Although cell lineage information is fundamental to understanding organismal development, very little direct information is available for humans. We performed high-depth (250×) whole-genome sequencing of multiple tissues from three individuals to identify somatic single-nucleotide variants (sSNVs). Using these variants as “endogenous barcodes” in single cells, we reconstructed early embryonic cell divisions. Targeted sequencing of clonal sSNVs in different organs (about 25,000×) and in more than 1000 cortical single cells, as well as single-nucleus RNA sequencing and single-nucleus assay for transposase-accessible chromatin sequencing of ~100,000 cortical single cells, demonstrated asymmetric contributions of early progenitors to extraembryonic tissues, distinct germ layers, and organs. Our data suggest onset of gastrulation at an early progenitor stage of about 170 cells and about 50 to 100 founders for the forebrain. Thus, mosaic mutations provide a permanent record of human embryonic development at very high resolution.

Although recent strategies involving DNA editing have used molecular barcodes as clonal markers to map the developmental processes of proliferation, migration, and tissue formation (1), such methods are not applicable to understanding human development. Single-cell RNA-sequencing (RNA-seq) methods have been used to analyze transcriptional changes and cell differentiation during human development (2), but they are inadequate for lineage tracing, leaving global lineage patterns in humans still largely unexplored. Here, to examine developmental ancestries and clonal composition across the body, we characterized somatic single-nucleotide variants (sSNVs), which are suitable as lineage markers because they accumulate with each cell division (3) and most predictions are made to be functionally silent (4, 5).

High-depth whole-genome sequencing (WGS; >250× per sample) was performed for five bulk DNA samples from a 17-year-old male (ID: UMB1465) who died with no medical diagnosis (prefrontal cortex (PFC) section 2 gray matter (GM) and white matter (WM), heart, spleen, and liver (1250× total); Fig. 1A and table S1). Similarly, >250× WGS was also performed for PFC and two visual cortex samples (Brodmann area (BA)17 and BA18) from two additional individuals who also died with no medical diagnosis, a 15-year-old female (ID: UMB4638) and a 42-year-old female (ID: UMB4643). Applying MosaicForecast, a machine-learning algorithm (4), to bulk data and integrating with previously published single-cell WGS (6, 7), we identified 516 total sSNVs (8) (table S2). Among the 297 sSNVs detected in UMB1465, 65 (22%) were found across all tissues and 181 (61%) in at least two (Fig. 1B and table S2). All 65 widely shared sSNVs showed alternate allele frequency (AAF) >1%, with 38 (58%) showing >3% (Fig. 1B and table S2). Sensitivity estimates suggest that our approach achieved nearly 100% sensitivity for detecting sSNVs of 3 to 30% AAF (8) (Fig. 1C and fig. S1, A to C). Most sSNVs were predicted to be functionally neutral (only two of 297 sSNVs in UMB1465 were exonic; table S3) and thus represent unbiased lineage markers.

Clonal sSNVs in all organs showed similar base substitution patterns, with 55% being C>T substitutions (Fig. 1D and fig. S1, D and E). The trinucleotide context resembled that of sSNVs seen in proliferating tissues and cancer, e.g., clock-like Signature 1 in the COSMIC catalog (9), which likely reflects faulty repair of cytosine deamination in cycling cells (5, 7). Liver-specific variants were more common than heart- or brain-specific variants (n = 57, 33, and 19, respectively), consistent with known patterns of clonal amplification and replacement of hepatic units from resident stem cells (10), whereas spleen-specific variants were the least common (Fig. 1B and table S2). Amplicon-based targeted sequencing (~25,000× on average) of 94 samples from 17 organs (Fig. 1A and supplementary materials).
table S1) reidentified most sSNVs (>93%) when the same biopsy used for WGS was profiled (table S1); it identified slightly fewer when distinct tissue biopsies were profiled (81%), and, overall, 196 of 229 (86%) of targeted variants were validated (Fig. 1E, fig. S1F, and table S4).

Single-cell WGS data of 20 single neurons (6, 7) from UMB1465 resolved 82 of 297 sSNVs into branching clades or clones, producing a lineage tree that spans early postzygotic cell generations and traces the origin of each mutation back to the embryo (Fig. 2A, fig. S2A, and tables S2 and S5). As expected, earlier sSNVs showed higher mosaic fractions (MFs), which are the fractions of cells carrying the variant, defined as 2×bulk AAF for autosomal SNV, with the MFs from daughter clades summing to that of the mother clone. Similar patterns of early lineage were also identified in the two additional individuals based on bulk WGS and single-cell (7, 11) analysis (Fig. 2, B and C; fig. S2, B and C; and table S5). In UMB1465, we identified the first eight postzygotic progenitors corresponding to the third-cell generation (c1 to c8, with c5 to c6 not fully resolved and annotated as a second-generation clone), with the MFs of c1 to c8 summing to ≈100%, suggesting that all major early lineages were captured; we then traced their relative contributions to each organ (Fig. 2D and fig. S2D) (8). Contributions of c1 to c8 were highly unequal across organs, with c4 undetected in heart and spleen, and c3 and c8 together contributed >50% of the cellular content (Fig. 2D).

Changes in MFs across cell generations suggest highly asymmetrical segregation of the earliest progenitors between embryonic and extraembryonic tissues and in the several germ layers within the embryo. Instead of the expected twofold reduction of MFs with cell division, observed MFs for one branch (c8) barely decreased (30, 26, and 24%; P < 10⁻⁶, P < 10⁻²², and P < 10⁻⁵⁶, respectively; two-tailed binomial test); deviations from twofold reduction were also observed in other branches (Fig. 2, A and E, and fig. S2A) and in the two additional individuals (Fig. 2, B and C, and fig. S2, B and C). This pattern suggests unequal clonal partitioning during blastula formation, when extraembryonic tissues separate from embryonic tissue lineages (Fig. 1A). The observed MF asymmetries indicate that lineage segregation in human embryo might happen as early as the two-cell stage, as suggested in the mouse (12–14). To further test this hypothesis, we analyzed published (II) bulk WGS data (250×)...

Fig. 2. Asymmetric contribution of early embryonic clones to the human body. (A to C) Phylogenetic trees of individuals UMB1465 (A), UMB4638 (B), and UMB4643 (C). The cell-generation numbers for later sSNVs (fifth and sixth) are likely to be underestimates because of the limited number of cells used for lineage reconstruction and the reduced power of detecting very-low-MF sSNVs. (D) Third-cell-generation clones (c1 to c8) of UMB1465 showing unequal contributions to specific organs (P < 10⁻¹³, chi-square test), with the fraction of cells in each tissue contributed by clones c1 to c8 normalized by summing to 100% (see fig. S2D for non-normalized values). (E) Observed whole-body MFs for sSNVs from clades c1 to c8 across the two- to four-cell generations strongly deviate from expected values based on a symmetrical model of development. The 95% confidence intervals (95% CIs) calculated with binomial sampling are reported in table S2. (F) First-cell-generation clonal contributions are asymmetric and variable across 55 individuals (P < 10⁻¹³, Kolmogorov-Smirnov test for the null hypothesis of symmetry). Individuals UMB1465, UMB4638, and UMB4643 are marked with diamond symbols. (G) High intra-organ fluctuation of MFs for early-embryonic mosaic variants illustrated for chr11:40316580 C>T. (H) sSNVs restricted to one or two germ layers mark the beginning of gastrulation. A total of 196 validated sSNVs are ordered “chronologically” by their whole-body MFs (8). MFs in different germ layers are compared in four examples (two-tailed Wilcoxon rank sum test; ns, nonsignificant; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001).
from 74 individuals. Our maximum likelihood estimates (8) indicate overall asymmetric contributions of the first-cell-generation clones to the human body with strong interindividual variability, from a 50:50 symmetry in some individuals to a 20:80 asymmetry and potentially higher (Fig. 2F and table S6).

MFs of 196 sSNVs across 94 biopsies from 17 different organs (table S1) from UMB1465 also revealed asymmetric contributions of early lineages to embryonic germ layers during gastrulation (Fig. 1A; fig. S3, A to C; and table S4) (8). The relative contributions of several clades to organs of endoderm, ectoderm, and mesoderm varied up to several fold (fig. S3, B and C). Furthermore, multiple biopsies from the same organ showed noticeable intra-organ MF differences (Fig. 2G and fig. S3D). For example, MFs for sSNV chr11:40316580 (C>T) ranged from 5 to 26% across cerebral cortex samples, suggesting highly variable local clonal amplification in all tissues (Fig. 2G).

The tissue distribution of sSNVs identified the effective progenitor pool size at the onset of gastrulation. sSNVs with higher MFs were found in all organs and germ layers (8) (Fig. 2H, fig. S3E, and tables S4 and S7), but as MFs decreased past ~0.6%, many sSNVs became undetectable in one or two germ layers (Fig. 2H, fig. S3E, and table S7), reflecting lineage divergence during gastrulation. The effective cell number at the time of mutation occurrence can be inferred as ~1/MF; therefore, 0.6% MF corresponds to ~170 epiblast cells. Despite the asymmetries of clonal contributions to various tissues, multiple germ layer–restricted variants gave similar estimates (Fig. 2H), and our in vivo estimates are consistent with counts from cultured human embryos (15).

The earliest brain-specific sSNVs provide similar estimates for the number of brain founder cells. Fourteen sSNVs were present in at least one of 64 central nervous system (CNS) samples but not in 30 non-CNS samples (Fig. 3A and tables S1 and S8), with 10 sSNVs showing significantly higher MFs in the forebrain than in other CNS regions (Fig. 3A and table S8, e.g., P < 0.0001 for variant chr17:53347250 (A-G)). The earliest-occurring sSNVs were confirmed from analysis of 1228 single cortical cells (88% were from PFC section 2, so forebrain MFs were estimated from single cells may be biased (8); table S9), of which 791 were successfully placed in a lineage tree (Fig. 3B, figs. S4 and S5, and table S8). Early (first- to fourth-cell-generation) sSNVs were found in all rostral-caudal sections

Fig. 3. Brain-specific sSNVs estimate the number of forebrain founder cells. (A) MFs of 14 CNS-restricted sSNVs showing significant enrichment of some variants in forebrain-derived samples (two-tailed Wilcoxon rank sum test; significance levels are shown at the top). c8 and c1 (fig. 2A) and noncladed variants are indicated. chr17:53347250 A>G and chr7:17623547 C>T are the earliest brain-specific sSNVs in c8, based on average forebrain MFs (diamond symbols). The forebrain MFs between sSNVs were compared with the estimate of the likelihood that they arose at the same generation (two-tailed Wilcoxon rank sum test). (B) A total of 791 single cells (of 1228) were successfully assigned to lineage clades upon targeted sequencing of 37 sSNVs (8). NEUN+ and NEUN− cells are differentially distributed across clades (two-tailed Fisher’s exact test). (C) chr17:53347250 A>G and chr7:17623547 C>T were confirmed as the earliest lineage markers within c8 by single-cell genotyping (shown are the number of mutant cells over the number of cells with >10× coverage at the position). (D) Same as (C) but for c1. (E) Estimates of forebrain founder cells based on average MFs (25,000× sequencing).
(8) (Fig. 4B and fig. S6, A and B), although their widely varying mosaic fractions highlighted unexpectedly large local nonuniformities in clonal amplification (Fig. 4B and fig. S6, A and B). Later (fifth- and sixth-(or later)cell-generation) sSNVs showed progressive restriction to the frontal cortex (Fig. 4C and fig. S6, A and B) and finally the PFC, where they were discovered. Thus, whereas founder clones of the cortex show little topographic restriction for MFs of ~1% or higher, lower MF clones show evidence of broad differences in distribution from frontal to posterior regions, separated approximately by the Sylvian fissure and the central sulcus (Fig. 4D).

Single-nucleus RNA-seq (snRNA-seq) and single-nucleus assay for transposase-accessible chromatin sequencing (snATAC-seq) data reveal cell-type classification, but the clusters can also be linked to genotypes. Although limited by the per-cell coverage sparsity, snATAC-seq reads were more uniformly distributed across the genome compared with snRNA-seq reads (fig. S7A), suggesting that snATAC-seq may be better suited to detecting sSNVs genome wide (fig. S7). At the 297 sSNV positions, 5.6% of snRNA-seq cells (1933 of 34,325) and 12.8% of snATAC-seq cells (8356 of 65,199) obtained coverage over at least one of the 297 sSNV loci (table S10). To link cell lineage information with cell types, we classified all ~100,000 cells into seven groups (Fig. 4E and fig. S6, A and B), although a few trends of c8 contributions to the frontal cortex (8, 16, 19). Given the growing list of conditions associated with somatic mutations (20, 21), a deeper understanding of the patterns of cell lineage described here coupled with functional information will help to elucidate the origin and consequence of mosaicism in these diseases.

**REFERENCES AND NOTES**

8. Materials and methods are available as supplementary materials.
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SUPPLEMENTARY MATERIALS
science.sciencemag.org/content/371/6535/1249/suppl/DC1
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MDAR Reproducibility Checklist
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Landmarks of human embryonic development inscribed in somatic mutations
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Mutations provide an enduring record
Somatic mutations pepper our cells with change, but because they are not in the germline, they do not propagate to the next generation. Bizzotto et al. leveraged data on the distribution of somatic mutations in adults to take a backward look at the earliest moments of human development. Calculation of cellular lineages on the basis of shared somatic mutations shows the number of cells from which the body will develop when the human embryo gastrulates. The lineage for forebrain cells is identifiable, as are the asymmetrical fates spun out of many of the gastrula cells. Science, this issue p. 1249