Cell-autonomous correction of ring chromosomes in human induced pluripotent stem cells

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Ring chromosomes are structural aberrations commonly associated with birth defects, mental disabilities and growth retardation1–3. Rings form after fusion of the long and short arms of a chromosome, and are sometimes associated with large terminal deletions4. Owing to the severity of these large aberrations that can affect multiple contiguous genes, no possible therapeutic strategies for ring chromosome disorders have been proposed. During cell division, ring chromosomes can exhibit unstable behaviour leading to continuous production of aneuploid progeny with low viability and high cellular death rate5–8. The overall consequences of this chromosomal instability have been largely unexplored in experimental model systems. Here we generated human induced pluripotent stem cells (iPSCs)10–12 from patient fibroblasts containing ring chromosomes with large deletions and found that reprogrammed cells lost the abnormal chromosome and duplicated the wild-type homologue through the compensatory uniparental disomy (UPD) mechanism. The karyotypically normal iPSCs with isodisomy for the corrected chromosome outgrew co-existing aneuploid populations, enabling rapid and efficient isolation of patient-derived iPSCs devoid of the original chromosomal aberration. Our results suggest a fundamentally different function for cellular reprogramming as a means of ‘chromosome therapy’14 to reverse combined loss-of-function across different function for cellular reprogramming as a means of rapid and efficient isolation of patient-derived iPSCs devoid of the chromosomal instability that is of critical relevance to human development and disease.

We obtained fibroblasts from a Miller Dieker Syndrome (MDS) patient with ring chromosome 17, subsequently referred to as r(17). MDS is caused by heterozygous deletions of human band 17p13.3 (refs 14, 15) (Fig. 1a). This deletion alone leads to craniofacial dysmorphology, defective neuronal migration, abnormal cortical layering and nearly absent cortical folding with devastating neurological consequences such as mental retardation and intractable epilepsy16,17. However, in this case the 17p13.3 deletion was in a ring chromosome, and the patient had a typical MDS phenotype4. To separate the effects of r(17) from the 17p13.3 deletion, we obtained fibroblasts from two additional MDS patients with similar deletions but without r(17) (Fig. 1b).

Two critical genes deleted in MDS are PAFAH1B1 (encoding LIS1) and YWHAE (encoding 14–3–3ε) (Fig. 1a)18. Consistent with this, MDS fibroblasts MDS1r(17), MDS2 and MDS3 (Fig. 1c) expressed reduced PAFAH1B1 and YWHAE messenger RNA compared to control fibroblasts (Fig. 1d, e). MDS1r(17) fibroblasts had a 46,XY,r(17) karyotype in 95% of the cells (Figs 1f and Extended Data Fig. 1), with the remaining 5% exhibiting ring loss or secondary ring derivatives (Fig. 2b).

To investigate the behaviour of ring chromosomes in actively proliferating cells, we generated iPSCs using non-integrating episomal vectors17. All MDS iPSCs were morphologically indistinguishable from wild type (Fig. 1f) and expressed stem cell markers (Fig. 1g and Extended Data Fig. 2a–d). We confirmed that MDS iPSCs were free of exogenous factor integration (Extended Data Fig. 3a, b) and were functionally pluripotent, producing cell types of the three germ layers (Extended Data Figs 4 and 5).

We then analysed six early-passage MDS1r(17) iPSC clones for the presence of the ring (Fig. 1h) and, unexpectedly, found that four out of six clones did not have any detectable ring chromosomes (Fig. 1i), but grew well and had proper morphology (Extended Data Fig. 6a). In contrast, two other clones with detectable ring chromosomes (Fig. 1i) differentiated or stopped growing upon subsequent passaging (Extended Data Fig. 6b, c). Analysis of chromosome composition revealed that stable clones had 46 chromosomes and no ring in 85–100% of cells, in contrast to <15% of cells in unstable clones (Fig. 1i and Extended Data Fig. 6d, e). These results suggested that r(17) was incompatible with reprogramming and/or stem cell maintenance using our methods.

Further cytogenetic analysis of the first two MDS1r(17) iPSC clones demonstrated a normal 46,XY karyotype without r(17) (Fig. 2a–c). In addition, the deletion, which was readily detectable by Giemsa (G)-banding in MDS2 and MDS3 iPSCs, was not apparent in MDS1r(17) iPSCs (Fig. 2a and Extended Data Fig. 1). These findings could be explained by either clonal expansion of rare cells with a normal karyotype from mosaic fibroblasts; or repair or replacement of the ring chromosome during or after reprogramming. We reasoned that presence of a small fraction of cells with the deleted r(17) in predominantly corrected iPSC clones would confirm their origin from an abnormal fibroblast. To test this, we analysed interphase nuclei by fluorescence in situ hybridization (FISH) using a red probe to detect the sub-telomeric band 17p13.3 together with a green probe to detect the long-arm band 17q21.32 (Fig. 2d, e). Consistent with karyotype data, ≥80% of cells in iPSC clones 1 and 2 demonstrated a normal signal pattern (2R2G) (Fig. 2f), suggesting that they have two intact copies of chromosome 17. However, 10–20% of interphase iPSCs had a 1R2G signal pattern, indicative of r(17) with p13.3 deletion (Fig. 2f). These results revealed persistent mosaicism in MDS1r(17) iPSCs, confirming their origin from ring-containing fibroblasts and supporting the hypothesis that repair or replacement of the ring chromosome occurred during reprogramming. Of note, despite several attempts (>120 cells analysed in multiple experiments), no evidence of r(17) was found in metaphase iPSCs, suggesting that in the pluripotent state such cells may be terminal and non-dividing. The expectation is that remaining interphase cells with r(17) would be gradually depleted.

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Figure 1 | Reprogramming from fibroblasts with r(17) produces multiple iPSC clones that do not have the ring chromosome. a, Schematic of human chromosome 17 (chr 17), highlighting band 17p13.3, which is deleted in MDS. Encoded genes are listed on the right, and genes within the minimal critical MDS deletion are shown in red. b, Schematic of chr 17 status in wild type (WT) and MDS cells. c, List of fibroblast lines used in this study. d, e, Quantitative polymerase chain reaction with reverse transcription (RT-qPCR) for PAFAH1B1 (d) and YWHAE (e) mRNA levels in fibroblasts (n = 2). f, Images of WT, MDS1r(17) and MDS2 iPSCs. g, Western blot analysis for pluripotency markers in fibroblasts (Fib) and two iPSC clones. h, Examples of metaphase spreads with or without the ring chromosome. i, Percentage of mitotic cells with or without the ring chromosome in fibroblasts or MDS1r(17) iPSC clones.

Figure 2 | Karyotypically normal cells predominate in early passage iPSC clones derived from MDS1r(17) fibroblasts. a, Images of chr 17 pairs in MDS1r(17) fibroblasts and iPSC clones. The arrow is pointing to r(17) only found in the fibroblasts. b, c, Proportion of MDS1r(17) fibroblasts (b) and iPSC clones 1 and 2 (c) with various karyotypes (n = 20 each). d, Approximate position of FISH probes on chr 17. e, Signal patterns obtained with FISH probes in d, f. Proportions of cells with various signal patterns (n = 200 each). g, h, qPCR for genomic PAFAH1B1 DNA in fibroblasts (g) and iPSCs (h). AU, arbitrary units; n = 2. i, j, Western blot analysis for L1S1 and 14-3-3c in fibroblasts (i) (two samples per fibroblast line collected on different days) and iPSCs (two clones per line) (j).
Cyto genetic and FISH results suggested that the MDS-associated deletion was rescued in MDS1r(17) iPSC clones 1 and 2. Consistent with that rescue, PAFAH1B1 genomic DNA levels were restored from 50% of wild type in the original fibroblasts (Fig. 2g) to wild-type levels in iPSCs (Fig. 2h). Conversely, in MDS2 and MDS3 cells without r(17), PAFAH1B1 genomic DNA levels were consistently reduced before and after reprogramming (Fig. 2g, h). Similar results demonstrating rescue were obtained by western blot analysis for LIS1 and 14-3-3ε proteins (Fig. 2i, j). To investigate the status of the deleted locus before and after reprogramming in more detail, we performed single nucleotide polymorphism (SNP) microarrays. Analysis of total copy number for SNPs on chromosome 17 showed a clear decrease between positions 1 and 5,700,000 in MDS1r(17) fibroblasts, which was not observed in the corresponding iPSCs (Fig. 3a, b). In contrast, large terminal deletions were apparent in iPSCs derived from MDS2 and MDS3 fibroblasts (Fig. 3c, d). Of note, the extent of the deletion in MDS3 cells was exactly the same as in MDS1r(17) fibroblasts, making it an ideal control for the effect of the ring. Therefore, 17p13.3 deletion is recapitulated in iPSCs, the same as in MDS1r(17) fibroblasts, making it an ideal control for the effect of the corrected chromosome 17 (Fig. 3g).

Non-random gain of a third chromosome 17 occurs frequently in long-term culture of human pluripotent cells owing to a proliferative growth advantage. To test whether chromosomes not typically gained or implicated in advantageous stem cell growth can replace the corresponding ring chromosome in iPSCs, we reprogrammed fibroblasts from two additional cases involving ring chromosome 13, subsequently referred to as r(13), with large deletions (Fig. 4a, f) associated with multiple congenital anomalies. We confirmed the presence of r(13) in approximately 80–100% of the fibroblasts, with the remaining cells exhibiting...
45 chromosomes minus r(13) or non-clonal aberrations involving the ring structure (Fig. 4b, d and Extended Data Fig. 7a, d). Initial examination of iPSCs at passage six revealed 46,XY,r(13) karyotypes, although a fraction of cells had lost the ring chromosome (Fig. 4c, e). However, six out of nine clones that were examined after passage eight had a normal karyotype (Fig. 4a, c and Extended Data Fig. 7). In addition, GM00285 iPSC clone 4 transitioned from predominantly 46,XY,r(13) at passage six to 46,XY at passage twelve (Fig. 4c). These results demonstrate dynamic mosaic between clonally related cells, leading to preferential survival of karyotypically normal iPSCs in a matter of six passages, which did not occur in the fibroblasts (Fig. 4b, d). In agreement, FISH studies revealed normal signal patterns in the corrected iPSC clones (Fig. 4f–h). Finally, SNP microarray analysis confirmed rescue of the deleted regions (Extended Data Fig. 8a–e) and showed that corrected iPSCs are completely homozygous for chromosome 13 (Extended Data Fig. 8f, g). The repaired iPSCs expressed stem cell markers (Extended Data Fig. 2e–j), differentiated into three germ layers (Extended Data Fig. 3c, d). All of the established iPSC lines in this study were confirmed to be identical to their original fibroblasts by DNA fingerprinting (Extended Data Table 1).

Finally, to further rule out the possibility that repaired iPSC clones originated from rare 46,XY fibroblasts, we measured the reprogramming rates for the lines used in this study. If there is a bias against reprogramming from cells with ring chromosomes, then we would expect reduced reprogramming efficiency from ring-containing fibroblast populations. However, we found no significant difference between wild-type and MDS cells, while reprogramming efficiency from r(13) fibroblasts was increased compared to wild type (Fig. 4i). Therefore, r(17) or r(13) chromosomes do not interfere with episomal reprogramming. More likely, reprogramming increases cell divisions, increasing the probability that random nondisjunction compensates for the loss of the corresponding ring. Nondisjunction leading to chromosomal trisomy is the most commonly reported karyotypic change in cultured human ES cells and iPSCs, because mitotic checkpoints that ensure proper alignment and disentanglement of sister chromatids before separation are less efficient than in differentiated cells. In this case, isodisomic cells have a growth advantage over cells with ring chromosomes including large deletions; however, they are not exactly the same as wild type. UPD could lead to undesirable clinical consequences through homozgyosis for a recessive mutation or disruption of imprinting. Thus, expression of imprinted genes and presence of recessive mutations would have to be carefully monitored and/or corrected for any therapeutic applications.

Compensatory UPD and dynamic mosaicism occur in actively proliferating cells in vivo, including cases involving ring chromosome 21, in which patients’ lymphocytes transitioned from predominantly 46,XX,r(21) to 46,XX (with UPD21) several years later. These observations further suggest that taking advantage of this intrinsic property of iPSCs may be a feasible approach to correct combined loss-of-function and structural aberrations associated with additional ring chromosomes. Given that repair of large deletions involving multiple genes is not possible with current genome editing techniques, our results may have broad implications for regenerative medicine and advance the newly emerging concept of chromosome therapy. More broadly, our findings underscore the importance of studying chromosome repair in stem cells and establish a novel platform for investigating the mechanisms that regulate chromosome number during early human development.

**METHODS SUMMARY**

The following primary human cells were used in this study: MDS1r(17) (GM06047), MDS2 (GM06097), MDS3 (GM09208), GM00285 and GM05563 (Coriell Institute for Medical Research); B) and Detroit 551 (ATCC); HDF-1323 from a 48-year-old caucasian female (Cell Applications; 106-05a); HDF-WTc from a 30-year-old Japanese male, whose skin samples were collected at Gladstone. Human iPSCs were generated with the episomal Y4 plasmid mixture as described with some modifications (available in the online Methods). Established hiPSC lines were cultured in mTeSR1 medium (Stem Cell Technologies) on dishes coated with Matrigel (BD) or Synthemax (Corning). Chromosomal analyses were performed on 20 G-banded metaphase cells from each sample and FISH analyses were performed on two hundred interphase nuclei from each sample at Cell Line Genetics or the
University of California San Francisco cytogenetics laboratory. SNP genotyping to determine CNV presence and UPD status was performed using the Affymetrix Axiom EUR array platform by the Genomics Core Facility (GCF) at the University of California San Francisco for MDS samples and using the Agilent SurePrint G3 Human CGH + SNP 4 × 180K array platform by Cell Line Genetics for r(13) samples.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Cote, G. B. et al. The cytogenetic and clinical implications of a ring chromosome 2. Ann. Genet. 24, 231–235 (1981).
2. Kosztolányi, G. Does “ring syndrome” exist? An analysis of 207 case reports on patients with a ring autosome. Hum. Genet. 75, 174–179 (1987).
3. McClintock, B. The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. Genetics 23, 315–376 (1938).
4. Kristenmacher, M. L. & Punnett, H. H. Comparative behavior of ring chromosomes. Am. J. Hum. Genet. 22, 304–318 (1970).
5. Tommerup, N. & Lothe, R. Constitutional ring chromosomes and tumor suppressor genes. J. Med. Genet. 29, 879–882 (1992).
6. Jobanputra, V. et al. Changes in an inherited ring (22) due to meiotic recombination? Implications for genetic counseling. Am. J. Med. Genet. A 149A, 1310–1314 (2009).
7. Mantzouratou, A. et al. Meiotic and mitotic behaviour of a ring/deleted chromosome 22 in human embryos determined by preimplantation genetic diagnosis for a maternal carrier. Mol. Cytogenet. 2, 3 (2009).
8. Sodré, C. P. et al. Ring chromosome instability evaluation in six patients with autosomal rings. Genet. Mol. Res. 9, 134–143 (2010).
9. Zhang, H. Z. et al. Unique genomic structure and distinct mitotic behavior of ring chromosome 21 in two unrelated cases. Cytogenet. Genome Res. 136, 180–187 (2012).
10. Takahashi, K. et al. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Cell 131, 861–872 (2007).
11. Yu, J. et al. Induced pluripotent stem cell lines derived from human somatic cells. Science 318, 1917–1920 (2007).
12. Park, I. H. et al. Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451, 141–146 (2008).
13. Jiang, J. et al. Translating dosage compensation to trisomy 21. Nature 500, 296–300 (2013).
14. Dobyns, W. B. et al. Miller-Dieker syndrome: lissencephaly and monosomy 17p. J. Pediatr. 102, 552–558 (1983).
15. Cardoso, C. et al. Refinement of a 400-kb critical region allows genotypic differentiation between isolated lissencephaly, Miller-Dieker syndrome, and other phenotypes secondary to deletions of 17p13.3. Am. J. Hum. Genet. 72, 918–930 (2003).
16. Wynshaw-Boris, A. et al. Lissencephaly: mechanistic insights from animal models and potential therapeutic strategies. Semin. Cell Dev. Biol. 21, 823–830 (2010).
17. Okita, K. et al. A more efficient method to generate integration-free human iPS cells. Nature Methods 8, 409–412 (2011).
18. Robinson, W. P. Mechanisms leading to uniparental disomy and their clinical consequences. Bioessays 22, 452–459 (2000).
19. Speevak, M. D. et al. Molecular characterization of an inherited ring (19) demonstrating ring opening. Am. J. Med. Genet. A 121A, 141–145 (2003).
20. Hусsens, S. M. et al. Genome damage in induced pluripotent stem cells: assessing the mechanisms and their consequences. Bioessays 35, 152–162 (2013).
21. Moynahan, M. E. & Jasin, M. Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis. Nature Rev. Mol. Cell Biol. 11, 196–207 (2010).
22. Draper, J. S. et al. Recurrent gain of chromosome 17q and 12 in cultured human embryonic stem cells. Nature Biotechnol. 22, 53–54 (2004).
23. Baker, D. E. et al. Adaptation to culture of human embryonic stem cells and oncogenesis in vivo. Nature Biotechnol. 25, 207–215 (2007).
24. Spits, C. et al. Recurrent chromosomal abnormalities in human embryonic stem cells. Nature Biotechnol. 26, 1361–1363 (2008).
25. Azuhata, T. et al. The inhibitor of apoptosis protein survivin is associated with high-risk behavior of neuroblastoma. J. Pediatr. Surg. 36, 1785–1791 (2001).
26. Damelin, M. et al. Decatenation checkpoint deficiency in stem and progenitor cells. Cancer Cell 8, 479–484 (2005).
27. Wilton, L. Preimplantation genetic diagnosis for aneuploidy screening in early human embryos: a review. Prenat. Diag. 22, 512–518 (2002).
28. Vanneste, E. et al. Chromosome instability is common in human cleavage-stage embryos. Nature Med. 15, 577–583 (2009).
29. Petersen, M. B. et al. Uniparental sodiomidy due to duplication of chromosome 21 occurring in somatic cells monosomic for chromosome 21. Genomics 13, 269–274 (1992).
30. Bartsch, O. et al. “Compensatory” uniparental disomy of chromosome 21 in two cases. J. Med. Genet. 31, 534–540 (1994).

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Author Contributions M.B. conceived and designed the study, generated and characterized MDS iPS lines, performed experiments and analyzed data, created the figures and wrote the manuscript. Y.H. helped to generate MDS iPS lines, generated and characterized wild-type and r(13) iPS lines, designed the study, performed experiments and analysed data. G.D. and L.A.W. performed SNP array genotyping and CNV calling analyses for MDS samples. E.C.H., S.S. and K.M.T. provided technical support for various experiments. A.R.K. provided advice, access to equipment and laboratory space for M.B. S.Y. supervised the study, provided advice, laboratory space and financial support. A.W.-B. supervised the study, provided advice, helped with design and interpretation, and provided laboratory space and financial support. A.W.-B., Y.H., S.Y., A.R.K., E.C.H. and L.A.W. edited the manuscript. All authors read and approved the final version of the manuscript.

Author Information The microarray data reported in this paper have been deposited to NCBI GEO with the accession numbers GSE52585 and GSE52691. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.W.-B. (ajw168@case.edu) or S.Y. (syamana@gladstone.ucsf.edu).
Cell culture. Human iPSCs were generated from human dermal fibroblasts (HD3Fs) within six passages after receipt. The study involved use of existing specimens that are publicly available and provided without any identifiers linked to the subjects. All procedures were approved by the UCSF Committee on Human Research and the Ger and Embryonic Stem Cell Research Committee. Human fibroblasts and SNL feeder cells (Health Protection Agency Culture Collection) were cultured in DMEM containing 10% FBS, 1% GlutaMAX, 0.1 mM MEM non-essential amino acids (Invitrogen), 1 mM sodium pyruvate, and penicillin-streptomycin (all from Invitrogen). For passing, fibroblasts were washed once with Dulbecco’s PBS (DPBS, calcium and magnesium free) and incubated with Tryple Express (Invitrogen) at 37 °C. When cells were detached, the Tryple Express was removed, and the cells were washed with culture medium described above. The cells were collected into a 15-mL conical tube and spun down. The contents were transferred to a new dish. The split ratio was routinely 1:3 to 1:20. Established human iPSC lines were cultured in mTeSR1 medium (Stem Cell Technologies) on dishes coated with Matrigel (BD) or SyntheMAX (Corning). For passing, human iPSCs were washed once with DPBS and incubated with Accutase (Millipore) at 37 °C. When colonies at the edge of the dish started dissociating from the bottom, Accutase was removed, and mTeSR1 medium was added. Cells were scraped and replated into new dishes in mTeSR1 with 10 μM ROCK inhibitor (Y-27632: Millipore). The split ratio was routinely 1:3 to 1:20. The next day, the medium was replaced with mTeSR1 without ROCK inhibitor.

Generation of human iPSCs with episomal vectors. Human iPSCs were generated with episomal plasmids as described with some modifications. Three micrograms of expression plasmid mixtures (eCy4) were electroporated into 3 × 10^5 fibroblasts with a Neon Electroporation Device (Invitrogen) with a 100-μl kit, according to the manufacturer’s instructions. Conditions for electroporations were 1,650 V, 10 ms, and three pulses. Cells were detached within 1 week after electroporation and seeded at 1-3 × 10^5 cells per 10-cm dish onto irradiated or mitomycin-C-treated SNL feeder cells. The culture medium was replaced the next day with primate ESC medium (Reprocell) containing basic fibroblast growth factor (FGF). Colonies were counted 25 days after electroporation, and those colonies similar to human ESCs were selected for further cultivation and evaluation. For calculating repro-gramming efficiency, 1 × 10^5 electroporated cells were seeded per well of 6-well plate, and the number of TRA-1-60 positive colonies were counted on day 25. The generation and characterization of WT iPSC clones from BJ and HDF-1323 was previously described. These lines were free of episomal integration, maintained generation and characterization of WT iPSC clones from BJ and HDF-1323 was previously described. These lines were free of episomal integration, maintained

In vitro differentiation. For embryoid-body formation from human iPSCs, the cells were collected by treating with Accutase. The clumps of the cells were transferred to ultra-low-attachment dishes (Corning) in DMEM/F12 containing 20% knockout serum replacement (KSR, Invitrogen), 2 mM L-glutamine, 100 mM non-essential amino acids, 100 mM 2-mercaptoethanol (Invitrogen), and penicillin-streptomycin. The medium was changed every other day. After 8 days as a floating culture, embryoid bodies were transferred to gelatin-coated plate and cultured in the same medium for another 8 days.

Teratoma formation. Human iPSCs cells were dissociated by Accutase treatment. Cell pellets were resuspended in mTeSR1 complete medium with 10 μM ROCK inhibitor, Y-27632. One confluent well of cells from a 6-well plate was used for each injection into the tests of 8–10-week-old CB17 SCID male mice (Charles River). Tumor samples were collected 6.5–11 weeks after injection, fixed with PBS containing 4% paraformaldehyde, and processed for paraffin-embedded sections by the Gladstone Histology and Microscopy Core. All mouse studies were approved by the UCSF Institutional Animal Care and Use Committee.

Immunocytochemistry. For counting the TRA-1-60-positive colonies in hiPSC generation experiments, cells were fixed with PBS containing 4% paraformaldehyde and processed for paraffin-embedded sections by the Gladstone Histology and Microscopy Core. All mouse studies were approved by the UCSF Institutional Animal Care and Use Committee.

Bioinformatic analysis. A total of 11,000 MBPs were identified, which were further filtered based on the following criteria: (i) the Pearson’s correlation coefficient was greater than 0.8, (ii) the difference in expression levels between replicates was less than 2-fold, and (iii) the false discovery rate (FDR) was less than 0.05. The resulting MBPs were used as input for the Bioconductor package edgeR to perform differential gene expression analysis. The normalized expression values were used to identify differentially expressed genes (DEGs) between the two conditions. The false discovery rate (FDR) was set to 0.05 to control the rate of false positives. The resulting DEGs were then annotated using the DAVID (Database for Annotation, Visualization, and Integrated Discovery) tool to identify enriched pathways and gene sets. The DEGs were further analyzed using the ClueGO (Clustering of Functional Genes) and ClueSet (Clustering of Sets) plugins in Cytoscape to visualize the biological pathways and gene sets associated with the DEGs. The resulting network was used to identify potential regulatory networks and pathways involved in the regulation of drug resistance.

Quantitative PCR. Genomic DNA was isolated with QiAmp DNA micro kit (Qiagen). To isolate RNA, cells were lysed with TRIzol reagent. Following chloroform extraction, aqueous phase containing RNA was mixed with 70% ethanol, transferred onto a mini spin column, isolated with RNasey mini kit (Qiagen), and digested with DNase I (Qiagen). The quality and concentration of DNA and RNA were assessed using a Nanodrop 1000 spectrophotometer (Thermo Scientific). cDNA was prepared using the iScript cDNA synthesis kit (Biorad). qPCR was performed on genomic DNA isolated from iPS cells, using published primers. To measure endogenous genomic DNA levels by qPCR, the following primers sets were used: PAFAH1BI (set 1, forward 5'-ACCCCTCAGGGGTTCATT-3', reverse 5'-CATTGTGAGTAGCTCATT-3'); set 2, forward 5'-TTCCGACTTACCATTCTT-3', reverse 5'-GTGGTGCGCTGATTGAGGGA-3'); set 3, forward 5'-TGGTTACCTGGAATACCATGTC-3', reverse 5'-GAGGCCCCAGTCAAGGCTAC-3'), and B2M (forward 5'-TGGTGCCTCCTCTGAGAAA-3', reverse 5'-CTCACCTTTCACCAACCT-3'). For qPCR from cDNA, the following primers were used: PAFAH1BI (forward 5'-TGGTTACCTGGAATACCATGTC-3', reverse 5'-GAGGCCCCAGTCAAGGCTAC-3'). qPCR was done with ECL detection kits (Pierce) according to manufacturer’s instructions. Data were analyzed using the Pfaffl method, taking into account primer efficiency. The geometric mean of the primer sets was used to calculate the relative gene expression levels.

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in R. After quantile normalization and COPPER, we sum up the intensities on each axis, which represents a total copy-number estimate at each locus for each individual.

After the first iteration of this procedure, genome-wide variability in each individual’s copy number graph is manually assessed and individuals with excess variability are excluded. Typical individuals have variance of approximately 1, so individuals with variance across entire chromosomes >1 are excluded, leaving 87 samples in this case for the second run of quantile normalization and COPPER to perform CNV calling. To take into account the non-independence of consecutive SNPs, we utilize a 5-SNP median, as the copy number of a locus is estimated more accurately by including information from SNP probes on each side. We then apply a hidden Markov model to make the CNV calls, similar to QuantiSNP37, implemented in Python.

SNP genotyping to determine CNV presence and UPD status for r(13) samples was performed using the Agilent SurePrint G3 Human CGH + SNP 4 × 180K array platform by Cell Line Genetics, using standard protocols. Labelling reactions were prepared using the Agilent Enzymatic Labelling Protocol for aCGH (version 7.1) with 1 μg total DNA input. The protocol consists of two parts: labelling of the DNA and hybridization. First, 1 μg of DNA is fragmented with restriction enzymes and the test DNA labelled with the cyanine-5-labelled analogue of deoxyuridine triphosphate (5-dUTP) and reference DNA with cyanine 3-dUTP by ExoKlenow fragment. Labelled DNA is then purified using Millipore Amicon Ultracel-30 filters and the labelling efficiency determined by the NanoDrop spectrophotometer. Next, the labelled DNA is prepared for hybridization with human Cot1 DNA and placed on the Sureprint G3 human CGH + SNP 4 × 180k microarray and hybridized at 65 °C for approximately 24 h. Finally, the arrays are washed and scanned at 3 μM resolution on an Agilent G2565CA High Resolution Scanner. Data were processed through Agilent’s Feature Extraction software version 11.0.1.1 using the protocol CGH_1100_Jul11 and the 029830_D_F_20100916 grid file. Analyses of CNV estimation and visualization were performed using Agilent genomic Workbench 7.0 software.

**Karyotype and FISH analysis.** Non-banded chromosome number and ring status analyses were performed as described previously36. DNA was stained with DAPI in ProLong gold antifade reagent (Invitrogen) and mitotic chromosomes were visualized using a ×100 objective on a Leica TCS SP5 confocal microscope. Additional chromosomal analyses were performed on twenty G-banded metaphase cells from each sample at Cell Line Genetics or the UCSF cytogenetics laboratory. FISH analyses were performed on two hundred interphase nuclei from each sample at Cell Line Genetics as described previously36. A green probe to detect the pericentromeric long-arm band 13q12.12 together with a red probe to detect the subtelomeric band 13q34 were used for chromosome 13. A green probe to detect the subtelomeric long-arm band 17q21.32 together with a red probe to detect the subtelomeric short-arm band 17p13.3 were used for chromosome 17.

**DNA fingerprinting.** The short tandem repeat (STR) profile assays were performed at Cell Line Genetics. They used the Powerplex 16 (Promega) kit and followed the manufacturer’s recommendation to obtain the results. These samples were run in duplicate and blinded to the interpreter. All the established iPSC lines in this study were confirmed to be identical with their original fibroblasts by DNA fingerprinting (Supplementary Table 1).

31. Matsumoto, Y. et al. Induced pluripotent stem cells from patients with human fibrodysplasia ossificans progressiva show increased mineralization and cartilage formation. Orphanet J. Rare Dis. 8, 190–204 (2013).
32. Pfaffl, M. W. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, e45 (2001).
33. Hoffmann, T. J. et al. Next generation genome-wide association tool: design and coverage of a high-throughput European-optimized SNP array. Genomics 98, 79–89 (2011).
34. R: A language and environment for statistical computing. http://www.R-project.org. (R Foundation for Statistical Computing, 2008).
35. Bolstad, B. M. et al. A comparison of Normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 19, 185–193 (2003).
36. Weiss, L. A. et al. Association between microdeletion and microduplication at 16p11.2 and autism. N. Engl. J. Med. 358, 667–675 (2008).
37. Colella, S. et al. QuantiSNP: an objective Bayes hidden-Markov model to detect and accurately map copy number variation using SNP genotyping data. Nucleic Acids Res. 35, 2013–2025 (2007).
38. Meisner, L. F. & Johnson, J. A. Protocols for cytogenetic studies of human embryonic stem cells. Methods 45, 133–141 (2008).

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Extended Data Figure 1 | Karyotypes of MDS cells used in this study.

a, b, Representative karyotypes of MDS1r(17) fibroblasts (a) and two independent iPSC clones from each of the three MDS patients (b). Magnifications of chromosome 17 pairs (dotted squares) are shown in Fig. 2a for MDS1r(17) cells (boxes 1–3) and to the right of panel (a) for MDS2 and MDS3 iPSCs (boxes 4–7). c, Summary of karyotype data from G-banding analyses of MDS fibroblasts and iPSC clones 1 and 2 (n = 20 each).
Extended Data Figure 2 | Expression of pluripotency markers in iPSCs. a–j, Immunocytochemistry of MDS (a–d) and r(13)-derived (e–j) iPSCs with the antibodies indicated.
Extended Data Figure 3 | No evidence of episomal factor integration in iPSCs. qPCR from genomic DNA using primers specific for exogenous KLF4, SOX2, OCT4, L-MYC and LIN28, as described previously17. a, b, Relative levels of episomal factors in MDS fibroblasts on day 15 after electroporation with epiY4 mixture17 (positive control), WT and MDS iPSCs (passages 20–25), as well as WT human embryonic stem cell line HSF-1 (negative control) (n = 1). c, d, Episomal factor copy number per cell in r(13) fibroblasts electroporated with epiY4 mixture (positive control) and iPSCs (n = 1).
Extended Data Figure 4 | Embryoid-body differentiation from MDS iPSCs.

a–c, Immunocytochemistry showing expression of endoderm-derived cells positive for AFP (a), mesoderm-derived cells positive for SMA (b), and ectoderm-derived cells positive for TUJ1 (c) generated in vitro using the embryoid-body method with MDS1r(17), MDS2 and MDS3 iPSCs.
Extended Data Figure 5 | Teratoma formation from MDS iPSCs.
a–c, Histological sections from 6.5–11-week teratomas developed in the testis of SCID mice following injection with MDS1r(17), MDS2 and MDS3 iPSCs. Haematoxylin and eosin (H&E) staining reveals characteristic tissues from the mesoderm (a), endoderm (b) and ectoderm (c).
d, List of MDS iPSC lines that were injected and teratoma efficiency.
Extended Data Figure 6 | Cell morphology and chromosome distribution of additional MDS1r(17) iPSC clones. a–c, Cell morphology of well growing MDS1r(17) clones 3 and 6 at passage 5 on Matrigel (a), compared to the morphology of poorly growing clones 4 and 5 at passage 4 on SNL feeders (b, approximate colony borders indicated in white) and passage 5 on Matrigel (c). d, examples of metaphase spreads observed in MDS1r(17) clones. e, Quantification of cell populations in MDS1r(17) clones 3–6 with various chromosome compositions shown in (d).
Extended Data Figure 7 | Karyotypes of r(13) cells and morphology of corrected iPSC clones. a–c, Representative karyotypes of GM00285 fibroblasts (a) and corrected GM00285 iPSC clones 1 and 3 (b, c). d, e, Representative karyotypes of GM05563 fibroblasts (d) and corrected GM05563 iPSC clone 1 (e). f–h, Representative morphologies of corrected iPSC clones derived from r(13) fibroblasts cultured in feeder-free conditions.
Extended Data Figure 8 | Rescue of r(13)-associated deletions in corrected iPSC clones through compensatory uniparental disomy. a–e. Total copy number of SNPs across chromosome 13 in r(13) GM00285 fibroblasts (a), GM05563 fibroblasts (b), karyotypically normal GM00285 iPSC clones 1 (c) and clone 3 (d), and GM05563 iPSC clone 1 (e). The areas shaded in pink represent the deletions. f, g. Frequency of heterozygous (blue) or homozygous (red) SNPs in r(13) fibroblasts and karyotypically normal iPSC clones for chromosome 13 (f) and chromosome 12 (g). The corrected iPSC clones are completely homozygous for chromosome 13, supporting the compensatory UPD mechanism.
Extended Data Figure 9 | Embryoid-body differentiation from ring(13)-derived iPSCs. a–c, immunocytochemistry showing expression of endoderm-derived cells positive for AFP (a), mesoderm-derived cells positive for SMA (b) and ectoderm-derived cells positive for MAP2 (c) generated \textit{in vitro} using the embryoid-body method with corrected iPSC clones GM00285-1, GM00285-3 and GM05563-1.
Extended Data Table 1 | Summary of DNA fingerprinting for cells used in this study

| Locus       | MDS1r(17) FIB (p5) | GM00285 FIB (p6) | GM05563 FIB (p6) |
|-------------|--------------------|-----------------|-----------------|
| Amelogenin  | X                  | X               | X               |
| vWA         | 17                 | 17              | 15              |
|             | 18                 | 18              | 16              |
| D8S1179     | 11                 | 13              | 11              |
|             | 13                 | 15              | 14              |
| TPOX        | 9                  | 12              | 6               |
|             | 8                  | 8               | 8               |
| FGA         | 22                 | 23              | 23              |
|             | 22                 | 24              | 25              |
| D3S1358     | 15                 | 16              | 15              |
|             | 16                 | 17              | 17              |
| THO1        | 6                  | 7               | 9.3             |
|             | 7                  | 9.3             |                 |
| D21S11      | 28                 | 30.2            | 30              |
|             | 30                 | 32.2            | 31.2            |
| D18S51      | 12                 | 15              | 14              |
|             | 16                 | 19              | 16              |
| Penta E     | 7                  | 9               | 7               |
|             | 8                  | 13              |                 |
| D5S818      | 11                 | 10              | 11              |
|             | 11                 | 12              | 12              |
| D13S317     | 8                  | 11              | 10              |
|             | 9                  | 11              |                 |
| D7S820      | 9                  | 9               | 11              |
|             | 11                 | 10              |                 |
| D16S539     | 9                  | 11              | 9               |
|             | 11                 | 12              |                 |
| CSF1PO      | 11                 | 12              | 8               |
|             | 12                 | 10              |                 |
| Penta D     | 10                 | 12              | 9               |
|             | 12                 | 12              |                 |

| Confirmed Samples | MDS1r(17) iPSC-1 (p16) | GM00285 Fib (p15) | GM05563 Fib (p15) |
|-------------------|------------------------|------------------|------------------|
|                   | MDS1r(17) iPSC-2 (p16) | GM00285 iPSC-1 (p9) | GM05563 iPSC-1 (p8) |
|                   | GM00285 iPSC-2 (p9)  | GM00285 iPSC-3 (p9) | GM05563 iPSC-2 (p10) |
|                   | GM00285 iPSC-3 (p9)  | GM00285 iPSC-4 (p6) | GM05563 iPSC-3 (p6) |
|                   | GM00285 iPSC-4 (p12) | GM00285 iPSC-5 (p6) | GM05563 iPSC-5 (p12) |
|                   | GM00285 iPSC-5 (p6)  | GM00285 iPSC-5 (p12) |                 |

The origin of all the iPSC lines derived from fibroblasts with ring chromosomes was confirmed to be from the expected fibroblast source. All the patterns in these samples did not match the cell lines published in the American Type Culture Collection (ATCC), National Institutes of Health (NIH) or Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) websites.