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Data from Artificial Models of Mitochondrial DNA Disorders Are Not Always Applicable to Humans

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Mitochondrial DNA (mtDNA) mutations, a major cause of maternally inherited human diseases, are commonly characterized by the coexistence of mutant and wild-type mtDNA molecules within a cell (called "heteroplasmy" or "mutation load"). Usually, the higher the mutation load, the more severe the disease. Because of the high risk of recurrence in siblings and the absence of treatment options, couples at risk of transmitting mtDNA mutations often ask for prenatal diagnosis (PND) or preimplantation genetic diagnosis (PGD). These procedures are based on assessment of the mutation load (percentage of mutant mtDNA / total mtDNA) in either tissues (PND) or cells (blastomeres in a day 3 embryo). The predictive value of these procedures for postnatal outcome assumes that the mutation load is relatively stable among blastomeres at the 8-cell-stage embryo or in tissues throughout the entire embryofetal development.

By measuring mtDNA heteroplasmy levels in single blastomeres sampled from 8-cell-stage macaque embryos, Lee et al. reported a significant difference in heteroplasmy levels among cells from a given embryo, questioning the reliability of the PGD procedure in clinical practice (Lee et al., 2012). In their experiments, they removed half the cytoplasm of an oocyte derived from a female rhesus macaque and fused it with another halved oocyte containing the spindle-chromosomal complex recovered from a second female, thus generating artificially heteroplasmic embryos containing 50% of each mtDNA species. They observed a large variation of mtDNA mutation loads among blastomeres (mean of the standard deviations [SDs] of 15%, ranging from 3% to 28%). This feature increased dramatically during the first cleavages, and it was still present at the blastocyst stage. On the basis of these observations, Lee et al. concluded that heteroplasmy in one biopsied blastomere from cleaving embryos may not be predictive of total mutation load in the remaining blastomeres and the whole embryo. They therefore suggested that chorionic villus sampling could be more reliable than blastomere assessment for the prediction of embryonic mutation load.

Our experience of PGD for mtDNA mutations in humans does not support conclusions from these macaque experiments. Indeed, all studies on human heteroplasmic embryos hitherto conceived by in vitro fertilization have shown quite good stability of the mutation load among blastomeres, irrespective of the variant type: a mtDNA polymorphism (HV2 tract, Steffann et al., 2006) or mtDNA mutations (m.3243A>G, m.8993T>G, m.8344A>G, m.9185T>C, m.10197G>A, Steffann et al., 2006, Monnot et al., 2011, Treff et al., 2012, Vandewoestyne et al., 2012, Sallevelt et al., 2013). Figure S1C summarizes all published data and unreported personal observations regarding single-blastomere analysis on a total of 105 human embryos carrying various mtDNA mutations. At variance with the SD observed by Lee et al. (15%), the mean SD of the mutation loads was very low in our series (less than 2%), which can be ascribed to reproducibility of the technique (Steffann et al., 2006). In the less favorable cases, the SD was consistently below 15%, far less than the SD figure reported by Lee et al. (28%). For comparison between data from the macaque experiment and human clinical data, we plotted the rate of mutation in one randomly chosen blastomere (using a random number generator) against that in remaining cells of the same embryo (Figure S1). In rhesus macaque embryos, the test blastomere heteroplasmy was poorly predictive of the heteroplasmy observed in the remaining seven blastomeres ($R^2 = 0.066$, Figure S1A). Conversely, in human embryos, the test blastomere heteroplasmy was highly predictive of the mutation load in the remaining blastomeres ($R^2 = 0.98$, Figure S1B). The linear regression was more significant in human than in rhesus macaque embryos ($p < 0.0001$ and $p = 0.0013$, respectively).

Why results differ so markedly across species is an interesting question, especially given that mouse heteroplasmic embryos were reported to display very little variations in heteroplasmy levels among blastomeres (mean 2%, ranging from 0 to 6%, Dean et al., 2003). The difference may be due to the experimental procedure used to generate the heteroplasmic macaque model, which is likely to result in an mtDNA segregation pattern

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different from that occurring in nonmanipulated embryos. Because such embryos are formed by the merging of two distinct cytoplasts, mitochondria from donors and acceptors may be clustered in different cell areas, leading to a nonrandom distribution of mtDNA populations between the two daughter cells. Such a hypothesis is supported by the results of another study, carried out in the mouse, using a microsurgery approach similar to that of Lee et al. in the monkey (Meirelles and Smith, 1998). The variation of heteroplasmy among blastomeres was 10-fold higher in reconstructed embryos than in zygotes derived from heteroplasmic lineage (Meirelles and Smith, 1998). In their cases, the supposed "rapid mitochondrial DNA segregation" was ascribed to a partitioning of mtDNA genotypes occurring in the process of fusing the two egg cells, a feature that probably does not occur in naturally heteroplasmic oocytes.

These observations emphasize the risk of mis-segregation of mtDNA molecules in "artificially generated embryos." Lee's artificial model of mtDNA disorders might thus be more relevant to preventing the transmission of mtDNA disorders by nuclear transfer than by embryonic selection using PGD. However, it has to be emphasized that small amounts of mutant mtDNA are usually cotransferred with the karyoplast during nuclear transfer from affected to donor oocyte (Tachibana et al., 2009, Craven et al., 2010). Even if rapid segregation of mutant mtDNA occurs, the risk induced by the resulting heteroplasmy can probably be regarded as negligible. Technical improvements have indeed decreased the carryover heteroplasmy to levels below 1%, far less than the threshold of mtDNA disease.

Taken together, our data, as well as those from other groups, advocate a homogeneous distribution of wild-type and mutant mtDNAs in individual human blastomeres at the 8-cell stage, regardless of the mutation. They support the reliability of PGD procedures based on mutation load assessment on two different blastomeres sampled at day 3 (Monnot et al., 2011, Sallevelt et al., 2013, Vandewoestyne et al., 2012). Moreover, stability of the mutation loads between the inner cell mass and the trophectoderm has been reported at the blastocyst stage (Treff et al., 2012), at least for the m.3243A>G mutation, supporting the feasibility of PGD on a day 5 trophectoderm biopsy. The four children born to date after a PGD procedure had buccal cell or cord blood mutation loads very similar to the blastomere mutation loads (m.8993T>G: 0% versus 0% in the embryos [Steffann et al., 2006], m.3243A>G: 15% versus 12% [Treff et al., 2012] and 5% versus 5% and 13% [two transferred embryos; Monnot et al., 2011], m.8344A>G: 63% versus 53% and 59% [two transferred embryos; this report]). Although more data are certainly needed in order to document the stability of the mutation loads throughout human in utero development, these observations support the reliability of standard PGD procedures on human embryos at risk of carrying mtDNA mutations.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.05.005.

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