Induced pluripotent stem cells: A novel frontier in the study of human primary immunodeficiencies

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Background: The novel ability to epigenetically reprogram somatic cells into induced pluripotent stem cells (iPSCs) through the exogenous expression of transcription promises to revolutionize the study of human diseases.
Objective: Here we report on the generation of 25 iPSC lines from 6 patients with various forms of primary immunodeficiencies (PIDs) affecting adaptive immunity, innate immunity, or both.
Methods: Patients' dermal fibroblasts were reprogrammed by expression of 4 transcription factors, octamer-binding transcription factor 4 (*OCT4*), sex determining region Y-box 2 (*SOX2*), Krueppel-like factor 4 (*KLF4*), and cellular myelomonocytosis proto-oncogene (*cMYC*), by using a single excisable polycistronic lentiviral vector.
Results: iPSCs derived from patients with PIDs show a stemness profile that is comparable with that observed in human embryonic stem cells. After *in vitro* differentiation into embryoid bodies, pluripotency of the patient-derived iPSC lines was demonstrated by expression of genes characteristic of each of the 3 embryonic layers. We have confirmed the patient-specific origin of the iPSC lines and ascertained maintenance of karyotypic integrity.
Conclusion: By providing a limitless source of diseased stem cells that can be differentiated into various cell types *in vitro*, the repository of iPSC lines from patients with PIDs represents a unique resource to investigate the pathophysiology of hematopoietic and extrahematopoietic manifestations of these diseases and might assist in the development of novel therapeutic approaches based on gene correction. (J Allergy Clin Immunol 2011;127:1400-7.)

Key words: *Primary immunodeficiency, induced pluripotent stem cells, reprogramming*

Primary immunodeficiencies (PIDs) comprise more than 150 distinct disorders of immune system development, function, or both.[1] Dissection of the cellular pathophysiology of PIDs has been largely based on *in vitro* studies using patient-derived cells and on analysis of suitable animal models. Although largely successful, both of these approaches have important inherent limitations. In particular, many forms of PID are rare and severe and affect predominantly infants and young children. In these cases access to biological specimens from affected patients might be problematic. Furthermore, there is significant heterogeneity of clinical and immunologic phenotypes among patients with different mutations in the same gene, but limited information is available on this diversity at the cellular level.[2] Finally, studies that aim to define the cellular pathophysiology of human PIDs are usually performed on blood samples, occasionally on the bone marrow, rarely on lymphoid tissues (thymus, lymph nodes, and spleen), and almost never on nonhematopoietic tissues, yet many forms of PID also include extraimmune manifestations.[1,3] This is the case for immunodeficiency syndromes characterized by multisystem developmental defects (eg, DiGeorge syndrome[4] and cartilage hair hypoplasia[5]), broad expression of the disease-specific gene (eg, defects of DNA repair,[6] nuclear factor κB essential modulator deficiency,[7] hyper-IgE syndrome caused by signal transducer and activator of transcription 3 deficiency,[8,9] and adenosine deaminase deficiency[10]), or tissue-specific susceptibility to infections (eg, herpes simplex encephalitis[11-13]).

On the other hand, although murine models of PIDs have provided key insights, they also carry significant inherent limitations because of differences in immune system development and function between mice and humans and the relative lack of phenotypic variability and heterogeneity of mutations in murine models compared with PIDs in human subjects.

After the demonstration in 2006 by Takahashi and Yamanaka[14] that murine fibroblasts can be reprogrammed into induced

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Abbreviations used

bFGF: Basic fibroblast growth factor
cMYC: Cellular myelomonocytic proto-oncogene
hES: Human embryonic stem
iMEFs: Irradiated murine embryonic fibroblast
iPSC: Induced pluripotent stem cell
KLF4: Krueppel-like factor 4
OCT4: Octamer-binding transcription factor 4
PID: Primary immunodeficiency
QRT-PCR: Quantitative real-time polymerase chain reaction
RAG1: Recombinase activating gene 1
SCID: Severe combined immune deficiency
SOX2: Sex determining region Y-box 2
STEMCCA: Stem cell cassette

pluripotent stem cells (iPSCs) through transient forced expression of defined transcription factors, generation of iPSCs from terminally differentiated human cells has been recently reported.[15-17] Similar to embryonic stem cells, these cells hold the unique potential to differentiate into various tissue cell types, including neurons,[18-25] cardiomyocytes,[26-28] hepatic cells,[29-31] gastrointestinal cells,[32] thymic epithelial cells,[33] hematopoietic cells,[34,35] and many others.[36-41] Furthermore, iPSCs have also been used to correct genetic disorders in mice after gene targeting and homologous recombination.[42,43]

Over the last 10 years, we have established an extended repository of fibroblast cell lines from patients with various forms of PID. This repository is also representative of the diversity of the clinical and immunologic phenotype that is associated with different mutations occurring in the same gene. Using this collection of fibroblast cell lines, we now report on the successful generation and characterization of a series of PID-specific iPSCs that might serve as the foundation for future studies of disease pathophysiology and gene correction.

METHODS

Patients

Dermal fibroblast samples were obtained from 6 patients with PIDs carrying mutations in different genes, as detailed in Table I.[44,45] Informed consent was obtained from a parent or guardian. Study protocols were approved by the Children's Hospital Boston Institutional Review Board.

Cell lines and culture

A previously reported human iPSC line[17] obtained by reprogramming dermal fibroblasts with retroviral vectors encoding octamer-binding transcription factor 4 (OCT4), sex determining region Y-box 2 (SOX2), Krueppel-like factor 4 (KLF4), and cellular myelomonocytosis proto-oncogene (cMYC) transcription factors, was used as an internal control.

Patients' and healthy control subjects' fibroblasts were maintained in Dulbecco modified Eagle medium (high glucose and L-glutamine) containing 10% FBS, 1 mmol/L L-glutamine, and penicillin/streptomycin (hFib media).

iPSCs were maintained in human embryonic stem (hES) cell medium composed of Dulbecco modified Eagle medium/F12 (Invitrogen, Carlsbad, Calif) containing 20% KOSR (Invitrogen), 10 ng/mL basic fibroblast growth factor (bFGF; Gemini Bio-Products, West Sacramento, Calif), 1 mmol/L L-glutamine, 100 μmol/L nonessential amino acids, 100 μmol/L 2-β-mercaptoethanol, and penicillin/streptomycin. The cells were cocultured on CF1 irradiated murine embryonic fibroblasts (iMEFs; Globalstem, Inc, Rockville, Md), as previously described.[16,17] Expansion and splitting of the iPSC colonies was done by means of either mechanical passage or through the use of collagenase, as previously described.[46]

TABLE I. Patients and mutations

Disease phenotype	Gene	Mutation	Reference
SCID	*RAG1*	c.1228C>T; c.2332C>T	Unpublished data
Leaky SCID	*RAG1*	c.1180C>T; c.1180C>T	Unpublished data
OS	*RAG1*	c.256-257del; c.2164G>A	Cassani et al[44]
HSE1	*STAT1*	c.1928_1929 insA; c.1928_1929 insA	Chapgier et al[45]
HSE2	*TLR3*	c.1660C>T; c.2236G>T	Guo Y et al, unpublished data
CHH	*RMRP*	c. 27G>A; c. 27G>A	Unpublished data

CHH, Cartilage hair hypoplasia; *HSE*, herpes simplex encephalitis; *OS*, Omenn syndrome; *RAG1*, recombinase activating gene 1; *RMRP*, RNA component of the mitochondrial RNA processing endoribunuclease; *STAT1*, signal transducer and activator of transcription 1; *TLR3*, Toll-like receptor 3.

iPSC differentiation into embryoid bodies was achieved by transferring iPSC colonies into low-adhesion plates free of feeder cells and using a bFGF-free hES cell medium, as previously described.[16,17,47]

Lentiviral reprogramming vector production

Lentiviruses containing the previously described[48-51] polycistronic lentiviral vector stem cell cassette (STEMCCA)–LoxP were produced by using a 5-plasmid transfection system, as previously described.[52]

Reprogramming of fibroblasts and human iPSC generation

Fibroblasts were infected with the lentiviral reprogramming vector in hFib media supplemented with 5 μg/mL protamine sulfate (Sigma, St Louis, Mo) for 24 hours. After 72 hours, cells were transferred onto iMEFs in hES cell media. iPSC colonies with hES-like morphology started to appear after 3 to 5 weeks. Colonies were picked, subcloned, and expanded by means of mechanical transfer into new plates containing fresh and adhered iMEFs, as previously described.[16] Several clones were derived and characterized from each fibroblast line (see Table E1 in this article's Online Repository at www.jacionline.org).

Immunohistochemistry

iPSC colonies were stained for OCT4, NANOG, TRA-1-60, TRA-1-81, SSEA3, and SSEA4, as previously described.[47] Images were acquired with a Pathway 435 bioimager equipped with a 10× objective (BD Biosciences, San Jose, Calif).

Quantitative real-time PCR

The mirVana RNA isolation kit (Ambion, Austin, Tex) was used for total RNA extraction and reverse transcription preformed with qScript cDNA supermix (Quanta Biosciences, Gaithersburg, Md), according to the manufacturer's instructions.

Quantitative real-time PCR (QRT-PCR) in PowerSYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, Calif) was performed on an AB 7500 Real-Time PCR system (Applied Biosystems). Primer sequences used for amplifying *OCT4*, *SOX2*, *NANOG*, *REX1*, *GDF3*, *hTERT*, *KLF4*, *cMYC*, *RUNX*, *AFP*, *GATA4*, *Brachyury*, *NESTIN*, *NCAM*, and the β-actin gene *(ACTB)* were as previously described.[16,17,47]

Results were normalized to *ACTB* expression, and relative expression was calculated by using the delta-delta-Ct (ddCt) method relative to expression levels in the individual parental cell lines by using SDSv1 software.

Mutation analysis

Genomic DNA was isolated from dermal fibroblasts and iPSCs by using the QiAMP DNA Kit (Qiagen, Valencia, Calif). Genes known to be mutated in the

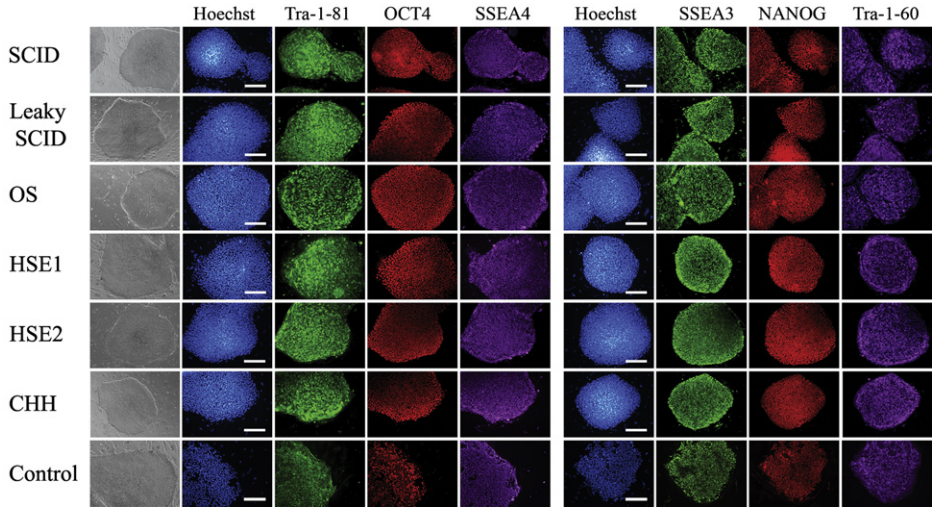

FIG 1. PID-specific iPSCs express markers of pluripotency. PID-specific iPSCs have morphology similar to that seen in hES cells *(left column)* when grown in coculture with iMEFs and express pluripotency markers, including Tra-1–81, OCT4, SSEA4, SSEA3, NANOG, and Tra-1-60, as demonstrated by means of immunohistochemistry. Nuclear staining with Hoechst 33342 is shown in the *second* and *sixth columns* to indicate the total cell content per image. *CHH*, Cartilage hair hypoplasia; *HSE*, herpes simplex encephalitis; *OS*, Omenn syndrome; *SCID*, severe combined immune deficiency.

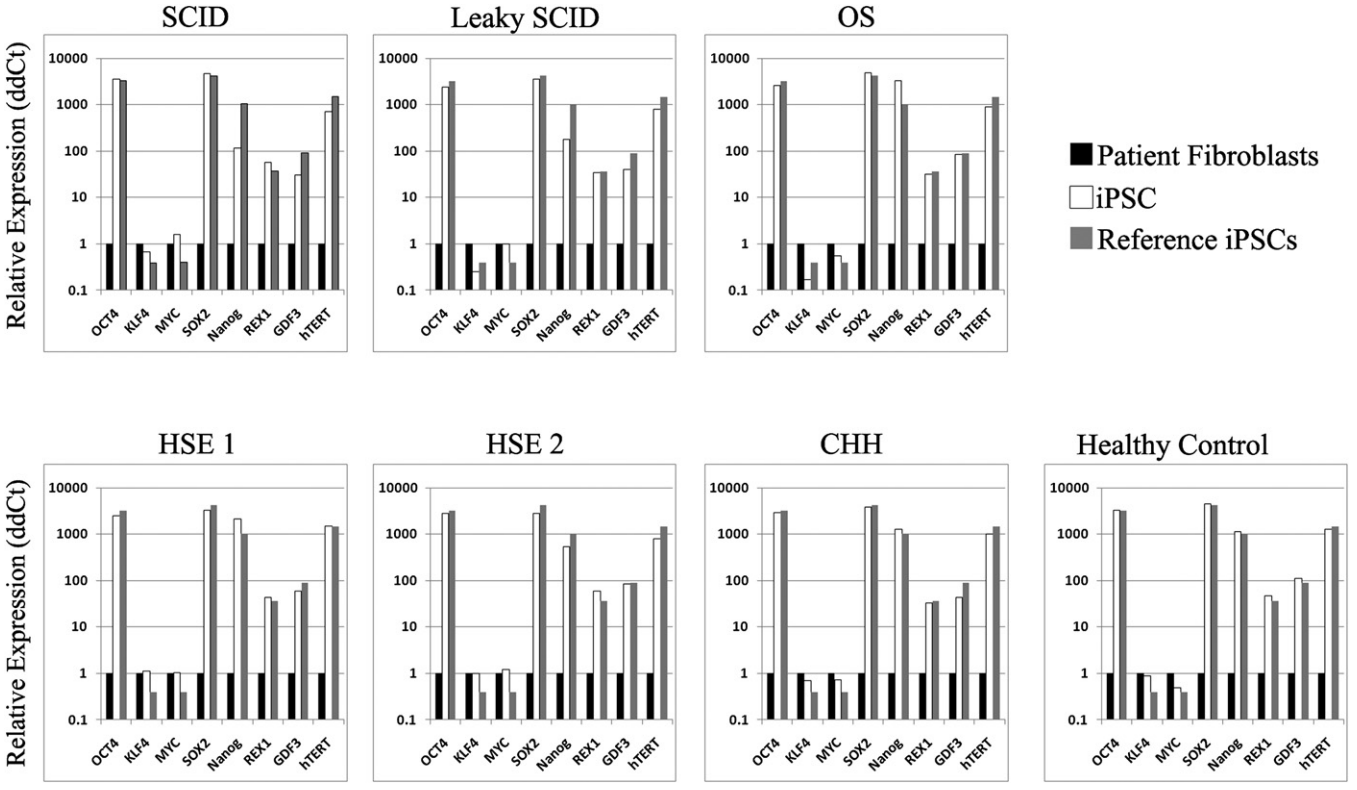

FIG 2. PID-specific iPSCs show a gene expression panel similar to that of reference iPSCs derived from a healthy control subject. Quantitative real-time PCR (QRT-PCR) assay for gene expression of *OCT4*, *KLF4*, *MYC*, *SOX2*, *NANOG*, *REX1*, *GDF3*, and *hTERT* was performed in iPSCs derived from healthy control subjects and from patients with PID and compared with the pattern observed in a previously established control human iPSC line obtained by reprogramming with 4 different retroviral vectors.[17] QRT-PCR reactions were normalized against β-actin *(ACTB)*. Expression was calculated by using the ddCt method relative to expression levels in the individual parental fibroblast cell lines for the normal and PID-specific iPSC or normal control human fibroblasts for the human embryonic stem cells. *CHH*, Cartilage hair hypoplasia; *HSE*, herpes simplex encephalitis; *OS*, Omenn syndrome; *SCID*, severe combined immune deficiency.

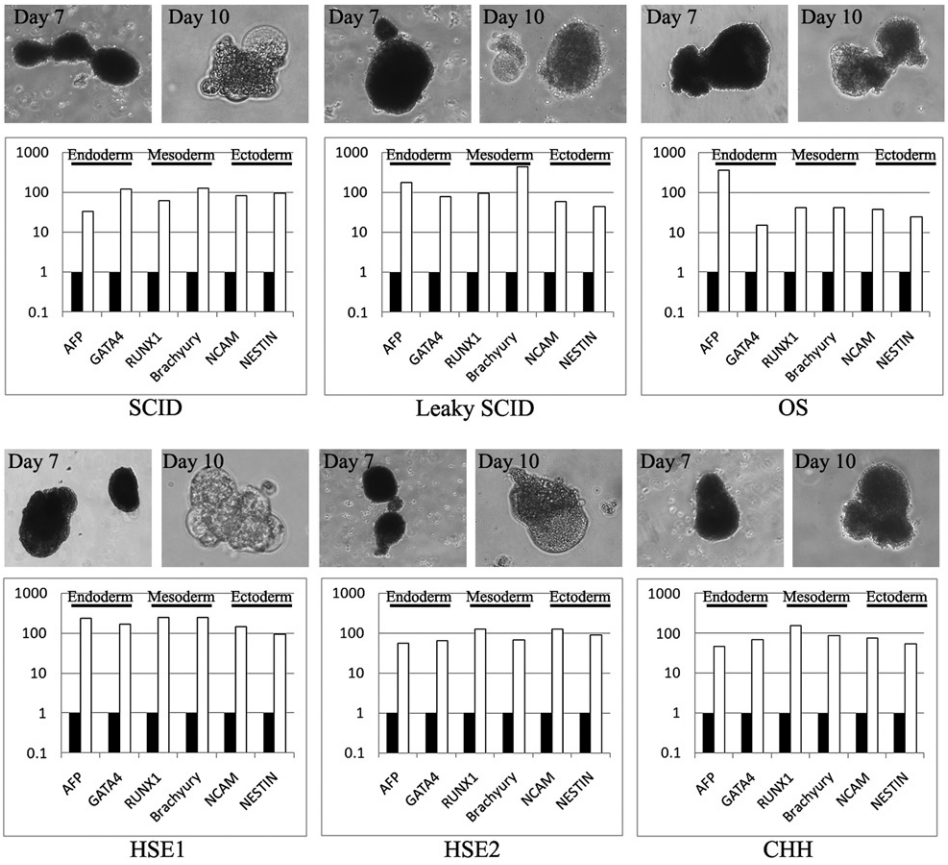

FIG 3. Differentiation of PID-specific iPSCs reveals lineage-specific gene expression. PID-specific iPSC were allowed to differentiate into embryoid bodies by means of culture in a bFGF-free hES medium and without coculture with feeder cells. Robust formation of tight and well-formed cell clusters was detected by day 7 and became cystic by day 10 (*upper row* in each cell-specific panel). Quantitative real-time polymerase chain reaction (QRT-PCR) gene expression analysis of the derived embryoid bodies after 10 days shows increased expression of lineage-specific markers from each of the 3 embryonic germ layers, including *AFP* and *GATA4* (endoderm), *RUNX1* and *Brachyury* (mesoderm), and *NCAM* and *NESTIN* (ectoderm). QRT-PCR reactions were normalized against β-actin *(ACTB)*. Expression was calculated by using the ddCT method relative to expression levels in undifferentiated iPSCs. *Black* and *white bars* identify undifferentiated iPSCs and day 10 embryoid bodies, respectively. *CHH*, Cartilage hair hypoplasia; *HSE*, herpes simplex encephalitis; *OS*, Omenn syndrome; *SCID*, severe combined immune deficiency.

patients were amplified by means of PCR, as previously described, by using primer sets as detailed in Table E2 in this article's Online Repository at www.jacionline.org. Amplified products were purified with QIAquick PCR purification kit (Qiagen) and sequenced by the DF/HCC DNA Sequencing Facility. Sequences were analyzed with Sequencher 4.8 software (Gene Codes Corporation, Ann Arbor, Mich).

In some of the compound heterozygote patients, PCR products were cloned with the TOPO TA Cloning Kit with pCR2.1-TOPO vector (Invitrogen). Cloning products were amplified in competent bacteria, purified (QiaPrep Miniprep Kit, Qiagen), and later sequenced as described above.

Karyotype analysis

Karyotyping and G-banding were performed as previously described (see the Methods section in this article's Online Repository at www.jacionline.org) in a blinded fashion by Cell Line Genetics (Madison, Wis).

RESULTS

We have established a repository of dermal fibroblast cell lines from more than 60 patients with various forms of PID that are representative of defects in various components of the immune system. This repository of PID-specific fibroblast lines has been used to establish a pipeline for the systematic production of PID-specific iPSCs.

The initial cohort of iPSCs was derived from patients with Toll-like receptor 3 deficiency, immune deficiency associated with systemic disorders (cartilage hair hypoplasia), and early defects in T- and B-cell development (recombinase activating gene 1 *[RAG1]* mutations, Table I). For the latter, we sought to derive iPSCs from patients with a different clinical and immunologic phenotype (severe combined immune deficiency [SCID], leaky SCID, and Omenn syndrome) associated with null or hypomorphic mutations in the same gene *(RAG1)*. In parallel, iPSCs were also derived from healthy control subjects' fibroblasts.

Various strategies have been described for reprogramming somatic cells to pluripotency. We have made use of an excisable, human STEMCCA–containing single polycistronic lentiviral vector that allows transduction of the 4 reprogramming factors *OCT4*, *SOX2*, *KLF4*, and *cMYC* (see Fig E1 in this article's Online Repository at www.jacionline.org). After infection with the STEMCCA lentivirus, patient- and control subject–derived fibroblasts were maintained under stringent hES cell–supporting culture conditions, as previously described.[16,17,47] After 3 to 5

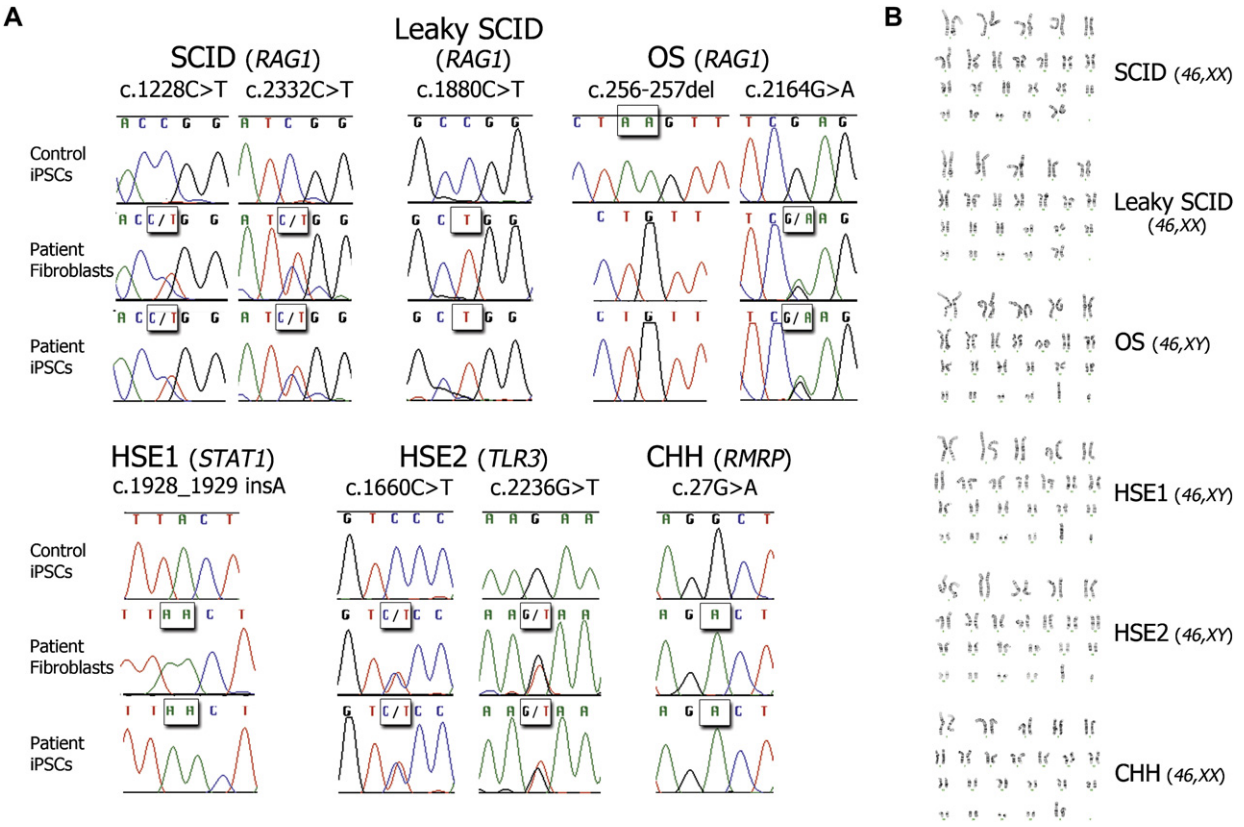

FIG 4. Patient's origin and chromosomal integrity of the PID-specific iPSCs. **A**, PCR amplification followed by DNA sequencing of genomic DNA derived from the PID-specific iPSCs and their parental fibroblasts was performed by using specific primers corresponding to the disease-causing mutations for each of the lines and demonstrated that the PID-specific iPSC lines carry the same disease-causing mutations as their parental fibroblasts. In the case of the first allele (c.256-257del) of the *RAG1*-mutated patient with Omenn syndrome, genetic identity between patient-derived iPSCs and fibroblasts was demonstrated on cloning and sequencing of the specific product. **B**, PID-specific iPSCs were analyzed for chromosomal integrity by using G-banding karyotyping. *CHH*, Cartilage hair hypoplasia; *HSE*, herpes simplex encephalitis; *OS*, Omenn syndrome; *SCID*, severe combined immune deficiency.

weeks of culture, ES-like colonies emerged and were picked and expanded. Several clones were derived from each of the fibroblast lines (see Table E1).

Human iPSC colonies were initially selected based on the similarity to hES cell colonies, with discrete and compact colony morphology (Fig 1, left column). The selected colonies were then expanded and studied for expression of stemness markers, including Tra-1–81, Tra-1–60, OCT4, NANOG, SSEA3, and SSEA4. As shown in Fig 1, all iPSC colonies demonstrated uniform expression of these pluripotency markers.

Expression of pluripotency-related genes was also evaluated by means of QRT-PCR. Compared with the parental fibroblast lines, patient-derived (and control-derived) iPSCs demonstrated robust expression of pluripotency-associated genes, such as *OCT4*, *SOX2*, *NANOG*, *REX1*, *GDF3*, and *hTERT* (Fig 2). As an internal control of the pluripotency gene expression profile, we used a previously reported control iPSC line[17] that had been generated with 4 retroviral vectors, each of which contained one of the 4 reprogramming factors. A similar profile of gene expression was demonstrated in the newly generated patient- and control subject–derived iPSCs and in reference iPSCs.

The PID-specific iPSC lines were allowed to differentiate *in vitro* into embryoid bodies, as previously described, to confirm pluripotency and the ability to support multilineage differentiation.[17,47] Similar to what was previously shown for hES cells, tight clusters of differentiating cells formed by day 7 and later cavitated, becoming cystic by day 10 (Fig 3). Both PID-specific and control iPSCs showed expression of markers of all 3 embryonic germ layers (ectoderm, mesoderm, and endoderm; Fig 3), thus confirming their ability to develop along multiple lineages.

To confirm patient-specific origin, we analyzed each of the iPSCs and the parental fibroblast lines for the specific gene mutation or mutations identified in each patient. In all cases genetic identity was observed between patient-derived fibroblasts and iPSCs (Fig 4, *A*).

It has been previously reported that reprogramming of somatic cells to pluripotency and prolonged culture of hES cells might result in clonal somatic chromosomal aberrations.[49,53,54] We tested representative PID-specific and control-derived iPSC lines for karyotypic integrity. At least 1 line was analyzed for each patient-specific iPSC line (see Table E1). For each line assayed, cytogenic analysis was performed on 20 G-banded metaphases. All but one of the various PID-specific iPSC lines that were analyzed demonstrated a normal karyotype (Fig 4, *B*). In one of the iPSC lines derived from a patient with *RAG1*-deficient SCID, 2 cells of the 20 analyzed carried a trisomy of chromosome

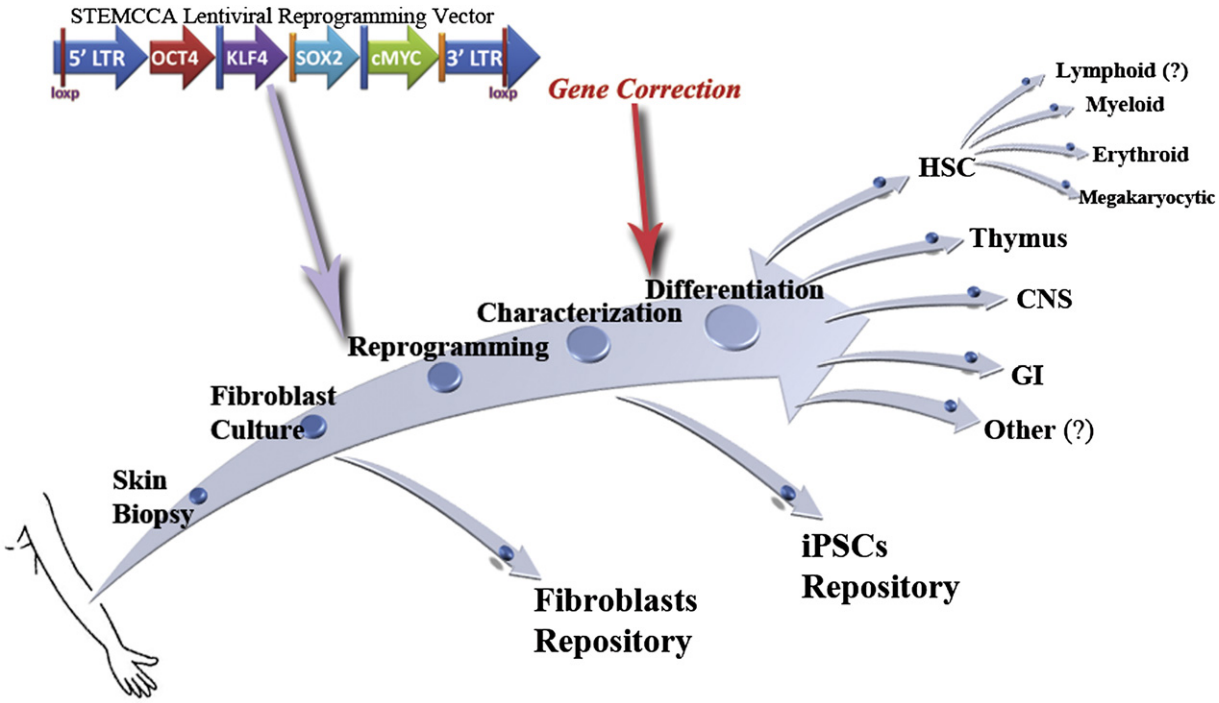

FIG 5. Schematic representation of the process of producing PID-specific iPSCs and their possible use in disease characterization and gene correction. *CNS*, Central nervous system; *GI*, gastrointestinal; *HSC*, hematopoietic stem cell; *LTR*, long terminal repeat.

7 (47,XX +7) and hence might represent a clonal chromosomal aberration. Two other lines derived from the same patient demonstrated a normal karyotype.

DISCUSSION

The field of iPSCs is rapidly growing as novel reprogramming strategies and protocols that allow differentiation of iPSCs into various cell types become available. In spite of this progress, variable efficiency of the nuclear reprogramming process, incomplete maintenance of iPSC stemness profile, failure to achieve transgene silencing, and integration-dependent effects on endogenous gene expression remain significant challenges.[55-57]

To generate iPSCs from patients with various forms of PID, we have chosen to use a single lentiviral vector expressing the 4 common reprogramming factors *OCT4*, *SOX2*, *KLF4*, and *cMYC*, which are coded on a single polycistronic cassette. Using this vector, we have succeeded in generating multiple iPSC clones for each of the fibroblast cell lines that we have infected. A very high efficiency of reprogramming had been also observed with a murine version of the STEMCCA.[50] Another potential advantage of this vector is that the STEMCCA is flanked by 2 inverted LoxP sites, thus permitting excision of the lentiviral sequences by transient expression of the Cre recombinase.[48,49] This strategy limits the residual genetic signature that is left after the reprogramming process to a minimum and removes all reprogramming transgenes.

We have demonstrated that the PID-specific iPSCs generated with this approach exhibit a robust stemness gene expression profile. Furthermore, iPSC lines expressed TRA-1-60, a marker that has been shown to identify fully reprogrammed cells capable of pluripotency.[58] In keeping with this, when cultured under appropriate conditions and allowed to differentiate into embryoid bodies, iPSC lines expressed genes specific for each of the 3 embryonic layers. Nuclear reprogramming is thought to result from transient expression of the *OCT4*, *SOX2*, *KLF4*, and *cMYC* transgenes, followed by induction of endogenous genes while the transgenes are silenced. Comparison of the pattern of expression of the *OCT4*, *SOX2*, *KLF4*, and *cMYC* genes in our series of PID-specific iPSCs is consistent with this notion. In particular, *KLF4* and *cMYC* were expressed at similar levels in patient-derived iPSCs, their parental fibroblasts, and in reference iPSC cells, whereas iPSCs maintained high levels of expression of *SOX2* and *OCT4*. Because transcription of all 4 transgenes contained in the STEMCCA is under control by the same promoter, these data suggest silencing of the transgenes and differential induction of the endogenous genes. This has been recently confirmed by using a modified version of the STEMCCA lentiviral vector also containing an m-Cherry reporter gene.[48]

Clonal chromosomal aberrations have been previously reported in aged hES cells.[53,54] Maintenance of karyotypic integrity is an important feature when considering use of patient-derived iPSCs to study the pathophysiology of the disease at the cellular level. With a single exception, all PID-specific iPSC lines tested retained a normal karyotype, demonstrating that genomic integrity is generally maintained at this level of resolution after reprogramming. However, assessment of genomic integrity remains an important test that should be performed on all newly generated iPSCs.

Before this study, generation of iPSCs had been reported only for one type of PID, namely adenosine deaminase deficiency,[47] using 4 retroviral vectors to allow transduction of the reprogramming factors. We have now shown that iPSCs can be generated with high efficiency from patients with various forms of PID that affect

different arms of the immune system. However, it is possible that some forms of PID remain resistant to reprogramming. In particular, use of integrating vectors might fail to induce reprogramming in fibroblasts from patients with defects in DNA repair because of toxicity and cell death associated with insertion of the vector. In this case alternative strategies could be considered to generate iPSCs, such as delivery of the reprogramming factors through nonintegrating vectors or transient correction of the cellular defect.

Generation of a repository of iPSCs from patients with various forms of PID provides unique research opportunities (Fig 5). *In vitro* generation of T lymphocytes has already been reported for hES cells cultured on stromal OP9-DL4 cells.[59-61] If a similar approach becomes available for human iPSCs, it would be possible to compare the cell-intrinsic potential of iPSCs carrying different mutations in the same gene to support T-cell differentiation. For example, use of the 3 patient-derived iPSC lines with mutations in the *RAG1* gene but with differing clinical phenotypes could provide a previously unforeseen experimental avenue to directly compare the efficiency and fidelity of human thymopoiesis.

Patient-derived iPSCs might also represent a unique tool to investigate in greater detail the pathophysiology of extrahematopoietic manifestations associated with PIDs. Indeed, we have been able to differentiate iPSCs derived from patients with genetically determined susceptibility to herpes simplex encephalitis into various mature cell types of the central nervous system and by this means dissect the cellular and molecular phenotype of the disease (Lafaille and Pessach et al, unpublished data). Similarly, it might become possible to study the differentiation and function of thymic epithelial cells and heart cells starting from iPSCs from patients with DiGeorge syndrome or inflammatory responses in various cell types obtained from iPSCs derived from patients with nuclear factor κB essential modulator deficiency. Finally, iPSCs can be used as a limitless source of stem cells in which novel strategies to achieve gene correction might be tested. In particular, they share with embryonic stem cells a higher susceptibility to homologous recombination and thus represent a promising tool to study the ability of zinc-finger nucleases, meganucleases, and sleeping-beauty transposons to mediate gene repair.[56,57]

In conclusion, we have reported on the successful generation and characterization of iPSCs from patients with various clinical PID phenotypes and underlying genotypes. The iPSC technology is still at its early days. Limitations and potential pitfalls of this approach include, among others, variability in the efficiency and validity of the reprogramming strategy and the possible introduction of genomic abnormalities that might lead to increased tumorigenesis. These problems must be addressed before considering use of these cells in clinical settings. Nevertheless, it can be anticipated that this novel technology will provide new insights into the pathophysiology of PIDs and facilitate development of novel and more effective forms of treatment for these disorders.

Clinical implications: iPSCs derived from patients with PIDs represent a unique resource to study the pathophysiology of and to develop novel therapeutic approaches for these disorders.

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METHODS

Lentiviral reprogramming vector production

Lentiviruses containing the previously described[E1-E4] polycistronic lentiviral vector STEMCCA–LoxP were produced by using a 5-plasmid transfection system, as previously described, with slight modification.[E5] 293T packaging cells were transfected with the plasmids coding the packaging proteins Gag-Pol, Rev, and Tat and the G protein of the vesicular stomatitis virus and with the STEMCCA–LoxP Vector by using *Trans*-IT 293 transfection reagent (Mirus Bio LLC, Madison, Wis). After 48 hours of incubation, viral supernatants were collected, and viral particles were concentrated by means of ultracentrifugation at 16,500 rpm for 1.5 hours at 4°C.

Reprogramming of fibroblasts and human iPSC generation

Fibroblasts were infected by culturing 10^5 cells in 1 well of a 6-well plate in the presence of the lentiviral reprogramming vector in hFib media supplemented with 5 μg/mL protamine sulfate (Sigma) for 24 hours. The viral supernatant was removed after 24 hours. Forty-eight hours later, the infected fibroblasts were transferred by means of trypsinization to a 10-cm tissue-culture dish containing iMEFs and cultured in hES media. iPSCs colonies with ES-like morphology started appearing after 3 to 5 weeks. Colonies were picked, subcloned, and expanded by means of mechanical transfer into new plates containing fresh and adhered iMEFs, as previously described.[E6] Several clones were derived and characterized from each fibroblast line (Table E1).

Immunohistochemistry

iPSC colonies were mechanically picked into 96-well plates that were coated with iMEF feeders. Two or 3 days after plating, cells were washed with PBS and fixed in 4% paraformaldehyde for 20 minutes. Cells were washed and treated with 0.2% Triton X in PBS for 30 minutes to allow nuclear permeation. Cells were stained in blocking buffer (3% BSA and 5% goat serum) with primary and conjugated antibodies at 4°C overnight, washed and stained with secondary antibodies and 1 μg/mL Hoechst 33342 in blocking buffer for 3 hours at 4°C, and protected from light. Images were acquired with a Pathway 435 bioimager equipped with a 10× objective (BD Biosciences, San Jose, Calif). Primary OCT4 and NANOG antibodies (Abcam, Cambridge, Mass) were used at 0.5 μg/mL, and an anti-rabbit IgG Alexa Fluor 555 (Invitrogen) was used as the secondary antibody (1:2000). The antibodies TRA-1-60–Alexa Fluor 647, TRA-1-81–Alexa Fluor 488, SSEA-4–Alexa Fluor 647, and SSEA-3–Alexa 488 (Millipore, Billerica, Mass) were used at 1:100 dilution.

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FIG E1. Somatic cell reprogramming to pluripotency using the STEMCCA lentiviral vector. Schematic representation of STEMCCA lentiviral vector coding for the expression of a "stem cell cassette" composed of a single polycistronic mRNA containing an internal ribosome entry site *(IRES)* element separating 2 fusion cistrons. The first cistron consists of *OCT4* fused to *KLF4* coding sequences through the use of intervening sequences encoding "self-cleaving" 2A peptides. The second cistron consists of the coding sequences of *SOX2* and *cMYC* fused in a similar manner. The polycistronic transcript is driven by a constitutive elongation factor-1α (*EF1a*) promoter and is flanked by LoxP sites enabling future Cre-mediated excision, as previously described.[E1-E4] *LTR*, Long terminal repeat; *PSI-RRE-cpPu*, packaging signal-rev response element-central polypurine track; *WPRE*, woodchuck hepatitis virus post-transcriptional regulatory element.

TABLE E1. Number of iPSC lines derived and analyzed for each patient

Patient	No. of lines derived	No. of lines analyzed for pluripotency and stemness	No. of lines analyzed for karyotypic integrity
SCID	4	3	3
Leaky SCID	4	2	1
OS	2	2	1
HSE1	4	2	1
HSE2	4	2	1
CHH	2	1	1
Healthy control subject	5	3	1
Totals	25	14	9

Several lines were derived from each of the patients' and control subjects' fibroblast cell lines that were reprogrammed. After the reprogramming process, part of the lines were analyzed for pluripotency and stemness by means of immunohistochemistry and gene expression profiling. Selected lines were analyzed for karyotypic integrity by means of G-banding karyotyping.
CHH, cartilage hair hypoplasia; *HSE*, herpes simplex encephalitis; *OS*, Omenn syndrome.

TABLE E2. Primers used for mutation analysis

Gene	Disease-causing mutation	Amplification primers	Sequencing primers
STAT1	c. 1928_1929 insA	F: 5-ATTTTGGGATGTTCTATGGG-3 R: 5-AGACTGTGCCACGCTGTT-3	F: 5-ATTTTGGGATGTTCTATGGG-3 R: 5-AGACTGTGCCACGCTGTT-3
TLR3	c.1660C>T	F: 5-CTTTCAACAGCATTACAGAG-3 R: 5-AATGACTTTAGAGACACCTG-3	F: 5-CCTACAACAAGTACCTGCAG-3 R: 5-CCTCAACTGGGATCTCGTC-3
TLR3	c. 2236G>T	F: 5-ACTGATGCTCCGAAGGGTG-3 R: 5-AGAGCAGGACTCCATCTC-3	F: 5-CCCAGTGAGACTTTTTGATAC-3 R: 5-CACAATGTTTACCTACC-3
RAG1	c.1180C>T	F: 5-CCATCTCCTGCCAGATCTGT-3 R: 5-GCCTTCCAAGATGTCTTCTTC-3	F: 5-CCATCTCCTGCCAGATCTGT-3 R: 5-GCCTTCCAAGATGTCTTCTTC-3
	c.1228C>T	F: 5-GTGCACATTAATAAAGGGGGC-3 R: 5-GCCTTCCAAGATGTCTTCTTC-3	F: 5-GTGCACATTAATAAAGGGGGC-3 R: 5-GCCTTCCAAGATGTCTTCTTC-3
	c.2332C>T	F: 5-GAGGCTTCTGGCTCAGTCTA-3 R: 5-ATGATGATCGCCATACTGGT-3	F: 5-GAGGCTTCTGGCTCAGTCTA-3 R: 5-ATGATGATCGCCATACTGGT-3
	c.256-257del	F: 5-GGTACCTCAGCCAGCATG-3 R: 5-AGATGTCGAAGGTTGGCTTG-3	F: 5-GGTACCTCAGCCAGCATG-3 R: 5-AGATGTCGAAGGTTGGCTTG-3
	c.2164G>A	F: 5-GCAAAGAGGTTCCGCTATGA-3 R: 5-TTTTGAGAGGCTTCCAGACG-3	F: 5-GCAAAGAGGTTCCGCTATGA-3 R: 5-TTTTGAGAGGCTTCCAGACG-3
RMRP	c. 27 G>A	F: 5-GGAGGATACAGGCGAGTCAG-3 R: 5-GCAGAATAGCTAATAGACAC-3	F: 5-GGAGGATACAGGCGAGTCAG-3 R: 5-GCAGAATAGCTAATAGACAC-3

F, Forward primer; *R*, reverse primer; *STAT1*, signal transducer and activator of transcription 1; *TLR3*, Toll-like receptor 3.