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Supplemental Information

Balancing serendipity and reproducibility: Pluripotent stem cells as experimental systems for intellectual and developmental disorders

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SUPPLEMENTAL INFORMATION

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SUPPLEMENTAL METHODS

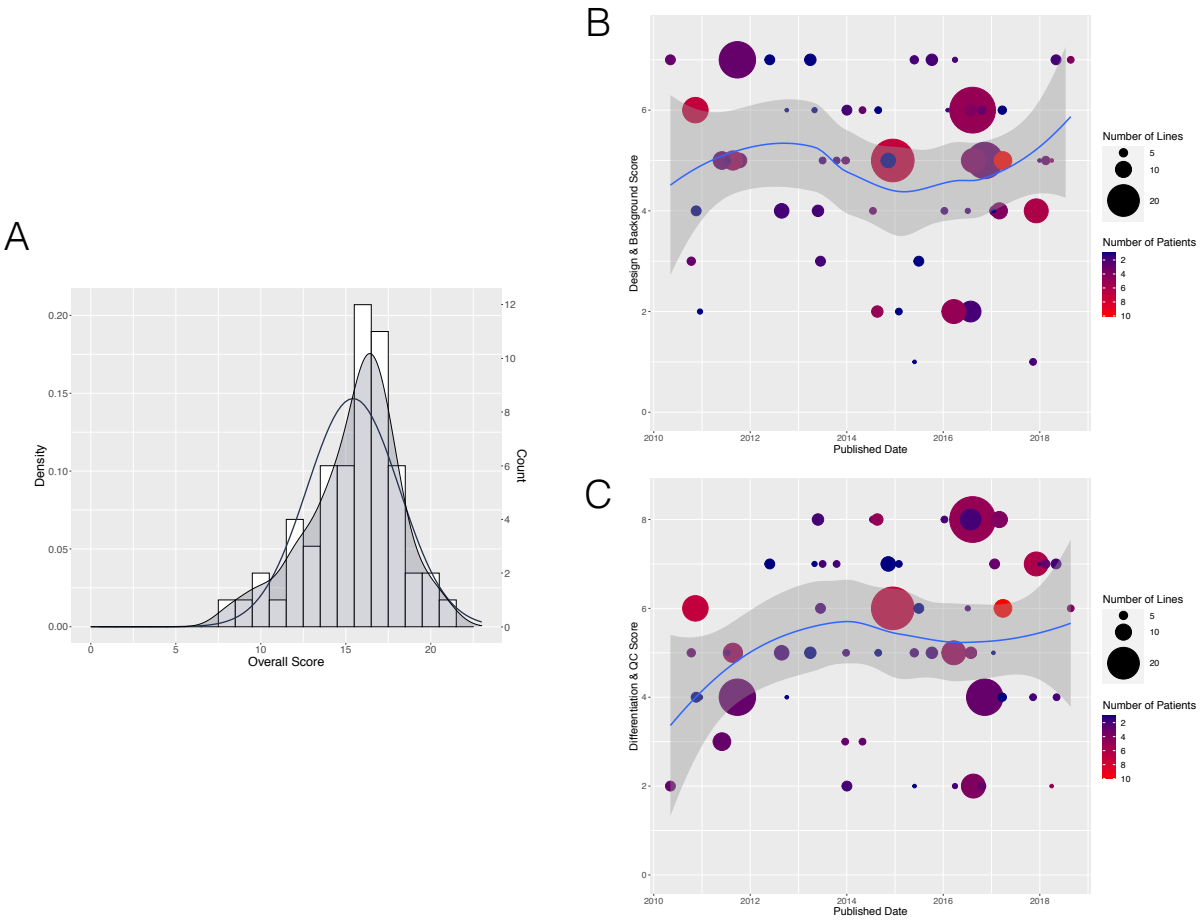
Literature Search

Papers were identified using the following PubMed search terms:
“(Christianson[All Fields] OR Pitt-Hopkins[All Fields] OR ("angelman syndrome"[MeSH Terms] OR ("angelman"[All Fields] AND "syndrome"[All Fields]) OR "angelman syndrome"[All Fields]) OR ("CDKL5 deficiency disorder"[Supplementary Concept] OR "CDKL5 deficiency disorder"[All Fields] OR "cdkl5 deficiency disorder"[All Fields]) OR ("down syndrome"[MeSH Terms] OR ("down"[All Fields] AND "syndrome"[All Fields]) OR "down syndrome"[All Fields]) OR ("epilepsies, myoclonic"[MeSH Terms] OR ("epilepsies"[All Fields] AND "myoclonic"[All Fields]) OR "myoclonic epilepsies"[All Fields] OR ("dravet"[All Fields] AND "syndrome"[All Fields]) OR "dravet syndrome"[All Fields]) OR (FOXG1[All Fields] AND ("syndrome"[MeSH Terms] OR "syndrome"[All Fields])) OR ("fragile x syndrome"[MeSH Terms] OR "fragile x syndrome"[All Fields]) OR ("Telomeric 22q13 Monosomy Syndrome"[Supplementary Concept] OR "Telomeric 22q13 Monosomy Syndrome"[All Fields] OR "phelan mcdermid syndrome"[All Fields]) OR ("prader-willi syndrome"[MeSH Terms] OR ("prader-willi"[All Fields] AND "syndrome"[All Fields]) OR "prader-willi syndrome"[All Fields] OR ("prader"[All Fields] AND "willi"[All Fields] AND "syndrome"[All Fields]) OR "prader willi syndrome"[All Fields]) OR ("rett syndrome"[MeSH Terms] OR ("rett"[All Fields] AND "syndrome"[All Fields]) OR "rett syndrome"[All Fields]) OR (16p11.2[All Fields] AND ("dna copy number variations"[MeSH Terms] OR ("dna"[All Fields] AND "copy"[All Fields] AND "number"[All Fields] AND "variations"[All Fields]) OR "dna copy number variations"[All Fields] OR ("copy"[All Fields] AND "number"[All Fields] AND "variation"[All Fields]) OR "copy number variation"[All Fields])) OR ("tuberous sclerosis"[MeSH Terms] OR ("tuberous"[All Fields] AND "sclerosis"[All Fields]) OR "tuberous sclerosis"[All Fields] OR ("tuberous"[All Fields] AND "sclerosis"[All Fields] AND "complex"[All Fields]) OR "tuberous sclerosis complex"[All Fields]) OR ("williams syndrome"[MeSH Terms] OR ("williams"[All Fields] AND "syndrome"[All Fields]) OR "williams syndrome"[All Fields] OR ("williams"[All Fields] AND "beuren"[All Fields] AND "syndrome"[All Fields]) OR "williams beuren syndrome"[All Fields])) AND ("induced pluripotent stem cells"[MeSH Terms] OR ("induced"[All Fields] AND "pluripotent"[All Fields] AND "stem"[All Fields] AND "cells"[All Fields]) OR "induced pluripotent stem cells"[All Fields] OR "ipsc"[All Fields]) AND ("humans"[MeSH Terms] OR "humans"[All Fields] OR "human"[All Fields]) AND ((Journal Article[ptyp] OR Classical Article[ptyp] OR Letter[ptyp]) AND English[lang]) AND ("0001/01/01"[PDAT] : "2019/07/12"[PDAT]).”

Scoring

Papers that provided details regarding fibroblast plating density and passage number, and information related to iPSC selection and karyotyping received higher scores than those that did not. Likewise, papers that utilized isogenic controls, familial or sex matched/age matched controls received higher scores than papers that did not. Inclusion of patient demographic information (whether in the manuscript or a citation of a previously published case report) resulted in a higher score. Finally, the quality of the differentiation protocol was evaluated with a higher score assigned to papers that either: (1) fully outlined the experimental procedures used, or (2) cited a previously published methods paper with a step-by-step protocol. Publications that indicated differentiation was carried out as previously described by other publications with slight modifications received an intermediate score if modifications were clearly outlined and a lower score if: (1) no modifications were outlined and/or no detailed experimental procedure was provided or (2) the citation referenced did not provide a detailed protocol and/or referred to yet another paper. For differentiation quality control, papers are scored for data provided on RNA marker expression, protein marker expression, and functional assay. See also Figure 1 and Supplemental Table S1. We did not include the number of lines or number of patients in scoring, because these parameters are often study-dependent, and we were not able to score in a quantitative and objective manner. However, these two parameters are indicated in the size (number of lines) and color (number of patients) of each data point.

SUPPLEMENTAL FIGURES



Supplemental Figure 1. Analysis of 58 papers using iPSCs as experimental systems for IDDs. Research articles were evaluated based on the following three categories: (1) iPSC derivation and quality control, which encompass both a detailed protocol for iPSC derivation and a detailed description of iPSC clonal selection and quality control (QC) assays. (2) Experimental design and background information, including a description of experimental controls and demographic information about the patients from whom iPSCs were derived. (3) Neural differentiation and quality control, including the differentiation protocol outlined, RNA and protein marker expression, and description of neuronal functional activity. **(A)** Overall score distribution (x-axis) of 58 primary articles published presented in density plot (grey area, left y-axis) and paper count (white bars, right y-axis). The gray line indicates theoretical symmetric distribution. Publications were plotted based on published date (x-axis), each publication was evaluated on **(B)** a scale of 0 to 7 (y-axis) for experimental design and background, or **(C)** a scale of 0 to 8 (y-axis) for differentiation and quality control. The number of iPSC lines is indicated by the size of the circles, with larger sphere indicating a greater number of iPSC lines. The number of patients is indicated by the color of the sphere. A smooth regression line (blue) and a confidence band (grey shade) are shown using geom_smooth function through ggplot2 in R. This figure is related to **Figures 1-2** in the main text.

Supplemental Table Legends

Supplemental Table S1. Analysis of 58 papers using iPSCs as experimental systems for IDDs. Papers were identified using the search terms outlined in this Supplemental Document. All selected papers generated patient-derived iPSCs and utilized them to study a particular genetically defined IDD. Column A: List of the 58 papers analyzed. Column B & C: Description of the scoring categories and items evaluated from each *publication. Column D: Minimum score assigned to each publication. Column E: Maximum score assigned to each publication.*

Supplemental Table S2. Deriving glial cells from iPSCs and their use as experimental systems to model IDDs. (A) References describe approaches for deriving glial cell types from iPSCs including astrocytes, oligodendrocytes, and microglia. (B) Examples of studies modeling IDDs in iPSC-derived glia are shown. The IDD, mutation(s) modeled, glial cell type, and references are indicated.

Supplemental Table S3. Summary of gene editing systems to reduce unwanted genomic modifications Table summarizes Cas9 variants that reduce nuclease activity at undesired genomic loci and identifies the relevant mutated amino acids in each variant and whether the specific variant modifies the protospacer adjacent motif (PAM) binding sequence. Systems that do not introduce a double-strand break (DSB) in the DNA are detailed with the protein component responsible for DNA targeting, the protein component responsible for DNA modification, and the resultant outcome for each system.

Supplemental Table S4. Directed differentiation into cortical excitatory neurons. Technical variables that differ by laboratory and protocol during the specification of human pluripotent cells as cortical excitatory neuron progenitors and their differentiation and maturation into glutamatergic excitatory neurons were assessed. For neural progenitor specification, these include: (Column B) the signaling cues used, (Column C) the basal media used, (Column D) whether differentiation was performed in adherent or floating three dimensional embryoid bodies (aEB/EB) or in monolayer culture (ML), (Column E) days of specification, and (Column F) the marker expression assessed (green text), with (Column G) indicating the percentage of cells expressing the marker indicated in green in Column F. During progenitor differentiation and maturation into functional excitatory neurons, similar technical variables were assessed, as were the molecular and functional criteria used to assess the neurons generated. Marker indicated in green was used to quantify the percentage of excitatory neuron progenitors (Specification Section) or neurons (Differentiation Section) obtained, as determined by immunocytochemistry or FACS analysis. Abbreviations and definitions are in **Supplemental Table S8.**

Supplemental Table S5. Directed differentiation into cortical inhibitory interneurons. Technical variables that differ by laboratory and protocol were assessed during the specification of human pluripotent stem cells as medial ganglionic eminence-like (MGE) interneuron progenitors and their differentiation and maturation into GABAergic cortical inhibitory neurons. For MGE progenitor specification, these include: (Column B) the signaling cues used, (Column C) treatment with a SHH agonist from initiation of the protocol versus during a later time, (Column D) the basal media used, (Column E) specification in monolayers versus in suspended or adherent embryoid bodies (ML, EB, aEB), (Column F) days of specification, and (Column G) marker expression assessed (green text), with (Column H) indicating the percentage of cells expressing the marker shown in green in Column G. During progenitor differentiation and maturation into functional inhibitory neurons, similar technical variables were assessed, as were the molecular and functional criteria used to assess the neurons generated. Marker indicated in green was used to quantify the percentage of interneuron progenitors (Specification Section) or inhibitory neurons (Differentiation Section) obtained, as determined by immunocytochemistry or FACS analysis. Functional analysis of the neurons generated is also indicated, if performed. In some cases (indicated), a reporter line with GFP knock-in to the *NKX2-1* locus was also used to enrich for specified progenitors by FACS. Abbreviations and definitions are in **Supplemental Table S8.**

Supplemental Table S6. NGN2-induced differentiation into cortical excitatory neurons. Technical variables that differ by laboratory were assessed by using published NGN2-induced iN protocols. Technical variables include: (Column B) transcription factor used, (Column C) starting cell type, (Column D) PSC culture conditions prior to differentiation, (Column E) plating density, (Column F) Doxycycline concentration used for induction, (Column G) Timing of doxycycline treatment, (Column H) timing for drug selection, (Column I) drug selection dosage, (Column J) timing for glial coculture, (Columns K-L) Ara-C use, concentration, and timing, and (Columns N-O) basal differentiation media and supplements. Note that one protocol used FACS to enrich for the CAMK2-expressing neuronal population (Column M). Also shown are the (Column P) earliest time point when neurons could be assayed, (Column Q) the molecular markers assessed, and (Column R) the functional assays performed. Abbreviations and definitions are in **Supplemental Table S8.**

Supplemental Table S7. Recommended reporting table. The table contains the recommendations for documentation and characterization at different stages of iPSC culture and differentiation, in a format to be completed by the researcher. This table is designed to help researchers track their progress and maintain best practices at each stage, with the goals of improving transparency, rigor, and reproducibility. (Y/N stands for Yes/No).

Supplemental Table S8. Abbreviations and definitions of terms used in Supplemental Tables S4-S6.

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