, 45], and Danon disease (an X-linked lysosome-associated membrane protein [LAMP2] deficiency) [46, 47]. Clinical characteristics, such as cardiomyocyte enlargement, high cytoplasmic to nuclear ratio, pleomorphic nuclei, increased cytoplasmic vacuoles, and cardiac electrophysiogical dysfunction [43, 45], may be assayed as in vitro surrogates of disease expression and may be amendable to screening for drug discovery. Whether these phenotypes track with clinically observed cardiovascular manifestations, however, is currently not known. The cardiovascular phenotype may also depend on non-cardiac cell types, such as hepatocytes, given that glycogen metabolism largely occurs in the liver. Two iPS cell model of GSD have been reported with recapitulation of disease phenotypes in hepatocytes and skeletal muscle (Table 1). The recapitulation of cardiovascular phenotypes was not investigated in these studies and remains an intriguing area for future research.

The first GSD to be modeled using iPS cells is GSD type 1a (von Gierke disease) [48]. GSD type 1a is characterized by a deficiency of glucose-6-phosphatase, the regulatory enzyme that hydrolyzes glucose-6-phosphate to glucose and phosphate in the terminal steps of gluconeogenesis and glycogenolysis (Table 1). Abnormally elevated intracellular glycogen and lipid and increased lactate production is typically observed in hepatocytes from patients with GSD type 1a and was recapitulated in diseased iPS cells. In addition, the hormonal responses from hepatocytes may also provide non-cell autonomous interactions that enhance phenotype manifestation in cardiomyocytes. Although marked hyperlipidemia is observed with GSD type 1a, its association with cardiovascular disease is not clear [49] and case reports have not yielded consistent conclusions regarding its role in cardiovascular complications of GSD type 1a [49, 50].

Pompe Disease

A murine iPS cell-based model of Pompe disease was described recently that demonstrated severe accumulation of glycogen in lysosomal vacuoles in skeletal muscle cells [51] (Table 1). A similar phenotype in cardiomyocytyes is expected but was not reported in this study. The development of a human iPS cell system of Pompe disease would enable the pursuit of translational applications such as drug screening and functional validation. iPS cell-based models of other GSD with well-established cardiac manifestations, such as Fabry, and Danon disease, have yet to be described in the literature (Table 2).

AMP-Activated Protein Kinase

Mutations in AMP-activated protein kinase (AMPK) cause inappropriate activation and accumulation of glycogen in most cell types, particularly within the heart, which eventually leads to well-described cardiac hypertrophy and conduction system disease [52, 53] (Table 2). AMPK functions to balance catabolic processes in order to meet the metabolic needs of the cell and can be thought of as a cell-level "energy gauge" [54]. At times of pathologic stress, such as hypertrophy and ischemia, AMPK is activated to upregulate maladaptive metabolic processes [53]. The cellular phenotype of cardiomyocyte glycogen accumulation can be assayed in vitro with functional phenotypes expected in

isolated cardiomyocytes. There are currently no reported iPS cell models of AMPK mutations. However, given the general interest in modeling hypertrophic cardiomyopathy in vitro, we anticipate a number of studies to be reported on this cardiometabolic disease model in the near future.

Neutral Lipid Storage Disease

Neutral lipid storage disease (NLSD) is characterized by excessive accumulation of neutral lipids in various cell types, including cardiomyocytes [55] (Table 2). The disease was first described in 1974–1975 as Chanarin-Dorfman Syndrome, a rare autosomal recessive inborn error of neutral lipid metabolism causing ichthyosis and typically accompanied by mild myopathy, hepatic steatosis, ataxia, opthalmopathy (cataract, nystagmus, and strabismus), hearing loss, and mild mental retardation [56, 57]. A clinically distinct variant of NLSD without the dermatological hallmark of ichthyosis but instead with severe cardiomyopathy was described [58], suggesting the presence of two separate clinical entities. More recently, two genes have been identified to cause these two forms of NLSD, one with ichthyosis (NSLD-I) caused by a mutation in comparative gene identification-58 (*CGI-58/ABHD5*) [59, 60] and another with myopathy (NSLD-M) caused by a mutation in the patatin-like phospholipase domain-containing protein 2 (*PNPLA2*) gene encoding adipose triglyceride lipase (ATGL) [61–63]. *CGI*-58 is a potent, insulin-dependent activator of ATGL [62, 64], which hydrolyze triacylglycerol (TAG) into diacylglycerol and free fatty acids. ATGL regulates non-redundantly this rate-limiting step in the breakdown of cellular lipid droplets to provide free fatty acid for cellular energy metabolism [65].

So far, a limited number of families worldwide have been reported with NLSD-M [59, 66, 67]. In all cases, excessive triglyceride accumulation was observed with patients often developing life-threatening cardiomyopathy and cardiac vasculopathy requiring heart transplantation. ATGL deficiency leads to TG accumulation in both myocardium and coronary arteries exhibiting triglyceride deposit cardiomyovasculopathy [66]. Of the 24 ATGL-deficient patients (nine men and 15 women) described so far, 14 of them carry unique mutations. They are globally dispersed throughout the United States, Europe, Africa, and Asia. All of these patients harbor homozygous or compound heterozygous ATGL mutations. All male patients suffered from adult-onset severe heart diseases such as congestive heart failure and fatal arrhythmias while six females out of 15 experienced cardiovascular symptoms, suggesting that ATGL mutations disproportionately affect men more severely than women. Four patients with cardiac involvement were identified post-mortem and two status-post heart transplantation. For the diagnosis of ATGL deficiency, the detection of lipid deposition in peripheral leucocytes known as Jordans' anomaly can be detected in blood smear before the development of cardiac and skeletal myopathy and may, thus, assist in screening individuals for NSLD-M. The phenotype of AGTL heterozygote deficiency is not known yet.

NLSD-M is well suited for iPS cell disease modeling given its known gene defect in *PNPLA2* and with clear and consistent manifestation in several energy-consuming organs such as cardiac and skeletal muscle [68, 69]. As expected, excessive intracellular TAG accumulation in multiple cell types, especially cardiac and skeletal myocytes is observed clinically [67, 70]. ATGL-knockout mice exhibit a similar phenotype, including massive fat accumulation in the heart that leads to fatal cardiomyopathy [65]. Such robust cellular phenotypes are likely to be expressed in vitro for assay by a number of modalities including intracellular lipid staining. Adaptation of a NLSD-M model for high throughput screening by colorimeteric assay may yield novel candidate therapeutics. These putative compounds can then be validated on the same platform using functional assays of high specificity, such as the suppression of fatty acid oxidation and induction of glycolysis, as would be predicted with normalization of intracellular TG levels and correction of the NLSD-M phenotype. These in vitro phenotypes will thus serve as convincing surrogates of the dominant clinical features of NLSD-M. Given the monogenic nature of this disease and the available family cohorts worldwide, NLSD-M is well positioned for human disease modeling using patient-specific iPS cell lines.

Conclusions

To be sure, human iPS cell models will not replace established in vivo disease models but will rather complement these platforms by providing human-based cell types that faithfully recapitulate the disease phenotype of interest. These models will be particularly important in studying cardiac arrhythmia and conduction system diseases given the cell intrinsic manifestation of human cardiac ion channel electrophysiology. Cardiometabolic diseases, such as glycogen storage disease and neutral lipid storage disease, are the logical choices for proof-of-principle studies using iPS cell technology. Establishment of such models will be a powerful platform on which drug discovery and functional validation studies can be based to accelerate development of targeted therapeutics, an area of urgency given that cardiovascular complications cause significant morbidity and are often life limiting.

Genome-wide association studies [34] and metabolomic profiling [27, 51] have provided myriad candidate mediators of cardiometabolic disease pathophysiology, diagnosis, and

therapy that require pre-clinical confirmation in human-based systems. Cardiometabolic diseases also present an opportunity to explore gene and cell-based curative therapies. Development of such therapeutics can first be evaluated using iPS cell models as demonstrated for sickle cell anemia [71] and alpha1-antitrypsin deficiency [72]. New genome editing tools such as zinc-finger nucleases [73, 74] are exciting strategies for curative therapy that can be validated and tested for safety in vitro prior to in vivo and eventual clinical studies. Insight gained from such investigation of basic cardiometabolic disease will inform the use of the technology for more complex diseases, such as recapitulating components of the metabolic syndrome.

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