Human-specific ARHGAPI11B increases size and folding of primate neocortex in the fetal marmoset

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The neocortex has expanded during mammalian evolution. Overexpression studies in developing mouse and ferret neocortex have implicated the human-specific gene ARHGAPI11B in neocortical expansion, but the relevance for primate evolution has been unclear. Here, we provide functional evidence that ARHGAPI11B causes expansion of the primate neocortex. ARHGAPI11B expressed in fetal neocortex of the common marmoset under control of the gene’s own, human, promoter increased numbers of basal radial glia progenitors in the marmoset outer subventricular zone, increased numbers of upper-layer neurons, enlarged the neocortex, and induced its folding. Thus, the human-specific ARHGAPI11B drives changes in development in the non-human primate marmoset that reflect the changes in evolution that characterize human neocortical development.

Evolutionary expansion of the human neocortex is linked to our cognitive abilities (1–6). The human-specific gene ARHGAPI11B (7, 8) is implicated in this neocortical expansion as it is expressed in the human progenitor cells giving rise to neocortical neurons and, when overexpressed in developing mouse and ferret neocortex, two evolutionarily distant mammals, can induce features associated with neocortical expansion (9, 10). ARHGAPI11B arose ~5 mya by partial duplication of ubiquitous ARHGAPIA, which encodes a Rho-GAP exhibiting nuclear localization (7–9, 11). However, due to a point mutation that presumably occurred after the partial gene duplication event and leads to a human-specific change in protein sequence, ARHGAPI11B lacks Rho-GAP activity in vivo and is localized in mitochondria, promoting proliferation of basal progenitors, the progenitors implicated in neocortical expansion, via glutaminolysis (11, 12). Here we tested ARHGAPI11B’s relevance for neocortical expansion in a non-human primate by expressing ARHGAPI11B under the control of its own human promoter in transgenic fetal marmosets.

To express human-specific ARHGAPI11B (7, 8) (fig. S1A) in the common marmoset, we constructed a lentiviral vector. In this functionally verified vector (fig. S1, B and C), a ~2.7 kb human genomic segment containing the ARHGAPI11B promoter drives expression of an EGFP reporter followed by the complete ARHGAPI11B protein coding sequence. The two proteins become separate polyepitides after translation due to the presence of a T2A self-cleaving sequence (fig. S1B). This expression vector was used to generate pregnant marmosets carrying ARHGAPI11B-transgenic fetuses, by following a previously established protocol (13) that involved microinjection into fertilized marmoset oocytes and transfer of in vitro developed embryos into foster mothers 3-5 days after ovulation (day of transfer being defined as day 0 of pregnancy; fig. S1D and table S1).

We confined our analyses to marmoset fetuses, because we anticipated that expression of this human-specific gene would affect neocortex development in the marmoset. In light of potential unforeseeable consequences with regard to postnatal brain function, we considered it a prerequisite – and mandatory from an ethical point of view – to first determine the effects of ARHGAPI11B expression on the development of fetal marmoset neocortex. To this end, we collected fetuses after Caesarian section at day 101 of the ~150-day gestation (fig. S1D), a stage when neocortical development shows both progenitor cell division and production of neurons (destined mostly to the upper layers) and which corresponds to fetal human neocortical development at ~16 weeks post conception. Of the seven EGFP- plus ARHGAPI11B-transgenic marmoset fetuses obtained (table S1), five expressed both EGFP and ARHGAPI11B in fetal neocortex whereas two expressed neither (Fig. 1). In the five transgenic fetuses exhibiting EGFP and ARHGAPI11B expression in neocortex, we found 3-4 lentivirus integration events at random genomic positions per animal (table S2). ARHGAPI11B mRNA expression in the marmoset neocortical wall resembled that in fetal human neocortex, with similar intensity and occurring preferentially in the germinal zones (ventricular zone (VZ), inner subventricular zone (iSVZ), outer subventricular zone (oSVZ) (14)) (9, 15), like EGFP, as revealed by in-situ-hybridization (fig. S1E) and RT-qPCR (fig. S1F).

First release: 18 June 2020
The ARHGAPI1B-expressing marmoset neocortex was larger and its cortical plate (CP) thicker than normal marmoset neocortex (Figs. 1B and 2A and fig. S1E) and, in contrast to the smooth surface of the normal marmoset brain, exhibited surface folds (Fig. 2A). Quantification of fetal marmoset neocortex as a whole indicated no statistically significant difference in width but a significant increase in length of ARHGAPI1B-expressing neocortex as compared to wild-type and ARHGAPI1B-non-expressing neocortex (Fig. 2B). To quantify cortical folding, we analyzed coronal sections of fetal marmoset neocortex along the rostro-caudal axis (Fig. 2C) for the gyrification index (GI) (fig. S2A), the ratio of tracing the de facto length of the (unfolded or folded) cortical surface (Fig. 2E, green) over a hypothetical minimal-length, i.e., smooth, tracing of the cortical surface (Fig. 2E, magenta) (16, 17). Applying this tracing to the entire dorso-ventral dimension of the coronal sections analyzed, wild-type and ARHGAPI1B-non-expressing neocortex exhibited a GI of nearly 1.0 (Fig. 2C), consistent with the essentially unfolded, near-lissencephalic nature of the marmoset neocortex (18, 19). The GI of ARHGAPI1B-expressing neocortex increased rostrally (Fig. 2C), and reached nearly 1.1 when the tracing was confined to the portion of the cortical surface where gyrus-like structures emerged (fig. S2, B and D). These structures did not arise by folding of a CP of equal thickness, but reflected local CP thickening (fig. S2, C to E), which in turn reflected a specific increase in upper-layer neurons as revealed by immunostaining for markers of specific neuron populations (fig. S2, D and F).

We then quantified CP thickness in wild-type, ARHGAPI1B-non-expressing and ARHGAPI1B-expressing marmoset neocortex, taking only regions where no gyrus-like structures emerged in the ARHGAPI1B-expressing neocortex. This revealed increased CP thickness for ARHGAPI1B-expressing neocortex as compared to wild-type and ARHGAPI1B-non-expressing neocortex (Fig. 3, A and B, and figs. S3 and S4A).

To understand the basis of this increase in CP thickness, we quantified CP nuclei positive for Tbr1 and Ctip2, two markers of deep-layer neurons, and CP nuclei positive for Satb2 and Brn2, which are expressed by upper-layer neurons (20, 21) (Fig. 3A and fig. S4B). We observed a nearly 40% and 50% increase in Satb2+ and Brn2+ neurons, respectively, but not in Tbr1+ and Ctip2+ neurons, in the CP of ARHGAPI1B-expressing marmoset neocortex as compared to wild-type and ARHGAPI1B-non-expressing neocortex (Fig. 3C and fig. S4, C and D).

In line with the developmental stage of our analyses (fig. S1D), we noted that a substantial proportion of the Satb2+ and Brn2+ neurons observed in the cortical wall were found in the subplate (fig. S5, A and B), consistent with these neurons migrating to the CP (22, 23). Accordingly, the numbers specifically of Satb2+ and Brn2+ neurons in the subplate were greater while subplate thickness was equal, for ARHGAPI1B-expressing marmoset neocortex compared to wild-type and ARHGAPI1B-non-expressing neocortex (fig. S5, C to F).

These data were consistent with an ongoing production of cortical neurons, mostly upper-layer neurons, at the developmental stage of our analyses (fig. S1D). We examined the germinal zones (VZ, iSVZ, oSVZ) and progenitors therein for wild-type, ARHGAPI1B-non-expressing and ARHGAPI1B-expressing marmoset neocortex (Fig. 4A). Analysis of the germinal zones showed increased oSVZ thickness for ARHGAPI1B-expressing neocortex compared to wild-type and ARHGAPI1B-non-expressing neocortex (Fig. 4B and fig. S7A). We observed an increase in mitotic basal progenitors that overall was ~2-fold in the iSVZ and ~3-fold in the oSVZ, but observed no difference in mitotic apical progenitors in the VZ (Fig. 4C and figs. S6 and S7, B and C).

At least half of the mitotic basal progenitors in the oSVZ of ARHGAPI1B-expressing neocortex exhibited a basal process and hence were basal (or outer) radial glia (24–27), whereas this proportion was less (~40%) for wild-type and ARHGAPI1B-non-expressing neocortex (Fig. 4D and figs. S8, A to D), in line with previous data (28, 29). ARHGAPI1B expression increased mitotic basal radial glia ~3-fold (Fig. 4E and fig. S8E). A significant increase in basal radial glia due to ARHGAPI1B expression was also observed when these cells were quantified in interphase using the marker Hopx (6) (fig. S9). More than 99% of the mitotic basal radial glia in oSVZ were Sox2+ (fig. S9F), and about half lacked expression of Tbr2 (Fig. 4, D and E, and fig. S8G). Hence, the cells amplified upon ARHGAPI1B expression in fetal marmoset neocortex exhibited a marker signature consistent with the identity of basal radial glia (5, 6, 9).

In conclusion, we here examined physiologically relevant expression of human-specific ARHGAPI1B (7, 8) in the fetal neocortex of a non-human primate, the common marmoset, by using the human ARHGAPI1B promoter, in contrast to previous studies that used a strong constitutive promoter (9, 10). This expression increased fetal neocortex size, CP thickness, upper-layer neurons, oSVZ size (14), and basal progenitors, including basal radial glia, the progenitor type thought to drive development of the mammalian neocortex (2–6, 14, 30). Our results suggest that the human-specific ARHGAPI1B gene may have caused neocortex expansion in the course of human evolution.

REFERENCES AND NOTES


ACKNOWLEDGMENTS

We would like to apologize to all researchers whose work could not be cited due to space limitations. We are grateful to Dr. Junko Okahara (Okahara Lab in RIKEN CBS) for providing wild-type marmosets and to Dr. Jun-ichi Hata (Okahara Lab in RIKEN CBS) for MR imaging of fetal marmosets brains. We would like to thank (I) Prof. Dr. Rüdiger Behr (German Primate Center) for providing...
marmoset fibroblasts for initial construct/virus tests; (ii) D. Gerrelli, S. Lisgo, and their teams at the HDBR for the invaluable support from this resource; (iii) the Light Microscopy Facility, a Core Facility of the CMCB Technology Platform at TU Dresden, for scanning of cryosections; (iv) the Histology Facility of the CMCB Technology Platform at TU Dresden for cryosectioning; (v) Jan Peychl and his team of the Light Microscopy Facility at MPI-CBG for help with microscopy; (vi) Gayathri Nadar of the Scientific Computing Facility at MPI-CBG for help concerning data management; (vii) Franziska Friedrich and Kostas Margitudis for taking overview brain images, and (viii) Dr. Takashi Namba for providing the mouse monoclonal anti-ARHGAP11B IgG1 3758-A37-5. **Funding:** H.O. was supported by Brain/MINDS (JP20dm0207001) from AMED. E.S. was supported from the Strategic Research Program for Brain Science (JP17dm0107051) and Brain/MINDS (JP20dm0207065) from AMED. W.B.H. was supported by central funds of the Max Planck Society and by grants from the Deutsche Forschungsgemeinschaft (SFB 655, A2), the European Research Council (Advanced Grant 250197), and ERA-NET NEURON (MicroKin). **Author contributions:** Conceptualization, M.H., H.O. and W.B.H.; Investigation, M.H., C.H., A.M., Y.K. and H.S.; Writing – Original Draft, M.H. and W.B.H.; Writing – Review & Editing, M.H., A.M., H.O., E.S. and W.B.H.; Funding Acquisition, H.O., E.S. and W.B.H.; Resources, H.O. and E.S.; Supervision, H.O., E.S. and W.B.H. **Competing interests:** Authors declare no competing interests. Hideyuki Okano and Erika Sasaki are inventors on patent application (USA 8592643 2013/11/26, Europe 2246423 2013/11/26, China ZL20118006773.3 (2014/11/26), Japan 5374389/2013/09/27, Singapore 163739 (W02009/096101) 2013/09/30, Korea 10-1588474/2016/01/19) held by Keio University School of Medicine that covers “Method for introducing foreign gene into early embryo of primate animal”, and “Method for production of transgenic primate animal comprising the introduction method”. **Data and materials availability:** All data are available in the main text or the supplementary materials. Materials are available from M.H. or W.B.H. under a material transfer agreement and CITES permission.
Fig. 1. *ARHGAP11B* and EGFP expression in *ARHGAP11B*-transgenic marmoset 101-day fetuses. Genomic PCR for EGFP and *ARHGAP11B* using somatic cells (A) and absence (−) or presence (+) of EGFP protein and *ARHGAP11B* mRNA expression (see B) in neocortex (C) of 1 wild-type (WT) and 7 *ARHGAP11B*-transgenic marmoset fetuses. (B) *ARHGAP11B* mRNA *in situ* hybridization (left) and EGFP immunohistochemistry (right) of *ARHGAP11B*-non-expressing (TG2) and *ARHGAP11B*-expressing (TG6) neocortex of marmoset fetuses. Scale bars, 500 μm.
ARHGAP11B

**A**

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**B**

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**C**

Gyrification Index (GI)

**D**

Local GI

**E**

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Fig. 2. Size and gyrification index of wild-type and ARHGAP11B-non-expressing vs. ARHGAP11B-expressing 101-day fetal marmoset neocortex. (A) Wild-type brain (WT) and brain expressing ARHGAP11B in neocortex (TG3). Arrowheads, cortical folds; R, rostral; C, caudal. Scale bars, 1 mm. (B) Width and length (see cartoons) of 6 wild-type (black dots, white columns) plus 2 ARHGAP11B-non-expressing (green dots, white columns) vs. 5 ARHGAP11B-expressing (magenta dots, grey columns) neocortices. Mean ± SD; n.s., not significant; *, p<0.05 (two-tailed t-test). (C) Gyrification index (GI, see (E) and fig. S2A) of 3 wild-type plus 2 ARHGAP11B-non-expressing (white circles) vs. 5 ARHGAP11B-expressing (magenta circles) neocortices at 7 positions along the rostro-caudal axis (see cartoons). Data ± SD; *, p<0.05; **, p<0.01 (one-tailed t-test). (D) Local GI (see fig. S2B) of 3 wild-type (black dots, white column) plus 2 ARHGAP11B-non-expressing (green dots, white column) vs. 5 ARHGAP11B-expressing (magenta dots, grey column) neocortices. Mean ± SD; ***, p<0.001 (two-tailed t-test). (E) DAPI-stained coronal section of wild-type (WT) and ARHGAP11B-expressing (TG4) neocortex at position 3 (see C). D, dorsal; V, ventral. Green line, de facto length of cortical surface; magenta line, hypothetical minimal length of cortical surface. Scale bars, 500 μm.
Fig. 3. ARHGAP11B-expressing 101-day fetal marmoset neocortex shows increased CP thickness and elevated numbers specifically of upper-layer neurons. (A) Triple immunofluorescence for Tbr1 (yellow), Ctip2 (magenta) and Satb2 (green), combined with DAPI staining (white), of wild-type (WT, left) and an ARHGAP11B-expressing (TG3, right) neocortex (occipital lobe). Scale bars, 50 μm. (B, C) CP thickness (B) and Tbr1+, Ctip2+Satb2+ and Brn2+ neuron number in CP in 200 μm-wide field (C) of 3 wild-type (black dots, white columns) plus 2 ARHGAP11B-non-expressing (green dots, white columns) vs. 5 ARHGAP11B-expressing (magenta dots, grey columns) neocortices. For ARHGAP11B-expressing neocortex, quantification excluded gyrus. Mean ± SD; n.s., not significant; **, p<0.01; ***, p<0.001 (two-tailed t-test).
Fig. 4. ARHGAP11B-expressing 101-day fetal marmoset neocortex shows increased oSVZ thickness and elevated numbers of basal progenitors, notably basal radial glia. (A) Immunofluorescence for phosphohistone H3 (PH3, magenta), combined with DAPI staining (white), of wild-type (WT, left) and ARHGAP11B-expressing (TG6, right) neocortex (occipital lobe). Scale bars, 50 μm. (B and C) Germinal zone thickness (B) and PH3* cell numbers in germinal zones in 200 μm-wide field (C), of 3 wild-type (black dots, white columns) plus 2 ARHGAP11B-non-expressing (green dots, white columns) vs. 5 ARHGAP11B-expressing (magenta dots, grey columns) neocortices. Mean ± SD; n.s., not significant; *, p<0.05; **, p<0.01; ***, p<0.001 (two-tailed t-test). (D) Triple immunofluorescence for phospho-vimentin (pVim, magenta), Sox2 (green) and Tbr2 (yellow) showing mitotic bRG in oSVZ of ARHGAP11B-expressing neocortex. Arrowheads, basal process. Top bRG shown is Sox2* Tbr2+, bottom bRG is Sox2+ Tbr2−. Scale bars, 20 μm. (E) Numbers of total (left) and Sox2* Tbr2− (right) basal process-bearing pVim+ cells in oSVZ in 200 μm-wide field, of 4 wild-type (black dots, white columns) plus 1 ARHGAP11B-non-expressing (green dot, white columns) vs. 3 ARHGAP11B-expressing (magenta dots, grey columns) neocortices. Mean ± SD; ***, p<0.001 (two-tailed t-test).
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published online June 18, 2020