Relative Frequencies of Recurrent Acquired Karyotypic Abnormalities in hPSC from 2009 to 2016

Erik McIntire[1], Seth Taapken[1], Kimberly Leonhard[1], Benjamin Nisler[1], Anna Lisa Larson[1], Gustavo Velazquez[1] and Karen Dyer Montgomery[1,2]
[1]WiCell, Madison, WI, USA, [2]Stem Cell and Regenerative Medicine Center, University of Wisconsin, Madison, WI, USA

Introduction

Over nearly eight years, from January 2009 to November 2016, the WiCell Cytogenetics Lab performed routine g-banded karyotyping on approximately 7300 human pluripotent stem cell (hPSC) cultures. hPSC are known to acquire karyotypic abnormalities in culture, seven categories of which are recurrent. Specifically, gains of whole chromosomes X and 8, gains of the long (q) arm of chromosomes 1, 17, and 20, gain of the short (p) arm of chromosome 12, and loss of the long (q) arm of chromosome 18 occur[1]. Examples of these abnormalities are shown in Figure 1, with known minimal critical regions indicated. We inventoried the total number of recurrent acquired karyotypic abnormalities that occurred at our testing facility by year, and calculated the percentage of specific abnormality types. This data was collected from routine testing rather than through controlled experimentation. Ascertainment bias is likely present, but given both the quantity of samples and duration of data collection, the results are an indicator of the changing frequencies of specific recurrent abnormalities over time.

Materials and Methods

G-banded karyotyping was performed using standard cytogenetic protocols modified for use with hPSC. Metaphase preparations were digitally captured with Applied Spectral Imaging software and hardware. Results were reported in accordance with guidelines established by the International System for Cytogenetic Nomenclature 2016[2].

Results

We found a total of 1327 instances of recurrent chromosome abnormalities in 1189 of the 7300 cultures tested. Some cultures contained more than one recurrent abnormality type. Figure 2 shows annual relative frequencies for each of the seven types of recurrent karyotypic abnormalities over the eight year time period. Three striking trends emerge: 1) an increasing frequency of chromosome 1 long (q) arm gains, 2) an increasing frequency of chromosome 20q gains*, and 3) a decreasing frequency of chromosome 12 short (p) arm gains. Increases in chromosome 20q aberrations is the most striking; gains in 20q occurred in 6% of all recurrent abnormalities in 2009 but rapidly increased to a peak of 49% in 2014. No gains of chromosome 1q were observed in 2009, but by 2016, they comprised a total of 27% of all recurrent abnormalities that we found. The proportion of 12p gains steadily declined from 60% to 21% over the same period. It is notable that 1q, 12p, and 20q frequencies are the most dynamic of all recurrent abnormalities and appear to be inversely correlated, suggesting that these aberrations share a niche. Comparable trends are found between human embryonic (ESC) and induced pluripotent (iPSC) stem cell cultures (Figure 3). Mapping these frequency shifts gives critical information for designing targeted FISH and PCR screening evaluations of hPSC cultures, leading us to update our assays, especially for chromosomes 1q and 20q. This data raises the question of cause(s). As the methods for the derivation and culture of hPSC have changed over the time period documented here, this fluctuation in specific abnormal karyotypes prompts further research into factors that may affect genomic status.

* Subtle duplications of chromosome 20q11.21 (Figure 1D) are often detectable by karyotyping, but may require high-resolution testing to confirm and are therefore not included in this dataset.

References

1. International Stem Cell Initiative. "Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage." Nature biotechnology 29.12 (2011): 1132-1144.
2. McGowan-Jordan, J., Simons, A., and Schmid, M., eds. “ISCN 2016: An International System for Human Cytogenomic Nomenclature.” Reprint of: Cytogenetic and Genome Research 2016, Vol. 149, No. 1-2. Karger Medical and Scientific Publishers, 2016.
3. McIntire, Erik et al. “Recurrent Duplications of the Long Arm of Chromosome 1 in Human Pluripotent Stem Cell Lines.” Poster presented at: International Society for Stem Cell Research Annual Meeting; June 2016; San Francisco, California.

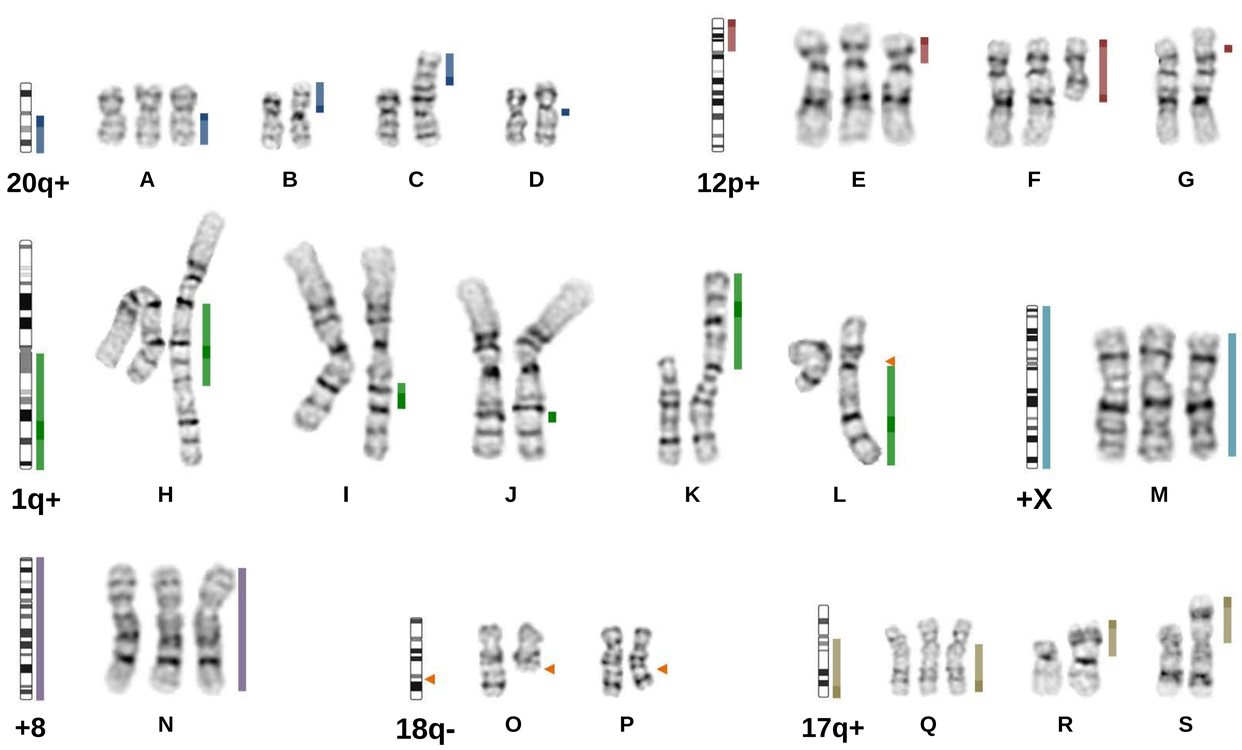

Figure 1. Recurrent acquired karyotypic abnormalities and their minimal critical regions (if known). Each of the seven different abnormality categories is marked with a distinct color that matches the color designations used in data plots (Figures 2, 3). Minimal critical regions are indicated by a darker shade for chromosomal regions 1q32[3], 12p13.31, 17q25, 18q21.2, and 20q11.21[1]. Examples of abnormalities are reported using ISCN: **(A)** +20 **(B)** i(20)(q10) **(C)** idic(20)(p12) **(D)** dup(20)(q11.2q11.2) **(E)** +12 **(F)** +i(12)(p10) **(G)** dup(12)(p13p13) **(H)** dup(1)(q21q44) **(I)** dup(1)(q25q32) **(J)** dup(1)(q32q32) **(K)** der(14)t(1;14)(q12;p11.2) **(L)** der(18)t(1;18)(q12;q21.1) **(M)** +X **(N)** +8 **(O)** del(18)(q21.1) **(P)** del(18)(q21.1q21.3) **(Q)** +17 **(R)** der(22)t(17;22)(q21.3;p11.2) **(S)** i(17)(q10)

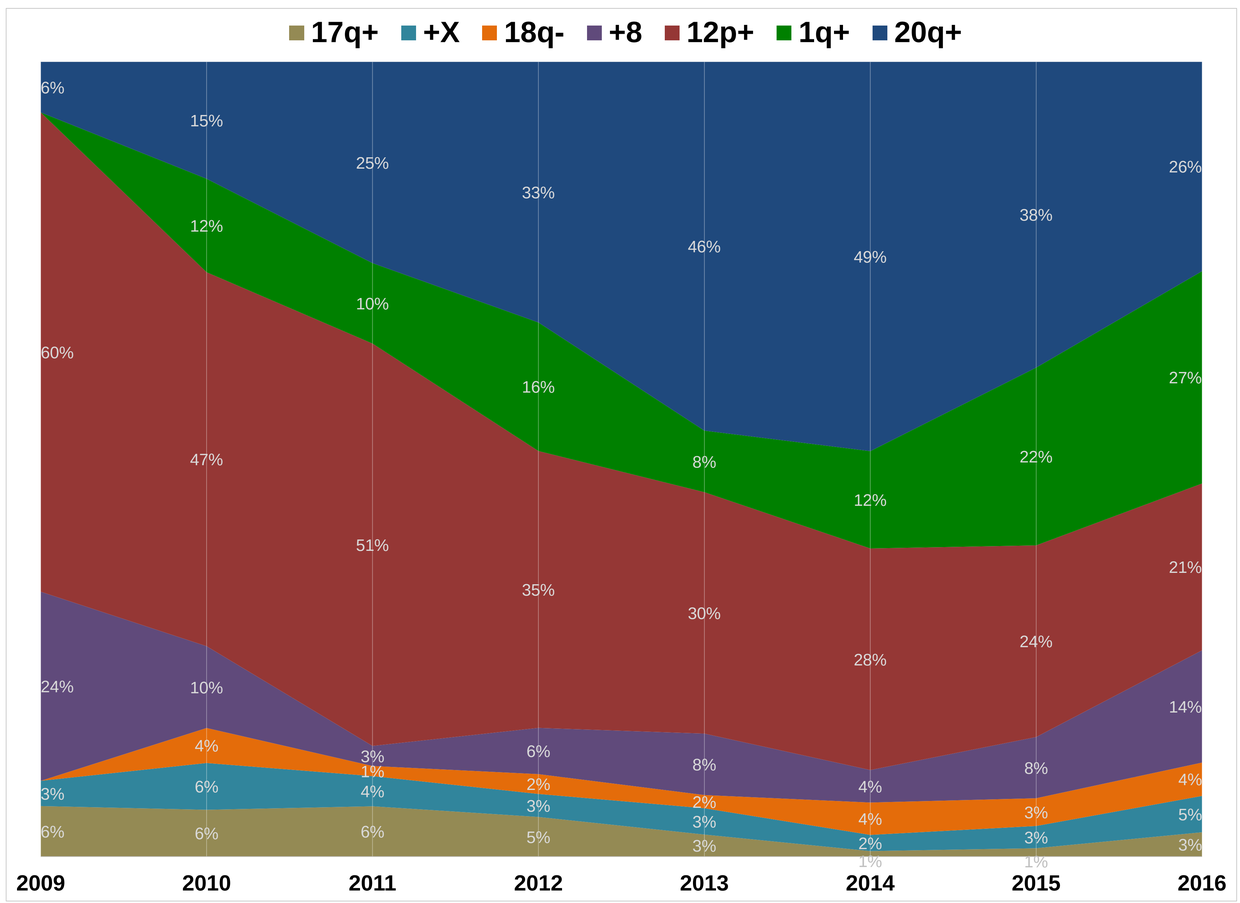

Figure 2. Relative frequencies of recurrent acquired karyotypic abnormalities detected in hPSC cultures from January 2009 to November 2016. We detected a total of 1327 instances of a recurrent abnormality occurring in 1189 specimens (some cultures contained more than one recurrent abnormality type). There are three striking trends: 1) an increasing frequency of chromosome 1 long (q) arm gains, 2) an increasing frequency of chromosome 20q gains, and 3) a decreasing frequency of chromosome 12 short (p) arm gains. These trends may reflect the adoption rates of new derivation and culture technologies; further research is needed to correlate such factors to genomic status.

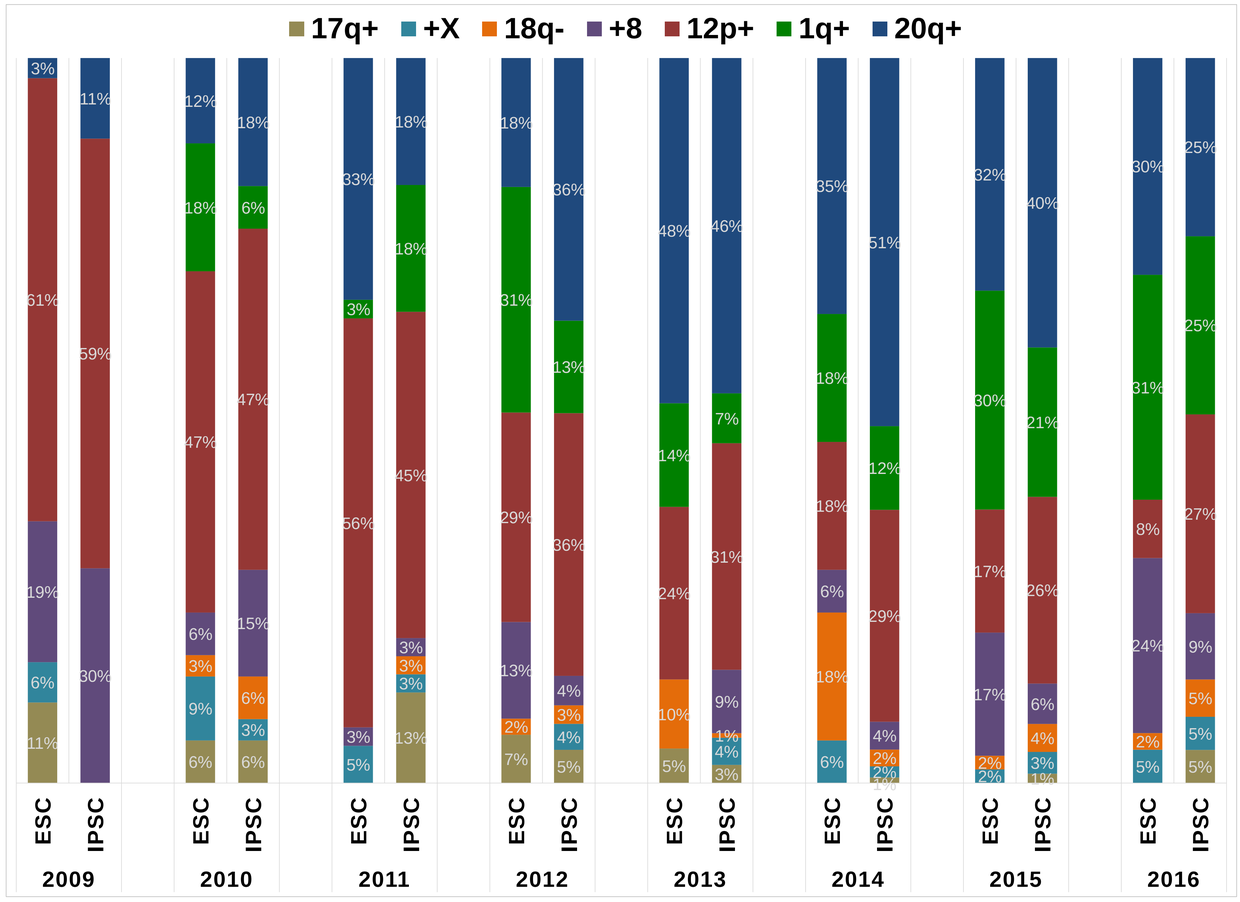

Figure 3. Relative frequencies of recurrent acquired karyotypic abnormalities detected in human ESC and iPSC cultures from January 2009 to November 2016. We detected a total of 1327 instances of a recurrent abnormality (332 from ESC and 995 from iPSC) occurring in 1189 specimens (280 ESC cultures and 909 iPSC cultures). Trends are comparable for both cell types.