Seeing cell to cell differences for first time explains symptoms of rare genetic disorders

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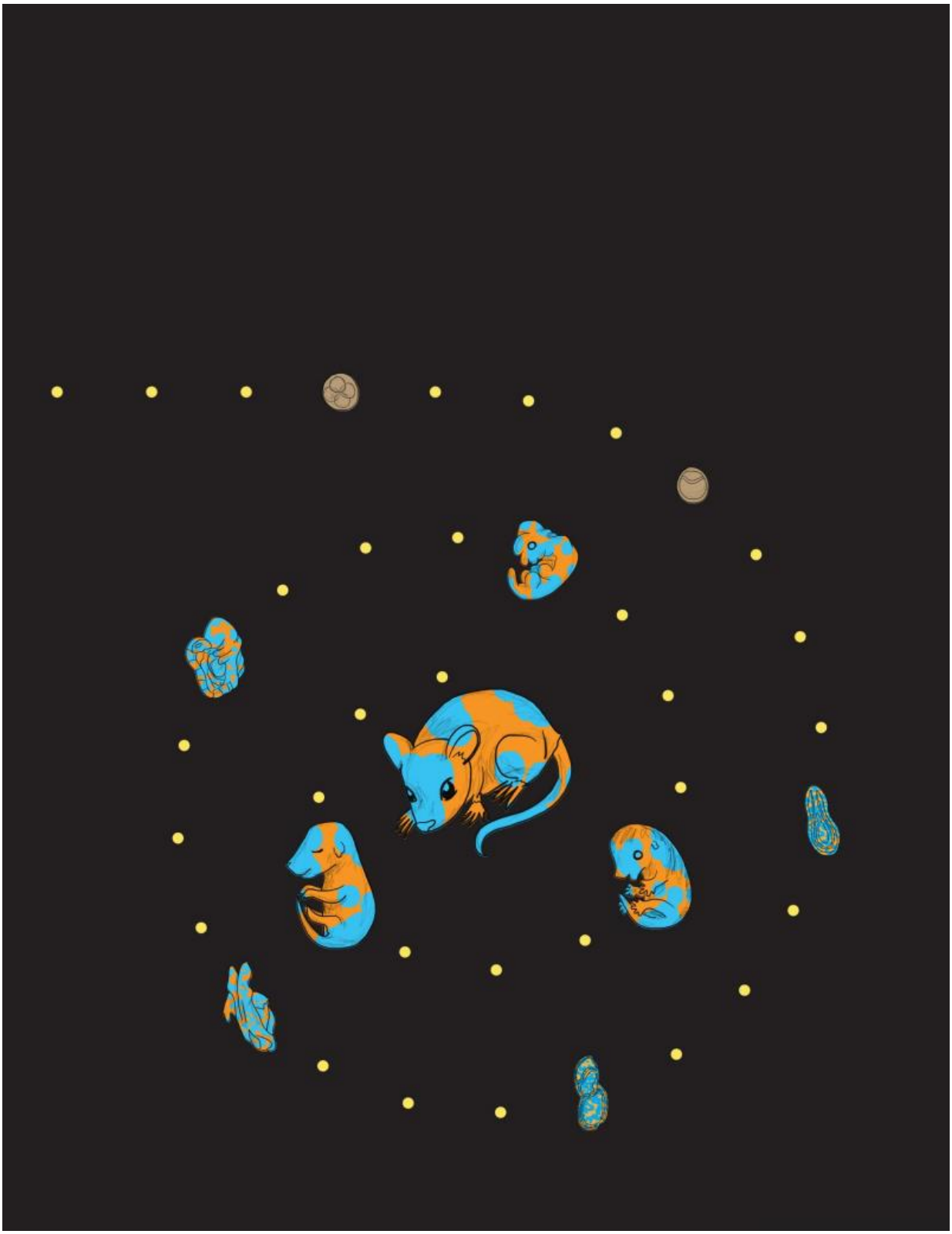

Epigenetic mosaicism occurs during mouse development in a mouse model of

epigenetic disease. Credit: Connie Jiang, M.D./Ph.D., student, Perelman School of Medicine, University of Pennsylvania.

Every cell in the body has two genomes, one from the mother and one from the father. Until now, researchers have lacked the tools to examine—in a single cell —the exact readout from each genome to make RNA. Using a new technology that allows researchers to do just that, an interdisciplinary University of Pennsylvania team examined a rare disease in which these two genomes are expressed differently throughout the body, even sometimes in the same organ. They found that at the single-cell level gene expression was highly variable and quite different than expected, which is now shedding light on the molecular causes of rare diseases and perhaps the complex nature of tumors.

"This is a great example of cross-school collaboration," said co-senior author Marisa Bartolomei, PhD, a professor of Cell and Developmental Biology in the Perelman School of Medicine. Her colleagues are co-first author Jennifer M. Kalish, MD, PhD, an expert in rare growth disorders from The Children's Hospital of Philadelphia, and co-first author Paul Ginart, an MD/PhD candidate, and co-senior author Arjun Raj, PhD, both from the School of Engineering and Applied Sciences at Penn, who devised the technology to image single genes in individual cells. The team published their findings in *Genes & Development*.

"With this new technique, we can now see which cells express which genome," Bartolomei said.

Silence of the Genes

For most genes, people inherit two copies, one maternal and one paternal. However, in the case of a phenomenon called imprinting,

offspring inherit only one working copy of a gene, and depending on its parental origin, expression of the gene is controlled by an added methyl group during egg or sperm formation to physically tighten DNA so it cannot be read to make RNA and ultimately protein.

A small number of genes are imprinted but are critical to normal development. Imprinted genes are normally turned on from either the maternal chromosome or the paternal chromosome, but not both. In human imprinting disorders, imprinted genes are either abnormally turned on for both the maternal and paternal genomes, or abnormally turned off from both genomes. Abnormal expression can be linked to genetic mutations and mistakes in the placement of the methyl group (a function of epigenetics).

Variability from cell to cell in which genes are expressed in the same organ may help explain why many diseases show a "mosaic" pattern, with different parts of the body exhibiting different degrees of disease. This hodge-podge can happen because of errors in imprinting.

Defects in imprinting can lead to inappropriate expression of the normally silenced version of the gene, but it remains unclear whether every cell in a given organ expresses the abnormal version. "If this were the case, it would have profound implications for human imprinting disorders," Kalish said.

Help from the Rare

Imprinting disorders are rare. Kalish works with two: Russell-Silver syndrome, an under growth disorder occurring in approximately 1 out of 50,000 to out of 100,000 births, with symptoms of small stature and limb, body, or facial asymmetry. The syndrome involves under-methylation of two genes, H19 and IGF2. H19 encodes a non-coding RNA that limits growth whereas IGF2 is a growth factor. In 50 percent

of cases, Russell-Silver syndrome is associated with an imprinting error on chromosome 11 in which the person receives two copies of the imprinting mark from the mother rather than one from each parent. Russell-Silver children have too much of the do-not-grow signal (H19) and too little of the grow signal (IGF2). The other imprinting disorder is Beckwith-Wiedemann syndrome, an overgrowth disorder usually present at birth, characterized by an increased risk of childhood cancer and such congenital features as a large tongue, large birth weight, and limb, body, organ, and facial asymmetry.

These syndromes display a range of symptoms, which happens because some cells in a given organ express maternal genes while others express paternal genes. "But the mom genes are the ones that limit growth of cells," Kalish said. "This means that the mosaic pattern is really a mix of normal cells and abnormal cells—with a disrupted balance between mom's do-not-grow signals and dad's grow signals, in the same tissue. This is what accounts for an enlarged or too-small organ in the same person." In some cases, patients may even have differently sized pairs of kidneys.

Using mice, the team compared cell genomes in normal mice versus Russell-Silver syndrome mice. At the cell population level, they asked which individual cells have correct maternal imprinting and which cells have abnormal maternal imprinting for the H19 gene. In Russell-Silver mice both maternal and paternal H19 is expressed.

Using Raj's new technology, they developed a molecular probe to detect a single nucleotide change in the RNA expressed in mom's versus dad's genomes to show which is expressed cell by cell. This method measured gene-variant specific expression in single cells. In the mouse model of Russell-Silver syndrome, they found that some cells had RNA from both the maternal and paternal version (abnormal—as seen in Russell-Silver patients) while other cells had only the maternal RNA (normal).

"We showed that mutant mouse embryo fibroblast cells are comprised of two subpopulations: those expressing both maternal and paternal H19 versions [abnormal] and those expressing only the maternal copy [normal]," Bartolomei said. "Only in the latter normal cell population is Igf2 expression detected."

In the Russell-Silver mice, all cells had the same genetic change but not all cells had the same DNA methylation pattern, explaining the mosaic arrangement of gene expression by epigenetics—the normal silencing of a version of a gene by the added methyl group during egg or sperm formation.

The two mutant subpopulations of cells exhibited distinct methylation patterns at the imprinting control region of their respective genomes. The cells that expressed H19 from just the maternal genome had the correct DNA methylation pattern while the cells that expressed H19 from both maternal and parental genomes exhibited a loss of H19 DNA methylation. Importantly, they also observed the same two subpopulations are also present within mouse heart tissue, showing the same defect in a living whole mouse.

"This is the first time that epigenetic mosaicism has been demonstrated at a single cell level," Bartolomei said. "What this means is that epigenetics—the balance of tightening or loosening of DNA to control which genes are expressed when—is the driver of mosaicism at the cell population level. Our results establish that imprinting disorders can display striking single-cell heterogeneity and suggest that such heterogeneity may underlie epigenetic mosaicism in human imprinting disorders," Bartolomei said.

"The epigenetic mosaicism shown in this work explains the spectrum of clinical features we see in our patients—it all makes sense," Kalish said. "Now we know what is going on."