Pluripotent stem cells in disease modelling and drug discovery

Yishai Avior, Ido Sagi and Nissim Benvenisty

Abstract | Experimental modelling of human disorders enables the definition of the cellular and molecular mechanisms underlying diseases and the development of therapies for treating them. The availability of human pluripotent stem cells (PSCs), which are capable of self-renewal and have the potential to differentiate into virtually any cell type, can now help to overcome the limitations of animal models for certain disorders. The ability to model human diseases using cultured PSCs has revolutionized the ways in which we study monogenic, complex and epigenetic disorders, as well as early- and late-onset diseases. Several strategies are used to generate such disease models using either embryonic stem cells (ES cells) or patient-specific induced PSCs (iPSCs), creating new possibilities for the establishment of models and their use in drug screening.

Aneuploidy
The occurrence of an aberrant number of chromosomes within a cell, including both chromosome additions and deletions.

Primary cell lines
Cultured cells derived directly from source tissues. Primary cells usually have a normal karyotype and a limited replicative potential unless immortalized.

Self-renewal
The ability of a cell to give rise to indefinite number of cells of the same type.

The Azrieli Center for Stem Cells and Genetic Research, Institute of Life Sciences, Hebrew University of Jerusalem, Givat-Ram, Jerusalem 91904, Israel. Correspondence to N.B.
nissimb@mail.huji.ac.il

doi:10.1038/nrm.2015.27
Published online 28 Jan 2016

A comprehensive view of the biological processes underlying human pathologies is crucial to devising strategies for their prevention and treatment. As the molecular basis of many diseases can be narrowed down to defined genetic loci, modelling these diseases can be facilitated by studying specific genotypes in a suitable experimental context.

As mammalian genomes are highly evolutionarily conserved, animal models such as mice, rats and non-human primates have emerged as invaluable tools for modelling human disorders by enabling the dissection of disease mechanisms at different developmental stages and in a variety of cell types *in vivo*. The advent of transgenesis and gene targeting has rendered the mouse particularly useful, making it one of the most frequently used model organisms in biomedicine. However, since the divergence of their ancestors around 10^8 years ago[1], humans and mice have acquired considerable developmental, genetic and physiological differences. For example, the two species vary in several aspects of embryonic development, particularly during gastrulation and organogenesis[2]. On the genomic level, although the majority of human and mouse genes are orthologous, about 20% do not have an identifiable singular orthologue, and 1% lack a homologue[1]. On the physiological level, mice and humans differ in many organ functions. For example, heart size and resting cardiac rate are substantially different[3]. The dissimilarities between the two species, many of which are phenotypically observable, can preclude the recapitulation of human disease phenotypes in the mouse. In extreme cases, the same genotype can be lethal in one species and viable in the other. For example, whereas mice with monosomy X are viable, this aneuploidy is usually embryonically lethal in humans[4]. Conversely, although mutations in the *BLM* gene in humans lead to Bloom syndrome, which is characterized by genomic instability and cancer, mutations in its mouse orthologue are fatal[5].

For these reasons, it is preferable to conduct biomedical research in humans, but this is almost always limited to *in vitro* systems. Culturing patient-derived cells is tremendously useful for studying disease aetiologies at the molecular and cellular levels, as well as for developing therapies. For example, cancer studies often rely on tumour cells that can be readily isolated from patients. Similarly, the modelling of genetic disorders can be facilitated by utilizing patient-derived immortalized cell lines originating from blood or tissue biopsies. However, disease phenotypes are frequently specific to cell types that are more difficult to isolate and that cannot be continuously grown in culture.

Human pluripotent stem cells (PSCs) outperform the aforementioned approaches, owing to a combination of three major advantages: they are normal primary cell lines, they have an intrinsic capability for indefinite self-renewal, and they have the potential to adopt virtually any cellular fate through differentiation. These properties enable us to study genotype–phenotype relationships in a broad range of human cell types and differentiation states, as well as to obtain large numbers of cells for additional purposes, including drug screening and cell therapy.

In this Review, we describe the ways in which human PSCs are generated and utilized for disease modelling

and as a platform for drug development. We discuss their suitability as models for the study of a plethora of different types of diseases — for example, monogenic disorders and complex disorders, and early-onset and late-onset diseases. We also discuss advantages and disadvantages of the use of human embryonic stem cells (ES cells) and induced PSCs (iPSCs) in disease modelling and the challenges that lie ahead. Furthermore, we describe the different strategies by which PSC-based models are being used for drug screening and highlight current trends in drug discovery for neurological diseases using patient-derived iPSCs.

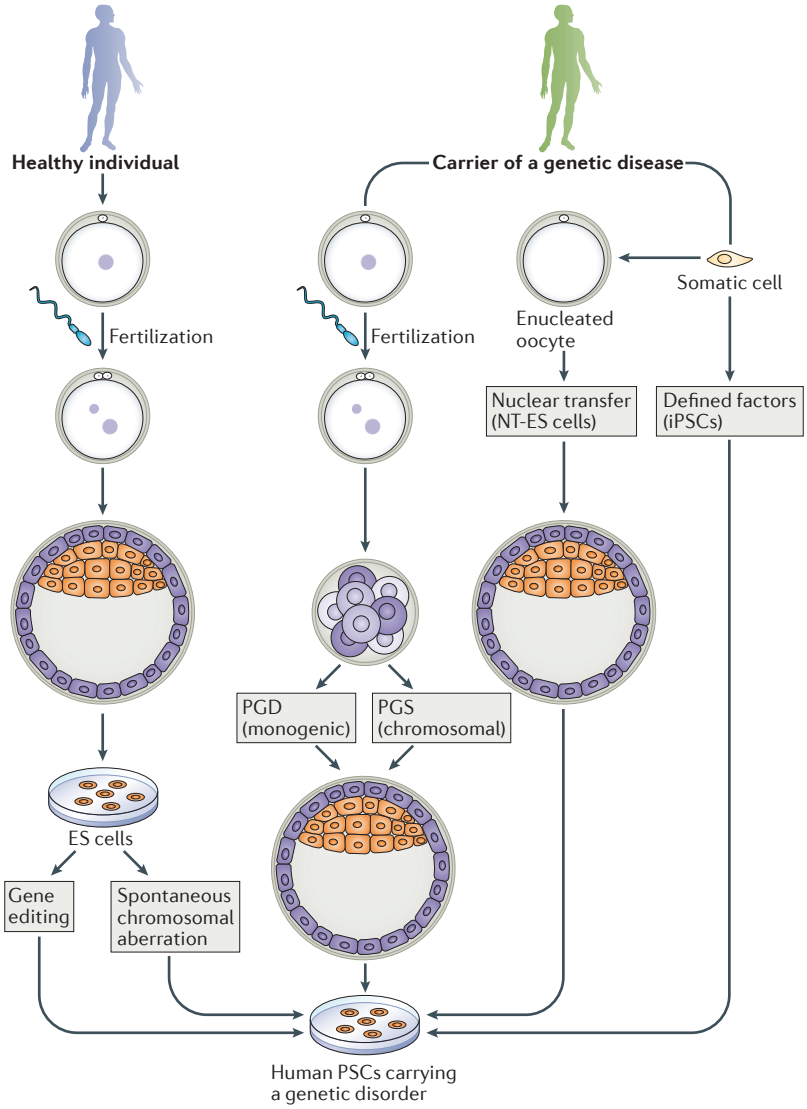

Figure 1 | **Strategies for generating disease models using human pluripotent stem cells (PSCs).** Human PSCs carrying a genetic disorder can be generated by utilizing healthy (left panel) or aberrant (right panel) cells. Isolated embryonic stem cells (ES cells) from healthy individuals can by genetically edited at a specific locus, generating *de novo* mutations. As ES cells acquire spontaneous chromosomal aberrations in culture, they can also be used to model chromosomal disorders such as Turner syndrome. Utilizing cells from a carrier of a genetic disorder provides other alternatives. Disease-specific ES cells can be identified during the in vitro fertilization (IVF) process by pre-implantation genetic diagnosis (PGD) or pre-implantation genetic screening (PGS). These cells can be readily cultured and can serve as models for monogenic or chromosomal disorders. Alternatively, somatic cells from patients can be reprogrammed into PSCs either by transferring their nucleus into an enucleated oocyte to generate nuclear transfer ES (SCNT-ES) cells or by the use of defined factors to generate induced PSCs (iPSCs). Each of these methods can be used to generate a platform to study and model genetic disorders.

Generation of disease models in human PSCs

All studies utilizing human PSCs for disease modelling begin by establishing cell lines carrying the molecular defect(s) of interest. These cells are then used to identify a robust disease phenotype in either the undifferentiated or differentiated state, and various methodologies can be employed to elucidate disease aetiology and devise novel therapies (for example, through drug screening). In this section, we outline the main strategies for achieving the crucial first step of generating disease-specific PSC lines and highlight the unprecedented contribution of cellular reprogramming.

Strategies to generate disease-specific human PSC lines. Models for human genetic disorders can be generated using different types of PSCs and various methodologies (FIG. 1). The enormous potential of deriving human ES cells[6] for disease modelling was quickly realized and utilized when normal ES cells were first used to target the hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) gene[7], the disruption of which causes Lesch–Nyhan syndrome, and to characterize the disease phenotype[8]. Normal human ES cells are also used to model chromosomal disorders by isolation of aneuploid cells that arise spontaneously in culture, as was shown for monosomy X, which is the cause of Turner syndrome[9]. Alternatively, human embryos carrying specific mutations or chromosomal aberrations can be identified by pre-implantation genetic diagnosis (PGD)[10,11] or pre-implantation genetic screening (PGS)[12], respectively. These embryos, which would otherwise be discarded, can be used as a source of ES cell models for monogenic or chromosomal disorders (see below). However, only a small range of disorders can be traced by PGD and PGS, limiting the potential to generate disease models in human ES cells. This limitation has been overcome by the ability to reprogramme somatic patient-derived cells to pluripotency.

Reprogramming of somatic cells to a pluripotent state through the expression of a defined set of transcription factors was first achieved with mouse cells[13] and later with human cells[14,15], and it has revolutionized studies of PSCs and their applications. The resulting reprogrammed cells are known as iPSCs[13–16]. Soon after the first reports of iPSCs, human iPSCs were used to generate models of human genetic disorders[17] (FIG. 1).

Recently, a long-awaited success with human somatic cell nuclear transfer (SCNT), by which a somatic cell nucleus can be reprogrammed by placing it into an enucleated oocyte, has led to the derivation of SCNT-ES cells from patient cells[18,19] (FIG. 1).

Nuclease-based genome editing techniques[20] have seen great improvements in recent years, becoming more versatile and thus making it popular again to use gene editing to generate disease models using human PSCs. These methodologies enable the introduction of site-specific genetic changes in PSCs, including gene knockout and gene correction in normal and disease

Table 1 | **Large-scale initiatives for derivation of diseased iPSCs**

Initiative	CIRM	StemBANCC	HiPSCi
Location	USA	EU	UK
Type of diseases	Mostly polygenic	Monogenic and polygenic	Mostly monogenic
Number of patients	3,000	500	500
Lines per patient	3	1–3	1
Cell type	• Fibroblasts • Blood	• Fibroblasts • Hair samples	Fibroblasts
Derivation technique	Episomal plasmids	Sendai virus	Sendai virus
Pluripotency assay	Pluripotency markers	• PluriTest • Embryoid bodies	• PluriTest • Directed differentiation
Further analyses	• SNP array • Episomal integration	• DNA-seq • SNP array • Proteome	• DNA-seq • RNA-seq • ChIP–seq • Methylome
Cell banking	Coriell	ECACC	ECACC

ChIP–seq, chromatin immunoprecipitation followed by sequencing; CIRM, California Institute for Regenerative Medicine; DNA-seq, DNA sequencing; ECACC, European Collection of Cell Cultures; HiPSCi, Human induced Pluripotent Stem Cells initiative; iPSCs, induced pluripotent stem cells; RNA-seq, RNA sequencing; SNP, single-nucleotide polymorphism; StemBANCC, Stem Cells for Biological Assays of Novel Drugs and Predictive Toxicology.

Monogenic disorders
Genetic diseases arising from a mutation in a single gene. Examples include cystic fibrosis (mutations in the *CFTR* gene) and Lesch–Nyhan disease (mutations in the *HPRT1* gene).

Complex disorders
Genetic diseases arising from alterations in several genes or that have an unclear genetic basis. Examples include forms of Alzheimer disease and diabetes.

Early-onset
Describes a disease in which phenotypes appear as early as fetal development or early childhood. Examples include Patau syndrome and fragile X syndrome.

Late-onset
Describes a disease in which phenotypes appear in adulthood. Examples include Alzheimer disease and Parkinson disease.

Chromosomal disorders
Diseases arising from either the loss or addition of chromosomes or subchromosomal regions. Examples include Down syndrome (trisomy of chromosome 21) and Turner syndrome (monosomy of chromosome X).

cell lines, respectively. This approach facilitates the generation of genetically matched (isogenic) human PSC lines, which are distinguished only by the alteration introduced within the targeted locus. Such cell lines provide a highly controlled system in which any phenotypic difference is more likely to result from that specific alteration. The growing use of genome editing for modelling both monogenic and complex disorders (see below) with human PSCs has been extensively reviewed elsewhere[21].

The impact of reprogramming on disease modelling. Reprogramming somatic cells to pluripotency has been largely motivated by concerns related to the immunological incompatibility of ES cells in future cell therapy and regenerative medicine. Importantly, reprogramming constitutes an exceptionally useful tool in translational research, as it enables — in principle — the modelling of essentially any human genetic disorder with patient-derived iPSCs. Although a complete understanding of the molecular mechanisms driving reprogramming by defined factors is still lacking, this process is extremely robust and enables the derivation of new iPSC lines using various methods[22], from multiple parental cell types (most commonly fibroblasts[14] or blood cells[23]) and a wide range of donor ages (from the fetal period[14] to old age[24]). Nonetheless, it is important to note that residual somatic epigenetic memory may sometimes persist in iPSCs[25–27], which can potentially affect their differentiation capacity and utility as disease models.

Reprogramming patient cells has several advantages over other strategies for generating disease-specific models with human PSCs. The derivation of iPSCs from multiple patients is usually straightforward, enabling the analysis of similar mutations in diverse genetic backgrounds. In addition, patient-derived iPSCs are more beneficial than genome editing in normal PSCs when modelling genetically complex disorders, which often involve multiple unknown loci. Finally, patient-specific iPSCs may be helpful in making therapeutic decisions in the context of personalized medicine.

It is thus understandable that national and international initiatives are already investing in major efforts to establish repositories of human iPSCs as models for human disorders (TABLE 1). These repositories are aimed at generating thousands of new cell lines, for both monogenic and complex disorders, using non-integrative reprogramming methods such as the use of Sendai viruses or episomal vectors; 1–3 cell lines are derived from each patient and stored in a public repository (reviewed in REF. 28). Along with consortia of multiple investigations within national and international projects, local initiatives such as that of the New York Stem Cell Foundation (NYSCF) have been founded. Specifically, the NYSCF enables automated, high-throughput derivation and differentiation of iPSCs to support and accelerate novel therapies[29]. The goal of these repositories is to provide cell lines for most genetic disorders, an achievement which should change the future of disease modelling and drug screening.

Modelling different disorders with human PSCs

Several criteria should be taken into account when choosing a stem cell-based model for the study of human diseases (TABLE 2). PSC-based models are ideal when studying diseases that arise from a single gene (monogenic disorders), show high penetrance, have an early onset during development and are associated with a clear cellular phenotype. These characteristics are sufficient to predict an *in vitro* observable phenotype in PSCs. By contrast, diseases that originate from a set of genes (complex disorders), show low penetrance, have a late onset (that is, they appear only in adult life) or are associated with phenotypes involving aberrant pattern formation of target organs are more challenging to model using PSCs. Furthermore, using PSCs is usually not advantageous when an established animal model is available or when cell-specific differentiation protocols are lacking. However, some of these challenges can be overcome in order to utilize PSCs in modelling certain diseases. For example, induced cellular ageing can be used for modelling late-onset diseases (as discussed below).

Modelling monogenic and complex disorders. The genetic basis of a disease is crucial for determining the ways in which it can be modelled, especially when using PSCs. Genetic disorders can be roughly divided into three classes: monogenic, chromosomal and complex. Monogenic diseases arise from alterations in a single gene, whereas chromosomal diseases originate from either the loss or the addition of chromosomes or subchromosomal regions. Diseases that arise from alterations in several genes or that have an unclear genetic basis are generally defined as complex. Despite these differences, human diseases from all three classes have been successfully modelled using PSCs: these include monogenic diseases

Table 2 | **Criteria for disease modelling using PSCs**

Criterion	Optimal (disease example)	Challenging (disease example)
Inheritance	Monogenic (fragile X syndrome)	Polygenic (autism spectrum disorder)
Penetrance	High penetrance (cystic fibrosis)	Low penetrance (Alzheimer disease)
Age of onset	Developmental disorder (Down syndrome)	Late onset (Parkinson disease)
Mouse model	Not recapitulated in mice (Lesch–Nyhan syndrome)	Good mouse model (diabetes)
Phenotype	Cellular phenotype (long QT syndrome)	Pattern formation (cleft palate)
Tissue	Accessible differentiation (glycogen storage disease)	Inaccessible differentiation (multiple myeloma)

The examples in this table illustrate diseases corresponding to a specific criterion. Many of them have already been modelled in human embryonic stem cells and/or induced pluripotent stem cells (iPSCs).

such as Lesch–Nyhan disease[8] and fragile X syndrome[10]; chromosomal diseases such as Down syndrome[30] and Turner syndrome[9,31]; and complex disorders such as autism spectrum disorder (ASD)[32] and schizophrenia[33]. It is important to note that although many diseases modelled using PSCs are neurological disorders, a substantial number of studies focus on other disease groups, such as cardiac disorders (reviewed in REF. 22).

Crucially, the three classes of genetic diseases differ in the means that are available to generate a relevant PSC model. Human PSC-based models for monogenic diseases can be obtained by mutagenesis of the disease-associated gene[7,34] (which has been facilitated by the improvement of gene-editing techniques), by isolation of ES cells from affected blastocysts following PGD[10] or by reprogramming somatic cells from the patient[17]. These techniques have all been extensively used, creating a growing number of cell repositories. Chromosomal diseases have been modelled using ES cells with chromosomal aberrations[9] or ES cells isolated from PGS embryos[12], or by reprogramming of somatic cells from patients with chromosomal disorders[17]. Complex diseases, which cannot be diagnosed prenatally or easily reproduced by gene editing, are now modelled using reprogramming of patient cells, either iPSCs[17] or SCNT-ES cells[19].

Another hurdle regarding complex diseases is their genetic correction. Although gene targeting and editing techniques (reviewed in REF. 35) are very efficient, they are still limited to a small number of known genes. Thus, complex diseases, which may involve mutations in genes that remain to be identified, cannot be corrected in the same way as monogenic diseases. Importantly, complex diseases are still being successfully modelled using human PSCs. However, in cases where the same disorder can arise from mutations in either a single gene or multiple genes, many investigators choose to model the rarer monogenic rather than the more prevalent complex trait, for practical reasons. Examples include analysing models of amyotrophic lateral sclerosis (ALS) with mutations in superoxide dismutase 1 (*SOD1*)[36] or models of Parkinson disease with mutations in parkin RBR E3 ubiquitin protein ligase (*PARK2*)[36,37].

Ongoing studies also compare monogenic and complex cases of the same disease. Studies of the complex genetic basis of Alzheimer disease, which is the most common neurodegenerative disorder, have identified mutations that predispose to such disease, as well as revealing protective haplotypes[38,39]. The sortilin-related receptor 1 (*SORL1*) gene encodes a neuronal apolipoprotein receptor that regulates the processing of amyloid precursor protein (APP) into amyloid-β (Aβ), which is the main component of the amyloid plaques that are found in the brains of patients with Alzheimer disease. Although loss of *SORL1* expression had been reported in Alzheimer disease, it was unknown how different *SORL1* variants affect the regulation of this gene[39]. Recently, iPSC-derived neurons from patients with Alzheimer disease with unidentified mutations have been used to examine the role of *SORL1* variants in complex Alzheimer disease[39]. In differentiated neurons of both healthy individuals and several patients with Alzheimer disease, brain-derived neurotrophic factor (BDNF) induced *SORL1* expression, consequently reducing Aβ levels. This response was correlated with the occurrence of a gene variant at the 5′ end of *SORL1*, which was therefore defined as a protective haplotype[39]. By contrast, a risk-associated *SORL1* haplotype did not respond to treatment with BDNF, halting its potential positive effect[39]. These observations demonstrated the complex genetic nature of Alzheimer disease, as the same variants appear in both healthy and diseased individuals. Furthermore, this study affirms the role of neurotrophins in Alzheimer disease, as they can directly alter the accumulation of Aβ peptides through *SORL1*.

In some diseases, different mutations in the same gene cause different disorders. For example, the cardiac arrhythmia disorders Brugada syndrome (BrS) and long QT syndrome 3 (LQT3) have been linked to different mutations in the *SCN5A* gene (which encodes a subunit of voltage-gated sodium channel). Moreover, a specific mutant allele of *SCN5A* can cause both syndromes[40]. Cardiomyocytes differentiated from iPSCs of a patient carrying this mutation recapitulated electrophysiological aberrations that are characteristic of both syndromes[40].

Modelling early- and late-onset disorders. Some human genetic disorders appear during fetal development or early childhood, whereas others arise during adulthood. As ES cells are derived from early pre-implantation human embryos, they are representative of an early embryonic cellular identity. Moreover, several cell types differentiated from PSCs resemble fetal rather than adult cells. Thus, PSC-based models are suitable for the study of developmental or early-onset disorders, some of which may not be viable. Examples include Turner and Down syndromes, which affect embryogenesis, causing a high level of miscarriages[5,41].

Many genetic diseases modelled using PSCs are diagnosed during early childhood. The fatal disease familial dysautonomia, which is caused by a mutation in the IKK complex-associated protein (*IKBKAP*) gene[42], causes widespread degeneration of sensory and

Pre-implantation genetic diagnosis
(PGD). Genetic profiling mainly of mutations within disease-causing genes in pre-implantation embryos produced by IVF. PGD is used to identify diseased embryos of parents with a predisposition for a specific disease.

Pre-implantation genetic screening
(PGS). Screening for chromosomal aberrations in pre-implantation embryos produced by IVF. PGS is used to identify embryos with chromosomal disorders, most commonly in cases of advanced maternal age or in women with multiple previous miscarriages.

Non-integrative reprogramming methods
Techniques that do not involve the insertion and persistence of ectopic reprogramming-inducing DNA sequences within the genome.

Penetrance
The proportion of individuals with a specific genotype who express it at the phenotypic level.

Haplotypes
Groups of adjacent genes and/or alleles that are usually inherited as clusters.

autonomic neurons, with symptoms appearing during early childhood. Patient-derived iPSCs revealed tissue-specific aberrant splicing of *IKBKAP*[42], and splicing-correcting drugs ameliorated neuronal differentiation and migration defects[43,44].

To model late-onset disorders, the embryonic nature of PSCs and their derivatives must be overcome, thus promoting cellular ageing *in vitro*[45]. In some cases, earlier-onset disease variants are available. Parkinson disease, which is one of the most common age-related neurodegenerative disorders, is characterized by progressive loss of dopaminergic neurons[46]. Although it is considered to have a complex genetic basis, some patients with Parkinson disease with a triplication of the α-synuclein (*SNCA*) gene develop early aggressive symptoms[47]. iPSCs derived from such patients enabled researchers to identify disease-specific phenotypes *in vitro* following iPSC differentiation towards dopaminergic neurons[47].

Long differentiation protocols can be used to obtain some mature cell types, including neurons and hepatocytes. However, even long (up to 6 months) and complex protocols to differentiate PSC-derived neurons can result in neurons with fetal features[48–50]. Nonetheless, modelling of many late-onset diseases relies on these differentiation protocols and reports disease-specific phenotypes *in vitro*. For example, neurons differentiated from iPSCs derived from patients with Parkinson disease carrying mutations in *PARK2* have aberrant morphology and reduced complexity of neuronal processes[37].

Stressors such as hydrogen peroxide, MG-132 and concanamycin A have also been used to induce ageing-like features of Parkinson disease in PSC models[51,52]. However, although control and diseased cells showed differential vulnerability to the stressors, it is unclear whether these treatments actually mimic cellular ageing, as most of the studies that used them did not measure age-related hallmarks[45].

An alternative approach for inducing ageing is to harness insights from premature ageing disorders to specifically target age-related markers. Progerin, which is a truncated version of the lamin A protein involved in Hutchinson–Gilford progeria syndrome, has recently been ectopically expressed to promote cellular ageing in Parkinson disease iPSC-derived neurons[53]. Cellular age-associated markers, such as nuclear morphology, expression of heterochromatin markers and DNA damage, were initially measured in fibroblasts from patients with Parkinson disease. These markers were abolished following reprogramming and did not reappear following differentiation towards neurons or fibroblasts[53]. However, these markers were induced after short-term exposure to progerin, suggesting cellular ageing[53]. Progerin expression caused Parkinson disease-related phenotypes, such as dendrite degeneration, expression of age-associated neuromelanin, and morphological features such as mitochondrial swelling.

Thus, several strategies are being used to overcome the limitation of inherently immature PSCs in the study of late-onset diseases. Together, these methods yield complementary phenotypes and insights into disease origins and progression.

Modelling epigenetic disorders. Whereas most inherited diseases are linked to genetic defects, certain disorders can be defined as predominantly or entirely epigenetic. The underlying basis for inherited epigenetic disorders often involves heritable changes in DNA sequences that are directly linked to epigenetic regulation. For instance, mutations in genes that affect DNA methylation may lead to widespread global effects in *trans*, as observed in immunodeficiency, centromeric instability and facial anomalies (ICF) syndrome type I, which is a disease caused by loss-of-function mutations in the *de novo* DNA methyltransferase 3B (*DNMT3B*) gene[54]. As expected from *DNMT3B* deficiency, patient-derived iPSCs and differentiated cell derivatives exhibit DNA hypomethylation in pericentromeric and subtelomeric regions, as well as altered gene expression profiles[55,56]; both of these characteristics are likely to be contributors to the genomic instability and physiological defects associated with this disease. Importantly, although *DNMT3B* is highly active in human PSCs, it is not essential for reprogramming and pluripotency[55–57], thereby enabling the study of the aetiology of ICF type I in the context of early development.

Another class of epigenetic disorders comprises those involving parental genomic imprinting[58], a process by which parent-specific epigenetic modifications occur differentially, causing allele-specific expression of genes in their proximity[59]. Parental imprints are marked and exert their function through differential DNA methylation at imprinting control regions (ICRs). Imprinting disorders can arise as the result of several types of genetic defects, including mutations localizing to specific imprinted genes or ICRs, as well as aberrations on a larger scale, such as subchromosomal deletions and translocations, that affect imprinted loci. Owing to the unique mode of transgenerational epigenetic inheritance that is intrinsic to imprinting, these disorders may also arise purely from epigenetic factors, including defective imprints and uniparental disomy (UPD).

Although the stability of imprinted regions can be variably influenced by prolonged culture and reprogramming, PSCs generally preserve normal imprinting signatures[60–64]. This notion enabled the generation of patient-derived iPSC models for two major neurobehavioral imprinting disorders, Prader–Willi syndrome (PWS) and Angelman syndrome, from individuals who carried distinct molecular defects in the PWS–Angelman syndrome region on chromosome 15q11–q13 (REFS 65–69). Of note, functional studies in PWS iPSC lines have enabled mechanistic exploration of imprinting in the PWS–Angelman syndrome region[66,69] and revealed that the long non-coding RNA *IPW*, which is absent in PWS, is involved in regulating the *DLK1–DIO3* imprinted locus in *trans*[67].

A more severe, global manifestation of abnormal imprinting is observed upon parthenogenetic development of human oocytes into ovarian teratomas[70]. The epigenome of parthenogenetic cells is entirely maternal in origin and is therefore incompatible with the expression of paternally inherited alleles. PSCs with only a maternal genome can be obtained through activation of an unfertilized egg generating parthenogenetic ES cells[71–73]

Parental genomic imprinting
A process by which parent-specific epigenetic modifications occur differentially in maternally and paternally inherited alleles.

Imprinting disorders
Disorders that originate from the aberrant regulation of imprinted genes. Examples include Prader–Willi syndrome and Angelman syndrome.

Parthenogenetic development
The development of an embryo from an unfertilized oocyte.

Organoids
Miniature organ-like structures generated in culture. Organoids vary in their complexity, but they are usually composed of several cell types and recapitulate three-dimensional organ development.

or by reprogramming of diploid ovarian teratoma cells into parthenogenetic iPSCs[74]. Such iPSCs have been utilized to identify novel imprinted genes[74] and new roles for imprinting in tumorigenesis[75].

Until recently, studying the epigenetic basis of human pathologies has largely relied on cells that harbour a pre-existing epigenetic state. In principle, these studies could also be performed in PSCs through 'epigenome editing', in which the same molecular tools that are utilized for genome editing are engineered to recruit chromatin modifiers and alter epigenetic modifications in a locus-specific manner[76]. With this approach, it may be possible to directly induce desired epigenetic alterations in a controlled setting (for example, in isogenic PSC lines), while avoiding the interference of unexpected epigenetic changes that might be conferred during culture or in reprogramming.

Box 1 | **Identifying disease-related phenotypes**

Modelling human diseases *in vitro* requires the identification of clear, disease-specific phenotypes. These phenotypes are defined using several different strategies and can be roughly divided into three categories: molecular, cellular and physiological.

Molecular phenotypes
These include expression analyses of RNA transcripts and proteins, and characterization of epigenetic markers such as DNA methylation and histone modifications. These molecular criteria are very often used to characterize diseases. Whereas some studies are performed at a global level (global gene expression and/or proteome profiles[104,105]), others analyse only the expression of the gene affected by the disease or its immediate targets (such as inactivation of the frataxin (*FXN*) gene in Friedreich ataxia[106]). Molecular phenotypes are especially useful in high-throughput drug screening, as they can be measured in an automated manner. In 2012, the ability to restore expression of IKK complex-associated protein (*IKBKAP*), which is the gene mutated in familial dysautonomia, was automatically evaluated by real-time quantitative reverse-transcriptase PCR (qRT-PCR) for over 6,000 compounds, identifying potential treatments for the disease[44].

Cellular phenotypes
These include aberrant morphology, protein aggregation or increased apoptosis, and are also frequently exploited to model diseases *in vitro*. Morphological changes are often visible in neurons differentiated from induced pluripotent stem cells (iPSCs) of patients with neurological disorders[107–109]. Cellular phenotypes are useful for the modelling of metabolic disorders. For example, patients with glycogen storage disease type 1a (GSD1a) are unable to maintain glucose homeostasis and have substantial metabolic defects, including glycogen and lipid accumulation. Hepatocytes differentiated from iPSCs of patients with GSD1a show similar phenotypes, as shown by periodic acid–Schiff and BODIPY staining[110]. Cellular features can also be evaluated on a large scale. Motor neuron survival, for example, was used in a small-molecule survival screen (an assay measuring the viability of cells in culture, which is highly useful for the evaluation of drug toxicity and cellular apoptosis) to identify potential treatments for amyotrophic lateral sclerosis (ALS)[111].

Physiological phenotypes
These phenotypes, which are the most challenging to evaluate, are particularly relevant to the study of neurological or cardiovascular diseases. Their evaluation includes measurements of ion exchange, channel activity and contractility. These measurements are carried out using multi-electrode arrays or patch-clamp analysis of neurons and cardiac muscles, as the activity of these cells depends on current and ion flux. Cardiomyocytes differentiated from iPSCs of patients with congenital long QT syndrome, for example, show prolonged action potentials and higher sensitivity to isoprenaline, which is a β-adrenoreceptor agonist[112]. Similarly, ALS patient-derived neurons were shown to be hyperexcitable and to have an increased spontaneous firing rate compared to control neurons[113].

It is important to note that differentiating PSCs in three-dimensional (3D) cultures may reveal additional phenotypic characteristics that can be used to model diseases. For example, brain organoids developed from iPSCs that were derived from patients with microcephaly[81] not only showed premature neural differentiation, but they were also substantially smaller than control organoids. Furthermore, these organoids showed aberrant radial glial spindle orientation, which is a complex phenotype that can be associated with specific disease symptoms. Such structural phenotypes can only be detected in 3D cultures.

Modelling with advanced culturing techniques

Although many diseases can be modelled using a single cell type *in vitro*, some diseases require more advanced culturing techniques. By modelling ALS using co-cultures of neurons and glial cells[77,78], it was shown that spinal motor neurons are selectively sensitive to the toxic effects of glial cells carrying a mutant *SOD1* gene. It is interesting to note that this effect was not apparent when culturing neurons with fibroblasts expressing mutant *SOD1*, confirming the specificity of the model[78].

The interactions between different cell types can be better understood by studying three-dimensional (3D) tissue structures. PSC-derived organoids of several tissues have been established in culture, allowing *in vitro* analysis of molecular, cellular and physiological phenotypes, as well as structural features related to development and disease (BOX 1). Some examples of 3D structures include those that recapitulate eye[79] and pituitary[80] development, and the generation of cerebral[81] and intestinal organoids[82]. The latter were also used to model microcephaly[81] and cystic fibrosis[82], respectively, uncovering disease-related phenotypes. Using intestinal organoids derived from iPSCs from a patient with cystic fibrosis, disease-specific phenomena such as aberrant swelling in response to forskolin were recapitulated[83]. This model enables *in vitro* diagnosis of cystic fibrosis and the development of personalized medicine. The exciting field of 3D-PSC organoids has been extensively reviewed elsewhere[84,85].

Comparison of models in ES cells and iPSCs

Models of human genetic disorders can be generated in all types of PSCs (FIG. 1). Although the first models were generated in ES cells, once iPSC technology became available, it rapidly became the preferred choice, given the limited availability of PGD-derived ES cells. Despite their different origins, iPSCs are very similar to ES cells, in that they express similar markers, possess self-renewal capacity and differentiate *in vitro* and *in vivo* into the three embryonic germ layers[15,16]. Nonetheless, there are several differences between these two types of PSCs, including the persistence of epigenetic memory from the somatic cells of origin in iPSCs[25–27], differential DNA methylation signatures[86,87] and a different extent of genetic aberrations (reviewed in REF. 88). Thus, it is sometimes worthwhile to generate models for the same disease in both ES cells and iPSCs, as studies have revealed phenotypic similarities and differences between them either before and/or following differentiation (reviewed in REF. 89).

The first comparison of ES cell and iPSC disease models was conducted for fragile X syndrome (FXS), which is the most prevalent cause of hereditary mental impairment in boys. In most patients, FXS is caused by an expansion of trinucleotide repeats at the 5′ untranslated region (UTR) of the fragile X mental retardation 1 (*FMR1*) gene, which causes silencing of *FMR1* transcription through epigenetic

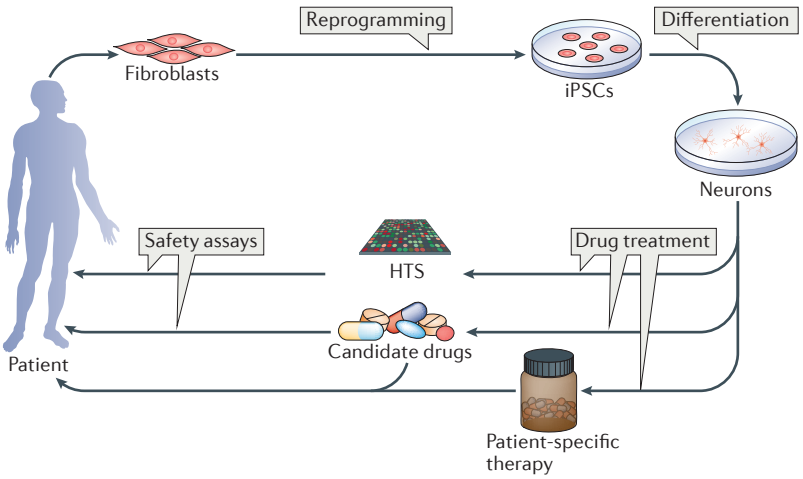

Figure 2 | **Drug development strategies using human induced pluripotent stem cells (iPSCs).** Patient-derived somatic cells (for example, fibroblasts) can be reprogrammed to generate iPSCs carrying a disease-specific genetic aberration. These cells can then be differentiated into the disease-affected cell type (for example, neurons in neurodegenerative diseases). After the establishment of a cellular disorder model with disease-specific phenotypes, three main strategies are commonly used: high-throughput screening (HTS) of drugs, the candidate drug approach or patient-specific therapy. In HTS, a very large number of compounds are tested on the differentiated cells, followed by phenotype re-evaluation. This method is extremely valuable for identifying novel therapies *in vitro*, by using large libraries of compounds. By contrast, both the candidate drug approach and the patient-specific therapy use a small number of potential drugs to attenuate the disease. These approaches are useful when the disease mechanism is known and potential therapies are available. Drugs found by both the HTS and candidate drug approaches usually require substantial safety assays before being prescribed to patients, whereas drugs already approved by regulatory agencies can be immediately prescribed for treatment.

modifications at the gene promoter. An FXS model in PSCs was first established in ES cells generated from an affected embryo following PGD[10]. Surprisingly, the mutated undifferentiated ES cells expressed *FMR1*, and the gene was silenced during differentiation. This model system revealed the developmental inactivation of the gene during embryogenesis and showed that the mutation in the 5′ UTR is necessary but not sufficient for *FMR1* silencing. The generation of a similar model in iPSCs by reprogramming somatic cells of patients with FXS produced different results[90]. The *FMR1* gene, which was silent in patient somatic cells, remained silent following reprogramming, suggesting an epigenetic memory from the somatic cells. However, while FXS iPSCs are unable to recapitulate *FMR1* activity in embryonic cells, they are very useful in analysing the molecular phenotype of FXS neurons[91] and in finding ways to activate this locus[92]. The comparison between FXS ES cells and FXS iPSCs carrying the same genetic mutation uncovered a difference in their epigenetic phenotype, suggesting that each system is useful for studying a different aspect of the disease.

Another informative comparison between models in ES cells and in iPSCs comes from the study of Fanconi anaemia. This disease involves a defect in DNA repair causing genomic instability, bone marrow failure and cancer. Initial attempts to model the disease in iPSCs failed, probably because of chromosomal aberrations in somatic cells and the possible involvement of the Fanconi anaemia pathway in the reprogramming process[93]. By contrast, Fanconi anaemia could be modelled in ES cells using lentiviral RNA interference[94]. Later attempts to model Fanconi anaemia in iPSCs were also successful, albeit with low reprogramming efficiency and the use of a modified reprogramming methodology[95]. This example indicates that the reprogramming process can be the cause of differences between ES cell and iPSC models. Although ES cell and iPSC models may vary for certain disorders, iPSC models for most disorders are expected to mimic ES cell models, thus highlighting the utility of reprogramming patient cells.

Drug therapy using PSCs

One of the main motivations for generating models for human diseases is to develop therapies enabling the diseases to be treated, alleviated or cured. Animal models are frequently used for drug screening; however, as detailed above, many disorders lack a suitable animal model. Moreover, the use of animal models for high-throughput screening (HTS) of small-molecule libraries is usually not feasible. Thus, the use of PSC disease models has become increasingly favoured for purposes of drug discovery.

Drug screening strategies using PSCs. Similar to studying animal models, the first step in therapy development using PSCs is to identify and define the phenotypes that are to be treated (BOX 1). This step is especially well illustrated in *in vitro* disease models, in which only a subset of the phenotypes are manifested, and which usually require differentiation towards a specific cell type in culture (FIG. 2). Once the phenotypes to be treated are identified, there are two main strategies to identify potential drugs: the candidate drug approach and the HTS approach (FIG. 2).

In the candidate drug approach, a small and well-defined group of compounds is tested on affected cells. This methodology is suitable when an abnormal cellular phenotype gives an indication that a specific pathway is responsible for such a phenotype, or when similar diseases have already been successfully modelled and treated *in vitro* using such drugs. The candidate drug approach is the cornerstone of personalized medicine, as once a patient-specific iPSC line has been established, the cells can be propagated and differentiated and used to test a defined set of drugs, with the aim of identifying the most potent therapy. This approach also includes the validation or confirmation of existing drugs in new iPSC models. The candidate drug approach makes it possible for drugs approved by regulatory agencies to be tested immediately on a new disease model, and thus to rapidly provide initial results before clinical use on patients (FIG. 2).

By contrast, HTS does not require previous knowledge to initiate drug discovery, as it enables the testing of over a million compounds per study[96]. However, HTS has the limitation of requiring a phenotype that can be automatically measured and quantified. This is useful when dealing with cell survival rates or with protein expression that can be easily made visible (for example, with fluorescent reporter genes), but it is less beneficial when the disease modelled affects a more complex phenotype (for example, electrophysiological defects). Furthermore,

High-throughput screening (HTS). A drug discovery strategy involving the analysis of a large array of compounds, which are chosen in an unbiased fashion. The effects of each compound on an aberrant phenotype are evaluated simultaneously.

Candidate drug approach A drug discovery strategy involving compounds that were previously shown to affect a specific pathway or phenotype and that are tested as potential therapies for a specific disease on the basis of this information.

a large number of cells are required to test a wide range of compounds. This is especially challenging when the affected cells require a long differentiation process or when the differentiation yield is low. The candidate drug approach requires a much lower number of cells and can be used to evaluate complex phenotype alterations.

Recent studies have used both HTS and focused candidate analysis and have drawn on the advantages of each approach. For example, Barmada *et al.*[96] first performed an extremely large HTS on diseased mouse neurons, and only then were selected compounds evaluated on motor neurons and astrocytes differentiated from ALS patient-derived iPSCs.

It is important to note that both approaches are merely the first step in drug development. As they utilize *in vitro* models, in most cases both HTS and novel candidate drug approaches require extensive safety assays before being applied in a clinical context.

Drug discovery for neurological disorders. Although many types of diseases have been modelled using iPSC-derived cells, neurological disorders are a prime example of the use of these cells in drug discovery. This diverse group of diseases includes different classes of genetic disorders, including both early- and late-onset diseases, disorders manifested in several cell types and disorders that affect both the central and the peripheral nervous systems. Furthermore, as the world's population ages, with the number of individuals over 60 expected to double in the next few decades, the growing prevalence of neural degeneration diseases calls for urgent solutions and novel therapies. Evaluating this spectrum of disorders provides a snapshot of current trends in iPSC-based disease modelling and drug discovery.

Here, we discuss studies that have used patient-derived (rather than ES cell-based) differentiated iPSCs to successfully treat the aberrant phenotype (FIG. 3; TABLE 3). The use of iPSC-derived neurons as a platform for drug development began only 2 years after the discovery of human fibroblast reprogramming, with pioneering papers published in 2009 (REF. 43), and the field has since seen a rapidly growing number of published studies. In TABLE 3, we list 25 neurological diseases for which iPSC-derived neural cells have been used to model the disorders and have then been screened for appropriate drug therapies.

Although differentiated neurons in culture are usually only comparable with immature embryonic neurons, almost half of these disease-modelling studies focused on late-onset diseases. In other words, although these diseases are manifested very late in life, aberrant cellular, molecular and electrophysiological phenotypes are detectable and treatable in culture. Interestingly, most studies have been performed on cells from patients bearing monogenic or chromosomal aberrations, and not from patients with complex genetic disorders (FIG. 3). Although most cases of Alzheimer disease and ALS do not have a monogenic basis, a considerable number of

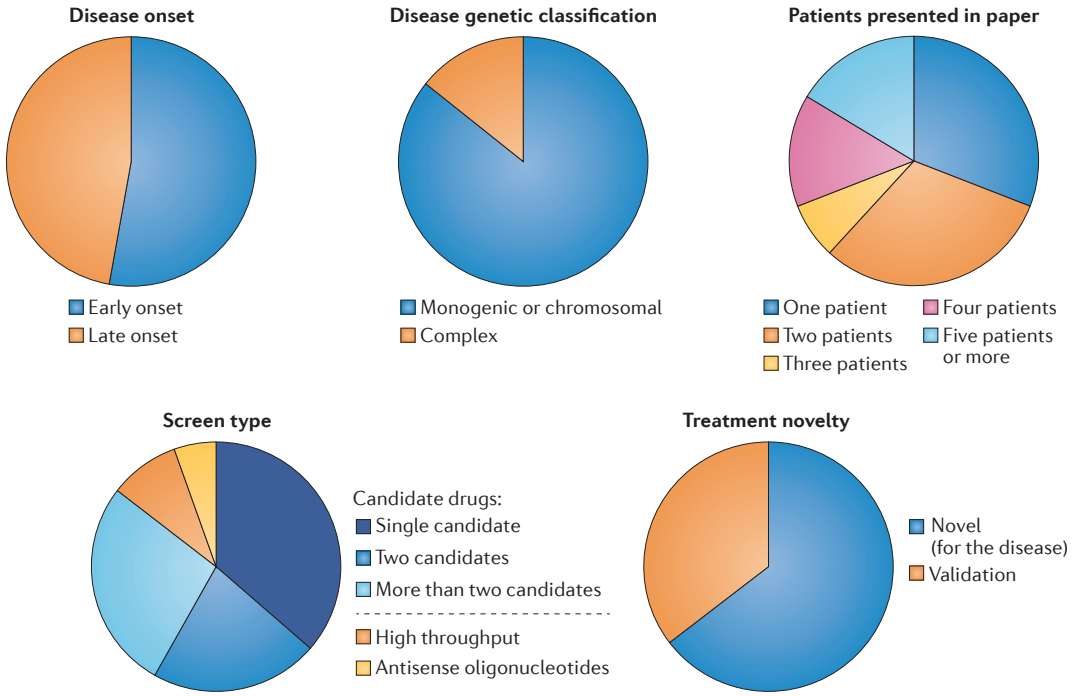

Figure 3 | **Evaluation of drug screening studies using patient-derived induced pluripotent stem cell (iPSC) models for neurological disorders.** Distributions of 55 studies reporting the identification of therapies for neurological disorders using human iPSCs are summarized. Shown in the upper panel from left to right are analyses of studies based on disease onset, the type of genetic classification of the disease, the and number of patients used to generate iPSCs in the study. From left to right, the bottom panel shows analyses based on type of drug screening and treatment novelty, that is, whether the therapy presented is novel or was previously shown *in vivo* or in other cell types modelling the same disease.

Table 3 | **Drug screening for neurological diseases using patient-derived induced pluripotent stem cell models**

Disease name (number of patients)	Genetic basis	Onset	Differentiated cell	Analysed and corrected phenotype*	Screening type	Identified drug	Refs
Adrenoleuko-dystrophy (2)	*ABCD1*	Early and late	Neurons and oligodendrocytes	Molecular and cellular	Confirmation	4-phenylbutyrate (4PBA), lovastatin	114
Amyotrophic lateral sclerosis (ALS) (1–16)	*SOD1*, *C9ORF72*, *FUS1*, *TDP43*, complex	Late	Neurons, motor neurons and astrocytes	Molecular, cellular and electro physiological	Confirmation, candidate (drugs and ASOs), HTS	Retigabine, kenpaullone, digoxin, lanatoside C, proscillaridin A, anacardic acid, methotrimeprazine, fluphenazine, ASOs	36, 96–98, 111,113, 115
Alzheimer disease (1–6)	*APP*, *PS1*, *PS2*, complex	Late	Neurons	Molecular and cellular	Confirmation, candidate	γ-secretase inhibitor, compound E, compound W, GSM-4, Si-II,OM99-2, docosahexaenoic acid (DHA)	116–120
Ataxia telangiectasia (2)	*ATM*	Early	Neurons and glia	Cellular	Candidate	Geneticin (G418)	121
Autism spectrum disorder (ASD) (1–5)	15q11-q13.1 duplications, (3;11) (p21;q22) translocation	Early	Neurons	Molecular, cellular and electro physiological	Candidate	Hyperforin with flufenamic acid (FFA), mithramycin	108,122
Bipolar disorder (2)	Complex	Early	Neurons	Molecular and cellular	Candidate	CHIR-99021	123
Down syndrome (1–2)	Trisomy 21	Early	Neural progenitor cells (NPCs), neurons, astroglia	Molecular and cellular	Candidate	Minocycline, epigallocatechin gallate (EGCG), F127–*N*-butylidenephthalide (BDPH)	124–126
Familial dysautonomia (2–3)	*IKBKAP*	Early	Neural crest cells	Molecular and cellular	Confirmation, HTS	SKF-86466 hydrochloride, kinetin	43,44
Fragile X syndrome (FXS) (1–3)	*FMR1*	Early	NPCs, neurons	Molecular	Candidate, HTS	5-azacytidine, several other compounds (not specified)	92,127
Friedreich ataxia (2)	*FXN*	Early	Neurons	Molecular and cellular	Confirmation, candidate	Forskolin, RG2833	106,128
GN1 gangliosidosis (1)	*GLB1*	Early	NPCs	Molecular and cellular	Candidate	Z-YVAD-FMK	129
Hereditary spastic paraplegias (HSP) (1)	*SPAST*	Early and late	Forebrain glutamatergic neurons	Cellular	Confirmation	Vinblastine	130
Hereditary transthyretin amyloidosis (1)	*TTR*	Early and late	Neurons	Cellular	Candidate	Flufenamic acid	131
Huntington disease (1–2)	*HTT*	Late	NPCs, neurons, GABAergic neurons and medium spiny neurons	Molecular and cellular	Confirmation, candidate	P110-TAT, KU-60019, X5050	109,132, 133
Machado–Joseph disease (4)	*ATXN3*	Late	Neurons	Cellular	Candidate	ALLN, calpeptin	134
Neuronal ceroid lipofuscinosis (2)	*TPP1*	Early	NPCs and neurons	Cellular	Candidate	PTC124	135
Niemann–Pick disease (1–4)	*NPC1*	Early	NPCs and neurons	Molecular and cellular	Confirmation, candidate	Rapamycin, carbamazepine, verapamil, trehalose, 2-hydroxypropyl-b-cyclodextrin (HPBCD), 2-hydroxypropyl-c-cyclodextrin (HPGCD), VEGF, δ-tocopherol with HPBCD or methyl-β-cyclodextrin (MBCD)	136–139
Parkinson disease (1–5)	*PARK2*, *SNCA*, *PINK1* and *LRRK2*	Late	Neurons, midbrain dopaminergic neurons and cortical neurons	Molecular and cellular	Confirmation, candidate, HTS	Taxol, isoxazole, NAB2, GW5074, co-enzyme Q10	37,52, 140,141

Table 3 (cont.) | **Drug screening for neurological diseases using patient-derived induced pluripotent stem cell models**

Disease name (number of patients)	Genetic basis	Onset	Differentiated cell	Analysed and corrected phenotype*	Screening type	Identified drug	Refs
Phelan–McDermid syndrome (PMDS) (2)	Complex	Early	Mature forebrain neurons	Molecular, cellular and electrophysiological	Confirmation, candidate	Insulin-like growth factor 1 (IGF1)	142
Rett syndrome (3–4)	*MECP2*	Early	Neurons and astrocytes	Cellular	Confirmation, candidate	Glypromate (GPE), IGF1, gentamicin	143,144
Schizophrenia (1–4)	Complex	Early and late	NPCs and neurons	Molecular and cellular	Confirmation	Loxapine, valproate (VPA)	33,145, 146
Spinal and bulbar muscular atrophy (SBMA) (1)	Androgen receptor	Late	Motor neurons	Cellular	Confirmation	Heat shock protein 90 (HSP90) inhibitor 17-AAG	147
Spinal muscular atrophy (SMA) (2)	*SMN1*	Early	Motor neurons	Molecular and cellular	Confirmation, candidate (drugs and ASOs)	Phosphorodiamidate morpholino oligonucleotides (PMOs), FasNT antibody, Z-DVED-FMK, salubrinal, guanabenz	99,102, 103
Timothy syndrome (2)	*CACNA1C*	Early	Cortical neurons	Molecular, cellular and electrophysiological	Candidate	Roscovitine	148
Wolfram syndrome (5)	*WFS1*	Early	Neurons	Cellular	Candidate	Dantrolene	149

ABCD1, ATP-binding cassette D1; *APP*, amyloid precursor protein; ASOs, antisense oligonucleotides; *ATM*, ataxia telangiectasia mutated; *ATXN3*, ataxin 3; *C9ORF72*, chromosome 9 open reading frame 72; *CACNA1C*, calcium channel subunit α1C; *FMR1*, fragile X mental retardation 1; *FUS1*, fused in sarcoma 1; *FXN*, frataxin; *GLB1*, galactosidase-β1; HTS, high-throughput screening; *HTT*, huntingtin; *IKBKAP*, IKK complex-associated protein; *LRRK2*, Leu-rich repeat kinase 2; *MECP2*, methyl-CpG-binding protein 2; *NPC1*, Niemann–Pick type C1; *PARK2*, parkin RBR E3 ubiquitin protein ligase; *PINK1*, PTEN-induced kinase 1; PS, presenilin; *SMN1*, survival of motor neuron 1; *SNCA*, α-synuclein; *SOD1*, superoxide dismutase; *SPAST*, spastin; *TDB43*, TAR DNA-binding protein 43; *TPP1*, tripeptidyl peptidase 1; *TTR*, transthyretin; VEGF, vascular endothelial growth factor; *WFS1*, Wolfram syndrome 1. *The different phenotypes that have been analysed and ameliorated by the drugs belong to three categories: molecular (gene and protein expression, and DNA methylation); cellular (morphology (foci, synapses), mitochondrial functions, apoptosis and toxicity-induced reactions); and electrophysiological (currents, action potentials and ion fluxes).

studies modelling these diseases still use fibroblasts from patients with mutations in a single gene. Moreover, most studies have reported reprogramming cells from only one or two patients, before differentiating them towards neurons (FIG. 3). This may change when more iPSC lines are available for each disease, as a result of the different repositories of diseased iPSCs (TABLE 1).

Most studies carry out a limited screen of candidate drugs on differentiated cells instead of running HTS. About a third of these studies have used iPSC-based models to validate the effects of existing drugs, rather than suggesting a novel one. One reason for this preference can be the potentially rapid transition from *in vitro* models to clinical trials when using regulatory approved drugs. Interestingly, alongside potential drugs and HTS of small molecules, modified oligonucleotides are used to target specific sequences[97–99]. In this approach, antisense oligonucleotides bind to complementary mRNA sequences of an aberrant gene, effectively inactivating it. This methodology is promising, as gene-specific sequences can be easily synthesized, and *in vivo* delivery methods are currently being developed and tested (reviewed in REF. 100).

From bedside to bench and back. Research on modelling human disorders using PSCs is already fulfilling its first mission of going from 'bedside to bench'. Somatic cells from patients are routinely reprogrammed to iPSCs, the cells are differentiated into a plethora of cell types and patient phenotypes are recapitulated *in vitro*. Moreover, in many cases, the cells are also utilized for drug screening and validation, thus promoting new drug discoveries. To truly fulfil the promise of disease modelling using iPSCs, we must move from 'bench to bedside' and, ideally, find a new therapy for the same patient who donated somatic cells for reprogramming and develop patient-specific therapies.

Many disorders already have multiple drug therapies, but not all drugs are effective for all patients. Instead of treating a patient with consecutive drug therapies until the most suitable one is found, all potential drugs can be tested in parallel on the patient's cells, facilitating prescription of the most effective drug (FIG. 2). Another approach is to test candidate drugs on the basis of our understanding of the pathology underlying the cellular phenotype (FIG. 2). If the tested drug is already approved for another disease by the regulatory agencies, clinical trials may be initiated at a fairly rapid pace. An example of such an approach was recently published for ALS[101]. The researchers discovered that patient iPSC-derived motor neurons displayed hyperexcitability with reduced survival. They showed that this phenotype could be corrected by a potassium channel agonist that is already FDA-approved for epileptic patients. Thus, they could immediately initiate Phase II clinical trials with this drug for ALS patients[101]. Amazingly, less than two years elapsed between the initial *in vitro* discovery and the approved clinical trial, without the need for preclinical

Clinical trials
Studies that evaluate potential treatments on human subjects. These trials are tightly regulated, have strict requirements and are composed of typical phases, evaluating the safety and efficacy of the treatment.

experiments on animals. Treatments for other neurological disorders, including familial dysautonomia and spinal muscular atrophy (SMA), are also at different stages on the road to the clinic[43,44,102,103].

The true challenge is finding new therapies in an unbiased fashion using HTS (FIG. 2). Such screens have already been initiated in several laboratories; however, the majority of such drugs will require the use of safety assays in cells and/or animals, and — if an animal model exists — even preclinical trials in animals. However, for disorders that cannot be recapitulated in animals, it may be reasonable to go directly from experiments on human PSCs to clinical trials, thus truly fulfilling the promise of going all the way from bedside to bench and back again.

Perspective

Despite the promising future of PSC-based therapies, there are still substantial hurdles between its potential and its fulfilment. In the future, we expect to have repositories of PSCs that will enable the modelling of practically any genetic disease, whether monogenic, chromosomal or complex. One step in this direction is the generation of isogenic cell lines with the induction or correction of relevant mutations, sometimes with different mutations in the same gene. In genetically complex disorders, work is likely to focus on using patient-derived iPSCs, alongside the induction of mutations in several genes that are suspected to be involved in the disease. The ability to model cell-specific disorders depends on efficient and robust differentiation protocols. Although we have the ability to obtain mature and functional cells for some cell types, this still constitutes a substantial impediment for others. Alongside the optimization of cell cultures and differentiation protocols, we expect the use of screening to flourish for therapies using small molecules, as well as for nucleic acid-based and amino acid-based drugs. We also expect to see many clinical trials with drugs identified in culture, especially using previously approved drugs. In cases in which the disease lacks appropriate animal models, it is likely that, after extensive safety assays, the therapy will be implemented directly from the dish to the patient. Although much additional work is required, recent advances and important results have confirmed the value of using PSCs in modelling and treating numerous diseases.

1. Mouse Genome Sequencing Consortium *et al.* Initial sequencing and comparative analysis of the mouse genome. *Nature* **420**, 520–562 (2002).
2. Strachan, T., Lindsay, S. & Wilson, D. I. (eds) *Molecular Genetics of Early Human Development* (Academic Press, 1997).
3. Hamlin, R. L. & Altschuld, R. A. Extrapolation from mouse to man. *Circ. Cardiovasc. Imaging* **4**, 2–4 (2011).
4. Saenger, P. Turner's syndrome. *N. Engl. J. Med.* **335**, 1749–1754 (1996).
5. Chester, N., Kuo, F., Kozak, C., O'Hara, C. D. & Leder, P. Stage-specific apoptosis, developmental delay, and embryonic lethality in mice homozygous for a targeted disruption in the murine Bloom's syndrome gene. *Genes Dev.* **12**, 3382–3393 (1998).
6. Thomson, J. A. *et al.* Embryonic stem cell lines derived from human blastocysts. *Science* **282**, 1145–1147 (1998).
This was the first report of human ES cell derivation, which laid the cornerstone for human PSC research.
7. Zwaka, T. P. & Thomson, J. A. Homologous recombination in human embryonic stem cells. *Nat. Biotechnol.* **21**, 319–321 (2003).
8. Urbach, A., Schuldiner, M. & Benvenisty, N. Modeling for Lesch-Nyhan disease by gene targeting in human embryonic stem cells. *Stem Cells* **22**, 635–641 (2004).
This was the first study to investigate a disease model in human ES cells.
9. Urbach, A. & Benvenisty, N. Studying early lethality of 45,XO (Turner's syndrome) embryos using human embryonic stem cells. *PLoS ONE* **4**, e4175 (2009).
10. Eiges, R. *et al.* Developmental study of fragile X syndrome using human embryonic stem cells derived from preimplantation genetically diagnosed embryos. *Cell Stem Cell* **1**, 568–577 (2007).
11. Mateizel, I. *et al.* Derivation of human embryonic stem cell lines from embryos obtained after IVF and after PGD for monogenic disorders. *Hum. Reprod.* **21**, 503–511 (2006).
12. Biancotti, J. C. *et al.* Human embryonic stem cells as models for aneuploid chromosomal syndromes. *Stem Cells* **28**, 1530–1540 (2010).
13. Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663–676 (2006).
This ground-breaking paper describes the successful reprogramming of mouse somatic cells to PSCs by forcing expression of specific factors.
14. Takahashi, K. *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861–872 (2007).
15. Yu, J. *et al.* Induced pluripotent stem cell lines derived from human somatic cells. *Science* **318**, 1917–1920 (2007).
References 14 and 15 both recapitulated the success of mouse somatic cell reprogramming in human fibroblasts.
16. Takahashi, T. & Yamanaka, S. A decade of reprogramming by transcription factors. *Nat. Rev. Mol. Cell Biol.* http://dx.doi.org/10.1038/nrm.2016.8 (2016).
17. Park, I. H. *et al.* Disease-specific induced pluripotent stem cells. *Cell* **134**, 877–886 (2008).
This report describes the first disease models in patient-derived human iPSCs.
18. Tachibana, M. *et al.* Human embryonic stem cells derived by somatic cell nuclear transfer. *Cell* **153**, 1228–1238 (2013).
This was the first report on the derivation of human PSCs using somatic nuclear transfer.
19. Yamada, M. *et al.* Human oocytes reprogram adult somatic nuclei of a type 1 diabetic to diploid pluripotent stem cells. *Nature* **510**, 533–536 (2014).
This study demonstrates the reprogramming of patient fibroblasts into PSCs using somatic cell nuclear transfer.
20. Kim, H. & Kim, J. S. A guide to genome engineering with programmable nucleases. *Nat. Rev. Genet.* **15**, 321–334 (2014).
21. Sterneckert, J. L., Reinhardt, P. & Scholer, H. R. Investigating human disease using stem cell models. *Nat. Rev. Genet.* **15**, 625–639 (2014).
22. Bellin, M., Marchetto, M. C., Gage, F. H. & Mummery, C. L. Induced pluripotent stem cells: the new patient? *Nat. Rev. Mol. Cell Biol.* **13**, 713–726 (2012).
This review presents the topic of disease modelling using iPSCs.
23. Loh, Y. H. *et al.* Generation of induced pluripotent stem cells from human blood. *Blood* **113**, 5476–5479 (2009).
24. Dimos, J. T. *et al.* Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* **321**, 1218–1221 (2008).
25. Kim, K. *et al.* Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. *Nat. Biotechnol.* **29**, 1117–1119 (2011).
26. Ohi, Y. *et al.* Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells. *Nat. Cell Biol.* **13**, 541–549 (2011).
27. Bar-Nur, O., Russ, H. A., Efrat, S. & Benvenisty, N. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. *Cell Stem Cell* **9**, 17–23 (2011).
28. Soares, F. A., Sheldon, M., Rao, M., Mummery, C. & Vallier, L. International coordination of large-scale human induced pluripotent stem cell initiatives: Wellcome Trust and ISSCR workshops white paper. *Stem Cell Rep.* **3**, 931–939 (2014).
This is a comprehensive report on initiatives to model diseases using iPSCs.
29. Paull, D. *et al.* Automated, high-throughput derivation, characterization and differentiation of induced pluripotent stem cells. *Nat. Methods* **12**, 885–892 (2015).
30. Briggs, J. A. *et al.* Integration-free induced pluripotent stem cells model genetic and neural developmental features of Down syndrome etiology. *Stem Cells* **31**, 467–478 (2013).
31. Li, W. *et al.* Modeling abnormal early development with induced pluripotent stem cells from aneuploid syndromes. *Hum. Mol. Genet.* **21**, 32–45 (2012).
32. DeRosa, B. A. *et al.* Derivation of autism spectrum disorder-specific induced pluripotent stem cells from peripheral blood mononuclear cells. *Neurosci. Lett.* **516**, 9–14 (2012).
33. Brennand, K. J. *et al.* Modelling schizophrenia using human induced pluripotent stem cells. *Nature* **473**, 221–225 (2011).
This study was the first to present an iPSC-based model for a psychiatric disease.
34. Ding, Q. *et al.* A TALEN genome-editing system for generating human stem cell-based disease models. *Cell Stem Cell* **12**, 238–251 (2013).
35. Cox, D. B., Platt, R. J. & Zhang, F. Therapeutic genome editing: prospects and challenges. *Nat. Med.* **21**, 121–131 (2015).
36. Burkhardt, M. F. *et al.* A cellular model for sporadic ALS using patient-derived induced pluripotent stem cells. *Mol. Cell. Neurosci.* **56**, 355–364 (2013).
37. Ren, Y. *et al.* Parkin mutations reduce the complexity of neuronal processes in iPSC-derived human neurons. *Stem Cells* **33**, 68–78 (2015).
38. Lambert, J. C. *et al.* Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat. Genet.* **45**, 1452–1458 (2013).
39. Young, J. E. *et al.* Elucidating molecular phenotypes caused by the SORL1 Alzheimer's disease genetic risk factor using human induced pluripotent stem cells. *Cell Stem Cell* **16**, 373–385 (2015).

40. Davis, R. P. *et al.* Cardiomyocytes derived from pluripotent stem cells recapitulate electrophysiological characteristics of an overlap syndrome of cardiac sodium channel disease. *Circulation* **125**, 3079–3091 (2012).
41. Morris, J. K., Wald, N. J. & Watt, H. C. Fetal loss in Down syndrome pregnancies. *Prenat. Diagn.* **19**, 142–145 (1999).
42. Slaugenhaupt, S. A. *et al.* Tissue-specific expression of a splicing mutation in the *IKBKAP* gene causes familial dysautonomia. *Am. J. Hum. Genet.* **68**, 598–605 (2001).
43. Lee, G. *et al.* Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. *Nature* **461**, 402–406 (2009).
44. Lee, G. *et al.* Large-scale screening using familial dysautonomia induced pluripotent stem cells identifies compounds that rescue *IKBKAP* expression. *Nat. Biotechnol.* **30**, 1244–1248 (2012).
45. Studer, L., Vera, E. & Cornacchia, D. Programming and reprogramming cellular age in the era of induced pluripotency. *Cell Stem Cell* **16**, 591–600 (2015).
46. Wirdefeldt, K., Adami, H. O., Cole, P., Trichopoulos, D. & Mandel, J. Epidemiology and etiology of Parkinson's disease: a review of the evidence. *Eur. J. Epidemiol.* **26**, 1–58 (2011).
47. Devine, M. J. *et al.* Parkinson's disease induced pluripotent stem cells with triplication of the α-synuclein locus. *Nat. Commun.* **2**, 440 (2011).
48. Wang, S. *et al.* Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. *Cell Stem Cell* **12**, 252–264 (2013).
49. Mariani, J. *et al.* Modeling human cortical development *in vitro* using induced pluripotent stem cells. *Proc. Natl Acad. Sci. USA* **109**, 12770–12775 (2012).
50. Roessler, R. *et al.* Detailed analysis of the genetic and epigenetic signatures of iPSC-derived mesodiencephalic dopaminergic neurons. *Stem Cell Rep.* **2**, 520–533 (2014).
51. Nguyen, H. N. *et al.* LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. *Cell Stem Cell* **8**, 267–280 (2011).
52. Cooper, O. *et al.* Pharmacological rescue of mitochondrial deficits in iPSC-derived neural cells from patients with familial Parkinson's disease. *Sci. Transl. Med.* **4**, 141ra90 (2012).
53. Miller, J. D. *et al.* Human iPSC-based modeling of late-onset disease via progerin-induced aging. *Cell Stem Cell* **13**, 691–705 (2013).
54. Xu, G. L. *et al.* Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. *Nature* **402**, 187–191 (1999).
55. Huang, K. *et al.* Selective demethylation and altered gene expression are associated with ICF syndrome in human-induced pluripotent stem cells and mesenchymal stem cells. *Hum. Mol. Genet.* **23**, 6448–6457 (2014).
56. Sagie, S. *et al.* Induced pluripotent stem cells as a model for telomeric abnormalities in ICF type I syndrome. *Hum. Mol. Genet.* **23**, 3629–3640 (2014).
57. Liao, J. *et al.* Targeted disruption of *DNMT1*, *DNMT3A* and *DNMT3B* in human embryonic stem cells. *Nat. Genet.* **47**, 469–478 (2015).
58. Peters, J. The role of genomic imprinting in biology and disease: an expanding view. *Nat. Rev. Genet.* **15**, 517–530 (2014).
59. Ferguson-Smith, A. C. Genomic imprinting: the emergence of an epigenetic paradigm. *Nat. Rev. Genet.* **12**, 565–575 (2011).
60. Adewumi, O. *et al.* Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. *Nat. Biotechnol.* **25**, 803–816 (2007).
61. Johannesson, B. *et al.* Comparable frequencies of coding mutations and loss of imprinting in human pluripotent cells derived by nuclear transfer and defined factors. *Cell Stem Cell* **15**, 634–642 (2014).
62. Pick, M. *et al.* Clone- and gene-specific aberrations of parental imprinting in human induced pluripotent stem cells. *Stem Cells* **27**, 2686–2690 (2009).
63. Rugg-Gunn, P. J., Ferguson-Smith, A. C. & Pedersen, R. A. Epigenetic status of human embryonic stem cells. *Nat. Genet.* **37**, 585–587 (2005).
64. Rugg-Gunn, P. J., Ferguson-Smith, A. C. & Pedersen, R. A. Status of genomic imprinting in human embryonic stem cells as revealed by a large cohort of independently derived and maintained lines. *Hum. Mol. Genet.* **16**, R243–R251 (2007).
65. Chamberlain, S. J. *et al.* Induced pluripotent stem cell models of the genomic imprinting disorders Angelman and Prader-Willi syndromes. *Proc. Natl Acad. Sci. USA* **107**, 17668–17673 (2010).
66. Martins-Taylor, K. *et al.* Imprinted expression of UBE3A in non-neuronal cells from a Prader-Willi syndrome patient with an atypical deletion. *Hum. Mol. Genet.* **23**, 2364–2373 (2014).
67. Stelzer, Y., Sagi, I., Yanuka, O., Eiges, R. & Benvenisty, N. The noncoding RNA *IPW* regulates the imprinted *DLK1–DIO3* locus in an induced pluripotent stem cell model of Prader–Willi syndrome. *Nat. Genet.* **46**, 551–557 (2014).
68. Yang, J. *et al.* Induced pluripotent stem cells can be used to model the genomic imprinting disorder Prader-Willi syndrome. *J. Biol. Chem.* **285**, 40303–40311 (2010).
69. Cruvinel, E. *et al.* Reactivation of maternal *SNORD116* cluster via SETDB1 knockdown in Prader-Willi syndrome iPSCs. *Hum. Mol. Genet.* **23**, 4674–4685 (2014).
70. Ulbright, T. M. Gonadal teratomas: a review and speculation. *Adv. Anat. Pathol.* **11**, 10–23 (2004).
71. Revazova, E. S. *et al.* Patient-specific stem cell lines derived from human parthenogenetic blastocysts. *Cloning Stem Cells* **9**, 432–449 (2007).
72. Mai, Q. *et al.* Derivation of human embryonic stem cell lines from parthenogenetic blastocysts. *Cell Res.* **17**, 1008–1019 (2007).
73. Brevini, T. A. *et al.* Cell lines derived from human parthenogenetic embryos can display aberrant centriole distribution and altered expression levels of mitotic spindle check-point transcripts. *Stem Cell Rev.* **5**, 340–352 (2009).
74. Stelzer, Y., Yanuka, O. & Benvenisty, N. Global analysis of parental imprinting in human parthenogenetic induced pluripotent stem cells. *Nat. Struct. Mol. Biol.* **18**, 735–741 (2011).
This paper describes the first analysis of an iPSC-based model for parental imprinting.
75. Stelzer, Y., Sagi, I. & Benvenisty, N. Involvement of parental imprinting in the antisense regulation of onco-miR-372-373. *Nat. Commun.* **4**, 2724 (2013).
76. Maeder, M. L. *et al.* Targeted DNA demethylation and activation of endogenous genes using programmable TALE-TET1 fusion proteins. *Nat. Biotechnol.* **31**, 1137–1142 (2013).
77. Di Giorgio, F. P., Carrasco, M. A., Siao, M. C., Maniatis, T. & Eggan, K. Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. *Nat. Neurosci.* **10**, 608–614 (2007).
78. Di Giorgio, F. P., Boulting, G. L., Bobrowicz, S. & Eggan, K. C. Human embryonic stem cell-derived motor neurons are sensitive to the toxic effect of glial cells carrying an ALS-causing mutation. *Cell Stem Cell* **3**, 637–648 (2008).
79. Eiraku, M. *et al.* Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* **472**, 51–56 (2011).
80. Suga, H. *et al.* Self-formation of functional adenohypophysis in three-dimensional culture. *Nature* **480**, 57–62 (2011).
81. Lancaster, M. A. *et al.* Cerebral organoids model human brain development and microcephaly. *Nature* **501**, 373–379 (2013).
82. Schwank, G. *et al.* Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell Stem Cell* **13**, 653–658 (2013).
83. Dekkers, J. F. *et al.* A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat. Med.* **19**, 939–945 (2013).
84. Huch, M. & Koo, B. K. Modeling mouse and human development using organoid cultures. *Development* **142**, 3113–3125 (2015).
85. Lancaster, M. A. & Knoblich, J. A. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* **345**, 1247125 (2014).
86. Lister, R. *et al.* Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature* **471**, 68–73 (2011).
87. Ruiz, S. *et al.* Identification of a specific reprogramming-associated epigenetic signature in human induced pluripotent stem cells. *Proc. Natl Acad. Sci. USA* **109**, 16196–16201 (2012).
88. Weissbein, U., Benvenisty, N. & Ben-David, U. Quality control: genome maintenance in pluripotent stem cells. *J. Cell Biol.* **204**, 153–163 (2014).
89. Halevy, T. & Urbach, A. Comparing ESC and iPSC-based models for human genetic disorders. *J. Clin. Med.* **3**, 1146–1162 (2014).
90. Urbach, A., Bar-Nur, O., Daley, G. Q. & Benvenisty, N. Differential modeling of fragile X syndrome by human embryonic stem cells and induced pluripotent stem cells. *Cell Stem Cell* **6**, 407–411 (2010).
This study was the first comparison of disease-specific models in ES cells and iPSCs.
91. Halevy, T., Czech, C. & Benvenisty, N. Molecular mechanisms regulating the defects in fragile X syndrome neurons derived from human pluripotent stem cells. *Stem Cell Rep.* **4**, 37–46 (2015).
92. Bar-Nur, O., Caspi, I. & Benvenisty, N. Molecular analysis of FMR1 reactivation in fragile-X induced pluripotent stem cells and their neuronal derivatives. *J. Mol. Cell. Biol.* **4**, 180–183 (2012).
93. Raya, A. *et al.* Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. *Nature* **460**, 53–59 (2009).
94. Tulpule, A. *et al.* Knockdown of Fanconi anemia genes in human embryonic stem cells reveals early developmental defects in the hematopoietic lineage. *Blood* **115**, 3453–3462 (2010).
95. Liu, G. H. *et al.* Modelling Fanconi anemia pathogenesis and therapeutics using integration-free patient-derived iPSCs. *Nat. Commun.* **5**, 4330 (2014).
96. Barmada, S. J. *et al.* Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models. *Nat. Chem. Biol.* **10**, 677–685 (2014).
97. Sareen, D. *et al.* Targeting RNA foci in iPSC-derived motor neurons from ALS patients with a *C9ORF72* repeat expansion. *Sci. Transl. Med.* **5**, 208ra149 (2013).
98. Donnelly, C. J. *et al.* RNA toxicity from the ALS/FTD *C9ORF72* expansion is mitigated by antisense intervention. *Neuron* **80**, 415–428 (2013).
99. Yoshida, M. *et al.* Modeling the early phenotype at the neuromuscular junction of spinal muscular atrophy using patient-derived iPSCs. *Stem Cell Rep.* **4**, 561–568 (2015).
100. Evers, M. M., Toonen, L. J. & van Roon-Mom, W. M. Antisense oligonucleotides in therapy for neurodegenerative disorders. *Adv. Drug Deliv. Rev.* **87**, 90–103 (2015).
101. McNeish, J., Gardner, J. P., Wainger, B. J., Woolf, C. J. & Eggan, K. From dish to bedside: lessons learned while translating findings from a stem cell model of disease to a clinical trial. *Cell Stem Cell* **17**, 8–10 (2015).
This is an elaborate and informative description of the rapid way this group moved from experiments on ALS patient-derived neurons differentiated from iPSCs to clinical studies in humans.
102. Ng, S. Y. *et al.* Genome-wide RNA-seq of human motor neurons implicates selective ER stress activation in spinal muscular atrophy. *Cell Stem Cell* **17**, 569–584 (2015).
103. Sareen, D. *et al.* Inhibition of apoptosis blocks human motor neuron cell death in a stem cell model of spinal muscular atrophy. *PLoS ONE* **7**, e39113 (2012).
104. Yang, J. *et al.* Validation of genome-wide association study (GWAS)-identified disease risk alleles with patient-specific stem cell lines. *Hum. Mol. Genet.* **23**, 3445–3455 (2014).
105. Kiskinis, E. *et al.* Pathways disrupted in human ALS motor neurons identified through genetic correction of mutant *SOD1*. *Cell Stem Cell* **14**, 781–795 (2014).
106. Soragni, E. *et al.* Epigenetic therapy for Friedreich ataxia. *Ann. Neurol.* **76**, 489–508 (2014).
107. Li, Y. *et al.* Global transcriptional and translational repression in human-embryonic-stem-cell-derived Rett syndrome neurons. *Cell Stem Cell* **13**, 446–458 (2013).
108. Griesi-Oliveira, K. *et al.* Modeling non-syndromic autism and the impact of *TRPC6* disruption in human neurons. *Mol. Psychiatry* **20**, 1350–1365 (2015).
109. Guo, X. *et al.* Inhibition of mitochondrial fragmentation diminishes Huntington's disease-associated neurodegeneration. *J. Clin. Invest.* **123**, 5371–5388 (2013).
110. Rashid, S. T. *et al.* Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. *J. Clin. Invest.* **120**, 3127–3136 (2010).
111. Yang, Y. M. *et al.* A small molecule screen in stem-cell-derived motor neurons identifies a kinase inhibitor as a candidate therapeutic for ALS. *Cell Stem Cell* **12**, 713–726 (2013).
112. Matsa, E. *et al.* Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation. *Eur. Heart J.* **32**, 952–962 (2011).

113. Wainger, B. J. *et al.* Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. *Cell Rep.* **7**, 1–11 (2014).
114. Jang, J. *et al.* Induced pluripotent stem cell models from X-linked adrenoleukodystrophy patients. *Ann. Neurol.* **70**, 402–409 (2011).
115. Egawa, N. *et al.* Drug screening for ALS using patient-specific induced pluripotent stem cells. *Sci. Transl. Med.* **4**, 145ra104 (2012).
116. Hossini, A. M. *et al.* Induced pluripotent stem cell-derived neuronal cells from a sporadic Alzheimer's disease donor as a model for investigating AD-associated gene regulatory networks. *BMC Genomics* **16**, 84 (2015).
117. Liu, Q. *et al.* Effect of potent γ-secretase modulator in human neurons derived from multiple presenilin 1-induced pluripotent stem cell mutant carriers. *JAMA Neurol.* **71**, 1481–1489 (2014).
118. Yagi, T. *et al.* Modeling familial Alzheimer's disease with induced pluripotent stem cells. *Hum. Mol. Genet.* **20**, 4530–4539 (2011).
119. Israel, M. A. *et al.* Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. *Nature* **482**, 216–220 (2012).
120. Kondo, T. *et al.* Modeling Alzheimer's disease with iPSCs reveals stress phenotypes associated with intracellular Aβ and differential drug responsiveness. *Cell Stem Cell* **12**, 487–496 (2013).
121. Lee, P. *et al.* SMRT compounds abrogate cellular phenotypes of ataxia telangiectasia in neural derivatives of patient-specific hiPSCs. *Nat. Commun.* **4**, 1824 (2013).
122. Germain, N. D. *et al.* Gene expression analysis of human induced pluripotent stem cell-derived neurons carrying copy number variants of chromosome 15q11-q13.1. *Mol. Autism* **5**, 44 (2014).
123. Madison, J. M. *et al.* Characterization of bipolar disorder patient-specific induced pluripotent stem cells from a family reveals neurodevelopmental and mRNA expression abnormalities. *Mol. Psychiatry* **20**, 703–717 (2015).
124. Chen, C. *et al.* Role of astroglia in Down's syndrome revealed by patient-derived human-induced pluripotent stem cells. *Nat. Commun.* **5**, 4430 (2014).
125. Hibaoui, Y. *et al.* Modelling and rescuing neurodevelopmental defect of Down syndrome using induced pluripotent stem cells from monozygotic twins discordant for trisomy 21. *EMBO Mol. Med.* **6**, 259–277 (2014).
126. Chang, C. Y. *et al. N*-butylidenephthalide attenuates Alzheimer's disease-like cytopathy in Down syndrome induced pluripotent stem cell-derived neurons. *Sci. Rep.* **5**, 8744 (2015).
127. Kaufmann, M. *et al.* High-throughput screening using iPSC-derived neuronal progenitors to identify compounds counteracting epigenetic gene silencing in fragile X syndrome. *J. Biomol. Screen.* **20**, 1101–1111 (2015).
128. Igoillo-Esteve, M. *et al.* Unveiling a common mechanism of apoptosis in β-cells and neurons in Friedreich's ataxia. *Hum. Mol. Genet.* **24**, 2274–2286 (2015).
129. Son, M. Y. *et al.* A novel human model of the neurodegenerative disease GM1 gangliosidosis using induced pluripotent stem cells demonstrates inflammasome activation. *J. Pathol.* **237**, 98–110 (2015).
130. Denton, K. R. *et al.* Loss of spastin function results in disease-specific axonal defects in human pluripotent stem cell-based models of hereditary spastic paraplegia. *Stem Cells* **32**, 414–423 (2014).
131. Leung, A. *et al.* Induced pluripotent stem cell modeling of multisystemic, hereditary transthyretin amyloidosis. *Stem Cell Rep.* **1**, 451–463 (2013).
132. Lu, X. H. *et al.* Targeting ATM ameliorates mutant Huntingtin toxicity in cell and animal models of Huntington's disease. *Sci. Transl. Med.* **6**, 268ra178 (2014).
133. Charbord, J. *et al.* High throughput screening for inhibitors of REST in neural derivatives of human embryonic stem cells reveals a chemical compound that promotes expression of neuronal genes. *Stem Cells* **31**, 1816–1828 (2013).
134. Koch, P. *et al.* Excitation-induced ataxin-3 aggregation in neurons from patients with Machado–Joseph disease. *Nature* **480**, 543–546 (2011).
135. Lojewski, X. *et al.* Human iPSC models of neuronal ceroid lipofuscinosis capture distinct effects of TPP1 and CLN3 mutations on the endocytic pathway. *Hum. Mol. Genet.* **23**, 2005–2022 (2014).
136. Maetzel, D. *et al.* Genetic and chemical correction of cholesterol accumulation and impaired autophagy in hepatic and neural cells derived from Niemann-Pick type C patient-specific iPS cells. *Stem Cell Rep.* **2**, 866–880 (2014).
137. Soga, M. *et al.* HPGCD outperforms HPBCD as a potential treatment for Niemann-Pick disease type C during disease modeling with iPS cells. *Stem Cells* **33**, 1075–1088 (2015).
138. Lee, H. *et al.* Pathological roles of the VEGF/SphK pathway in Niemann-Pick type C neurons. *Nat. Commun.* **5**, 5514 (2014).
139. Yu, D. *et al.* Niemann-Pick disease type C: induced pluripotent stem cell-derived neuronal cells for modeling neural disease and evaluating drug efficacy. *J. Biomol. Screen.* **19**, 1164–1173 (2014).
140. Ryan, S. D. *et al.* Isogenic human iPSC Parkinson's model shows nitrosative stress-induced dysfunction in MEF2-PGC1α transcription. *Cell* **155**, 1351–1364 (2013).
141. Chung, C. Y. *et al.* Identification and rescue of α-synuclein toxicity in Parkinson patient-derived neurons. *Science* **342**, 983–987 (2013).
142. Shcheglovitov, A. *et al.* SHANK3 and IGF1 restore synaptic deficits in neurons from 22q13 deletion syndrome patients. *Nature* **503**, 267–271 (2013).
143. Williams, E. C. *et al.* Mutant astrocytes differentiated from Rett syndrome patients-specific iPSCs have adverse effects on wild-type neurons. *Hum. Mol. Genet.* **23**, 2968–2980 (2014).
144. Marchetto, M. C. *et al.* A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell* **143**, 527–539 (2010).
145. Paulsen, B. S., Cardoso, S. C., Stelling, M. P., Cadilhe, D. V. & Rehen, S. K. Valproate reverts zinc and potassium imbalance in schizophrenia-derived reprogrammed cells. *Schizophr. Res.* **154**, 30–35 (2014).
146. Paulsen, B. S. *et al.* Altered oxygen metabolism associated to neurogenesis of induced pluripotent stem cells derived from a schizophrenic patient. *Cell Transplant.* **21**, 1547–1559 (2012).
147. Nihei, Y. *et al.* Enhanced aggregation of androgen receptor in induced pluripotent stem cell-derived neurons from spinal and bulbar muscular atrophy. *J. Biol. Chem.* **288**, 8043–8052 (2013).
148. Pasca, S. P. *et al.* Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. *Nat. Med.* **17**, 1657–1662 (2011).
149. Lu, S. *et al.* A calcium-dependent protease as a potential therapeutic target for Wolfram syndrome. *Proc. Natl Acad. Sci. USA* **111**, E5292–E5301 (2014).

Acknowledgements

The authors thank Eran Meshorer for his assistance in composing the glossary terms. I.S. is supported by the Adams Fellowships Program for Doctoral Students, and N.B. is the Herbert Cohn Chair in Cancer Research. This work was partially supported by the Israel Science Foundation (grant no. 269/12), by the Rosetrees Trust and by the Azrieli Foundation.

Competing interests statement

The authors declare no competing interests.

FURTHER INFORMATION

CIRM: https://cirm.ca.gov
Coriell Biorepository: https://catalog.coriell.org/
ECACC: http://www.phe-culturecollections.org.uk/collections/ecacc.jsp
HiPSCi: http://hipsci.org
StemBANCC: http://stembancc.org
New York Stem Cell Foundataion (NYSCF): http://nyscf.org

ALL LINKS ARE ACTIVE IN THE ONLINE PDF