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REVIEW

Neocortical neurogenesis in development and evolution—Human-specific features

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Abstract

In this review, we focus on human-specific features of neocortical neurogenesis in development and evolution. Two distinct topics will be addressed. In the first section, we discuss the expansion of the neocortex during human evolution and concentrate on the human-specific gene *ARHGAP11B*. We review the ability of ARHGAP11B to amplify basal progenitors and to expand a primate neocortex. We discuss the contribution of *ARHGAP11B* to neocortex expansion during human evolution and its potential implications for neurodevelopmental disorders and brain tumors. We then review the action of ARHGAP11B in mitochondria as a regulator of basal progenitor metabolism, and how it promotes glutaminolysis and basal progenitor proliferation. Finally, we discuss the increase in cognitive performance due to the ARHGAP11B-induced neocortical expansion. In the second section, we focus on neocortical development in modern humans versus Neanderthals. Specifically, we discuss two recent findings pointing to differences in neocortical neurogenesis between these two hominins that are due to a small number of amino acid substitutions in certain key proteins. One set of such proteins are the kinetochore-associated proteins KIF18a and KNL1, where three modern human-specific amino acid substitutions underlie the prolongation of metaphase during apical progenitor mitosis. This prolongation in turn is associated with an increased fidelity of chromosome segregation to the apical progenitor progeny during modern human neocortical development, with implications for the proper formation of radial units. Another such key protein is transketolase-like 1 (TKTL1), where a single modern human-specific amino acid substitution endows TKTL1 with the ability to amplify basal radial glia, resulting in an increase in upper-layer neuron generation. TKTL1's ability is based on its action in the pentose phosphate pathway, resulting in increased fatty acid synthesis. The data imply greater neurogenesis during neocortical development in modern humans than Neanderthals due to TKTL1, in particular in the developing frontal lobe.

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KEYWORDS
apical progenitors, ARHGAP11B, basal progenitors, lagging chromosomes, metaphase duration, Neanderthals, TKTL1

1 | INTRODUCTION

Understanding the cellular and molecular basis of neocortical neurogenesis in development and evolution has been the effort of numerous laboratories over the past years (for a treatise providing a near-comprehensive summary of these efforts, see Huttner, 2023). In this review, we will concentrate on human-specific features of neocortical neurogenesis in development and evolution, and will address two distinct aspects, in line with what was presented at the Cortical Evolution Meeting 2023 in Burgos, Spain. First, we will discuss key properties of the human-specific gene *ARHGAP11B* and its role in the expansion of the neocortex that occurred during human evolution. The *ARHGAP11B*-mediated increase in neocortical neurogenesis and, consequently, in neocortex size contributed to the evolutionary growth of the brain that distinguishes us from our closest living relatives, the chimpanzee and bonobo. Second, we will focus on the development of brains of modern humans versus Neanderthals, which are of comparable size, and discuss differences in neocortical neurogenesis between these two hominins that are due to a small number of amino acid substitutions in certain key proteins.

2 | HUMAN-SPECIFIC *ARHGAP11B* AND NEOCORTEX EXPANSION

2.1 | Neocortex expansion, basal progenitors, and *ARHGAP11B*

The primary cause of neocortex expansion is an increase in the production of cortical neurons and glial cells from cortical stem and progenitor cells (CSPCs). There are two classes of CSPCs: (i) apical progenitors (APs), whose cell bodies reside in the ventricular zone (VZ) and which comprise neuroepithelial cells (the primary stem cells of the central nervous system), apical radial glia (aRG; also called ventricular radial glia), and apical intermediate progenitors, and (ii) basal progenitors (BPs), whose cell bodies reside in the subventricular zone (SVZ) and which comprise basal radial glia (bRG; also called outer radial glia) and basal intermediate progenitors (see Taverna et al., 2014 and references therein). There is evidence that APs, notably neuroepithelial cells, may contribute to neocortex expansion (Benito-Kwiecinski et al., 2021), and also the interaction of aRG with meninges via their basal endfeet has been implicated in cortical development (Siegenthaler et al., 2009). However, our group has focused on the role of BPs in neocortex expansion. A rationale for this focus has been the consideration that AP mitoses in the VZ are confined to the ventricular surface, the apical-most region of this germinal layer; this constitutes a spatial restriction that limits the number of AP mitoses and hence the production of progeny. In contrast, BP mitoses in the SVZ can occur anywhere in this germinal layer, which increases in radial thickness in the course of human evolution, allowing for a massive increase in the production of progeny. The reason why AP mitoses are confined to the ventricular surface lies in an epithelial feature of these cells, specifically the presence of an apical primary cilium, which results in the tethering of the centrosomes—the organizers of the mitotic spindle—to the apical cell cortex. Such apical tethering no longer applies to BPs, as these cells have delaminated from the ventricular surface and hence no longer exhibit apical primary cilia but rather carry (baso)lateral primary cilia that usually emerge from the plasma membrane in the vicinity of the nucleus (Taverna et al., 2014; Wilsch-Bräuninger et al., 2012).

In light of these considerations, which are in line with the key role of BPs for neocortex expansion observed in developing mouse and ferret neocortex (Nonaka-Kinoshita et al., 2013), we conducted a screen for human-specific, protein-coding genes that are preferentially expressed in CSPCs and that increase BP proliferation and abundance. To this end, we developed a procedure to isolate aRG, bRG, and neurons from fetal human neocortical tissue and analyzed the transcriptomes of these distinct cell types. The gene most specifically expressed in aRG and bRG as compared to neurons was found to be *ARHGAP11B* (Florio et al., 2015).

As will be reviewed in detail in the sections below, *ARHGAP11B* can expand the fetal primate neocortex and increases BP abundance in chimpanzee cerebral organoids to a human-like level. However, evolutionary brain expansion also occurred in other, nonprimate, mammalian lineages, such as cetaceans. We therefore find it likely that other genes appeared in the evolution of these mammals that also caused brain expansion, a worthwhile topic of future investigations. In the context of evolutionary brain expansion, it is interesting to mention that a converse evolutionary process also exists, the secondary reduction of brain size as seen in the evolution of a lissencephalic mammal from a gyrencephalic ancestor (for a review, see Kelava et al., 2013).

2.2 | ARHGAP11B and BP amplification

As first shown by Evan Eichler and colleagues (Antonacci et al., 2014; Dennis et al., 2017; Sudmant et al., 2010), *ARHGAP11B* is a human-specific gene that arose about 5 million years ago by a partial segmental duplication of the widespread gene *ARHGAP11A*, which encodes a Rho GTPase Activating Protein (Rho-GAP) (Figure 1a). This origin of *ARHGAP11B* about 5 million years ago is after the lineage leading to the chimpanzee and bonobo, our nearest living relatives, segregated around 7 million years ago from the lineage leading to modern humans. However, this origin of *ARHGAP11B* is much before the lineage leading to modern humans segregated around 500,000 years ago from that leading to Neanderthals and Denisovans. Accordingly, *ARHGAP11B* is

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FIGURE 1 Summary of *ARHGAP11B* findings. (a) Origin of *ARHGAP11B* by partial gene duplication of *ARHGAP11A*, and its selective occurrence in hominins. (b) Effect of the single C→G nucleotide substitution in *ARHGAP11B* on mRNA splicing and protein structure in comparison to *ARHGAP11A*. (c) *ARHGAP11B* expression increases bRG abundance and, consequently, upper-layer neuron number; oSVZ, outer SVZ; iSVZ, inner SVZ; bIP, basal intermediate progenitor (the increase in bIP abundance by *ARHGAP11B* is not illustrated); CP, cortical plate. (d) Increase in bRG proliferation due to stimulation of glutaminolysis by ARHGAP11B, which provides αKG for a three-quarter TCA cycle. (e) Neocortex expansion and induction of cortical folding by physiological expression of *ARHGAP11B* in marmoset fetuses. (f) Increased cognitive performance in transgenic mice expressing *ARHGAP11B*.

found in all genomes of hominins for which genome data are available, that is, in modern human, Neanderthal and Denisova, but not in chimpanzee, bonobo, gorilla, orangutan, and other primates, such as gibbons and monkeys (Figure 1a).

The ARHGAP11B protein consists of the N-terminal quarter of ARHGAP11A and contains most of the GAP domain. However, due to a single C→G nucleotide substitution that presumably occurred after the gene arose and that creates a new splice donor site, 55 nucleotides are removed from the *ARHGAP11B* mRNA upon splicing. This in turn causes a reading frame shift and results in a new 47 amino acid-long C-terminal sequence starting at position 221 that is human specific and replaces the C-terminal part of the GAP domain (Florio et al., 2015, 2016). In line with the lack of the C-terminal part of the GAP domain, ARHGAP11B lacks GAP activity (Figure 1b).

The C→G point mutation in *ARHGAP11B* and the resulting new C-terminal sequence of ARHGAP11B are critical for function: ARHGAP11B with this sequence (referred to as “modern” ARHGAP11B) can amplify BPs upon expression in embryonic mouse neocortex; in contrast, the ARHGAP11B encoded by *ARHGAP11B* still containing the C rather than the G, which therefore lacks the new C-terminal sequence (referred to as “ancestral” ARHGAP11B), has no effect on BP abundance (Florio et al., 2016). Concomitant with BP amplification, ARHGAP11B expression in embryonic mouse neocortex can induce its folding, a hallmark of neocortex expansion (Florio et al., 2015). When *ARHGAP11B* is expressed in the developing neocortex of the ferret, a gyrencephalic carnivore, it increases bRG (the BP type thought to be key for neocortex expansion) and causes an extension of the neurogenic period and an increase in upper-layer neurons (Figure 1c). Thus, also in developing ferret neocortex, ARHGAP11B can elicit hallmarks of neocortex expansion (Kalebic et al., 2018). These findings rendered *ARHGAP11B* a prime candidate to underlie the expansion of the neocortex during human evolution.

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2.3 | *ARHGAP11B* can expand a primate neocortex

However, two key questions remained unanswered. First, the above-mentioned studies were limited to animals that are evolutionarily distant from humans. This led to the question if *ARHGAP11B* can actually expand the cerebral cortex of evolutionarily closer animals, like primates. Second, the mouse and ferret animal models used strong overexpression of *ARHGAP11B*. This led to the question if ARHGAP11B at physiological expression levels would still be able to expand the cerebral cortex.

To answer these two questions, we collaborated with the laboratories of Hideyuki Okano and Erika Sasaki—the pioneers in the generation of transgenic marmosets (Sasaki et al., 2009)—to generate *ARHGAP11B*-transgenic fetal marmosets (Heide et al., 2020). To achieve a "physiological-like" expression of *ARHGAP11B* in the marmoset, we created a lentiviral construct containing the protein-coding sequence of *ARHGAP11B* along with a 2.7-kb DNA fragment containing the sequence of the *ARHGAP11B* promoter. This construct was packaged into lentivirus particles, which were subsequently injected into fertilized marmoset oocytes. This resulted in the integration of the *ARHGAP11B* gene along with its regulatory DNA sequence. These oocytes were then transferred into surrogate mothers. After 101–102 days of pregnancy, the marmoset fetuses were collected by caesarean section for analysis. Interestingly, we found that the expression of ARHGAP11B at a near-physiological level was sufficient to enlarge the fetal marmoset cerebral cortex and to induce its folding (Heide et al., 2020) (Figure 1e). Furthermore, we showed that this is accompanied by an increased pool of BPs, especially of bRG, without an increase in AP abundance (Figure 1c), supporting the concept that increased BP abundance underlies the *ARHGAP11B*-mediated neocortex expansion (Heide et al., 2020). This in turn led to an increased production of specifically those neurons that form the outer layers of the neocortex (Heide et al., 2020). In summary, we demonstrated that expression of *ARHGAP11B* at a near-physiological level is sufficient to enlarge a primate cerebral cortex and to induce its folding.

2.4 | ARHGAP11B likely is a major contributor to neocortex expansion during human evolution

In light of these findings in *ARHGAP11B*-transgenic marmoset fetuses, two crucial questions remained unanswered. First, what would happen in the developing human brain if the function of ARHGAP11B would be inhibited? Second, which effect would ARHGAP11B have on the developing brain of our closest living relative, the chimpanzee? To answer the first question, we performed electroporation of human and chimpanzee cerebral organoids with a truncated version of ARHGAP11A (ARHGAP11A220), which had been shown to block the ability of ARHGAP11B to amplify BPs (Namba et al., 2020). Electroporation of *ARHGAP11A220* into human cerebral organoids resulted in a reduction in the number of BPs, which decreased to the level of chimpanzee organoids. In chimpanzee cerebral organoids, no difference in the number of BPs was detected, confirming the specificity of the ARHGAP11A220-mediated inhibition of ARHGAP11B, as ARHGAP11B does not exist in chimpanzee.

To answer the second question, we generated chimpanzee cerebral organoids and expressed ARHGAP11B by electroporation. This resulted in an increase in BPs, particularly in bRG, to a human-like level (Fischer et al., 2022). In summary, this study not only demonstrated that ARHGAP11B can ensure a BP abundance thought to be required for the evolutionary expansion of the human neocortex and therefore can be viewed as a major contributor to this expansion, but also established electroporation of chimpanzee organoids as a valuable tool for the functional analysis of human-specific genes.

2.5 | *ARHGAP11B*—Potential implications for neurodevelopmental disorders and brain tumors

In light of the presumably major contribution of *ARHGAP11B* to human neocortex expansion, it is likely that this gene is involved in disorders that affect the growth of the cerebral cortex. One possibility is that mutations affecting *ARHGAP11B* contribute to cortical malformations like microcephaly. In this context, *ARHGAP11B* may be involved in cases of the 15q13.3 microdeletion syndrome, which are associated with microcephaly (reviewed in Heide & Huttner, 2021). However, currently ARHGAP11B's contribution to this syndrome is unclear and needs further investigations. Another possibility is that ARHGAP11B contributes to the growth of glioblastoma. It was shown recently that glioblastoma contain an invasive cell population similar to bRG (Bhaduri et al., 2020). These bRG-like cells express *ARHGAP11B* (Wang et al., 2020). In light of these findings and ARHGAP11B's role in BP amplification, it is likely that ARHGAP11B has a similar effect on the proliferation of glioblastoma bRG-like cells. However, similar to ARHGAP11B's potential role in microcephaly, further investigations are needed.

2.6 | ARHGAP11B acts in mitochondria as a regulator of BP metabolism

The ARHGAP11B protein is imported into the mitochondrial matrix (Namba et al., 2020). In the matrix, ARHGAP11B interacts with adenine nucleotide translocator, a protein located in the mitochondrial inner membrane. This interaction allows ARHGAP11B to inhibit the opening or formation of the mitochondrial permeability transition pore (mPTP) through the human-specific C-terminal sequence of ARHGAP11B. Since the opening of mPTP induces Ca^{2+} efflux from mitochondria (Halestrap & Richardson, 2015), inhibition of mPTP by ARHGAP11B maintains an elevated level of mitochondrial [Ca^{2+}]. Mitochondrial Ca^{2+} is a major factor that regulates the activity of several mitochondrial dehydrogenases (Cannino et al., 2018; Lawlis & Roche, 1981; Rutter & Denton, 1989). One of these dehydrogenases is the alpha-ketoglutarate (αKG) dehydrogenase complex (KGDHC), one of the TCA (tricarboxylic acid) cycle enzymes. Upon higher [Ca^{2+}], KGDHC is

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activated, thus consuming αKG to produce succinyl-CoA. To replenish αKG levels, ARHGAP11B-expressing cells utilize glutaminolysis (glutamine-to-glutamate-to-αKG) to promote BP proliferation (Figure 1d). Since an inhibition of glutaminolysis reduces the number of BPs in the fetal human neocortex, ARHGAP11B acts in mitochondria to promote BP proliferation by induction of glutaminolysis.

These findings demonstrating that a change in mitochondrial metabolism underlies the ability of ARHGAP11B to increase BP abundance provide further support for the emerging concept that mitochondrial dynamics and metabolism exert key roles in CSPC fate decisions and cortical neurogenesis (Iwata et al., 2020; Khacho et al., 2016; Namba et al., 2021). In line with this concept, another recent study showed that the microcephaly-associated protein MCPH1 is localized on mitochondria of CSPCs and reinforces the interaction between mitochondria and the endoplasmic reticulum to support more Ca^{2+} influx into mitochondria (Journiac et al., 2020). Interestingly, cells with MPCH1 exhibit more glutaminolysis than cells without MCPH1 expression. These results lead to a key question: How does glutaminolysis promote BP proliferation?

2.7 | Glutaminolysis and BP proliferation

It has been suggested that in highly proliferative CSPCs, in particular radial glia, glycolysis is not the main source of acetyl-CoA supply for the TCA cycle (Khacho et al., 2016). In these cells, a major source of a metabolite to feed the TCA cycle appears to be αKG from glutaminolysis, the activity of which is promoted by ARHGAP11B. There would be two consequences of this. First, the TCA cycle would become independent of glycolysis and thus glucose could be used for other metabolic pathways such as cholesterol and fatty acid synthesis, 1C metabolism, and the pentose phosphate pathway (PPP). These pathways would then provide essential metabolites to fulfill certain demands of highly proliferating CSPCs. In this context, as will be discussed in greater detail below, it is interesting to note that bRG in the fetal neocortex of modern human, but not those predicted for the fetal neocortex of archaic human, have recently been reported to increasingly utilize the PPP to provide acetyl-CoA for lipid synthesis (Pinson et al., 2022). Second, the TCA cycle could stop at oxaloacetate (OAA) due to the limited supply of acetyl-CoA from pyruvate. In this scenario, only three quarters of the TCA cycle would be used, that is, from αKG to OAA (Namba et al., 2021) (Figure 1d). OAA could be converted into aspartate, which is an essential substrate for nucleotide de novo synthesis and thus is one of the rate-limiting amino acids for cell proliferation (Garcia-Bermudez et al., 2018). In conclusion, due to ARHGAP11B-induced glutaminolysis, human bRG could exhibit a pro-proliferative metabolism comprising several anabolic pathways. We previously proposed this scenario as the three-quarter TCA cycle concept (Namba et al., 2021). Comparative metabolomics would be needed to obtain further evidence for this concept in the future.

2.8 | ARHGAP11B-induced neocortical expansion increases cognitive performance

The wiring of neurons and the number of functional cortical areas likely influence cognitive functions in a major way. However, brain size has also been thought to be a prerequisite for higher cognitive functions because humans with brain-size abnormalities such as microcephaly and macrocephaly often exhibit intellectual disability (Jayaraman et al., 2018). Yet, the relationship between the size of the neocortex and higher cognitive functions, including spatial learning, reference memory, and long-term memory flexibility, is not fully understood. We recently developed a transgenic mouse line that expresses *ARHGAP11B* under the control of the endogenous *Arhgap11a* promoter (Xing et al., 2021). The neocortex of these *ARHGAP11B*-transgenic mice shows an increase by approximately 10% in the number of upper-layer neurons in adulthood. Interestingly, these *ARHGAP11B*-transgenic mice exhibit higher flexibility in long-term memory, albeit there are no changes in memory formation (Figure 1f). This phenotype is most likely due to the increase in the number of upper-layer neurons rather than to an action of ARHGAP11B in the adult neocortex, since there is no expression of ARHGAP11B in the neocortex of the adult transgenic mice (Xing et al., 2021).

When considering the observed increase in upper-layer neurons, it is important to realize that inhibitory interneurons have not yet been quantitated in these *ARHGAP11B*-transgenic mice. Will these mice exhibit an imbalance between excitation and inhibition, or is there a corresponding increase in inhibitory interneurons, given that *ARHGAP11B* is also expressed in the ventral telencephalon where inhibitory interneurons originate?

One of the neocortical regions responsible for memory flexibility is the prefrontal cortex (Logue & Gould, 2014). In line with this notion, the *ARHGAP11B*-transgenic mice at E18.5 possess a thicker neocortex in the rostral (frontal) region of the neocortex, but not in its caudal region (Xing et al., 2021). Therefore, the ARHGAP11B-induced neocortical expansion might provide a structural basis for cognitive flexibility. It would be interesting to know how these additional neurons form a circuitry that enhances memory flexibility. Does ARHGAP11B change dendritic arbor complexity or even synapse formation/plasticity? Comparison of the neuronal connectome between wildtype and *ARHGAP11B*-transgenic mouse neocortex will likely provide new insight into the structural basis of higher cognitive functions.

While the above section summarizes the data on the human-specific gene *ARHGAP11B* presented at the Cortical Evolution Meeting 2023 in Burgos, Spain, we would like to emphasize that there are, of course, important studies on other human-specific genes that impact brain expansion and evolution. In particular, the human-specific gene *NOTCH2NL* has emerged as a key player in this regard (Fiddes et al., 2018; Florio et al., 2018; Suzuki et al., 2018). Discussing these studies in detail, however, exceeds the scope of this review.

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3 | MODERN HUMAN VERSUS NEANDERTHAL DIFFERENCES IN NEOCORTICAL NEUROGENESIS

3.1 | Cell division differences between archaic and modern human neocortical progenitors

Recent work has identified differences between the CSPCs of humans and other primates, such as monkeys (Betizeau et al., 2013; Dehay et al., 2015; Kornack & Rakic, 1998; Lukaszewicz et al., 2005; Otani et al., 2016; Smart et al., 2002), as well as other great apes or hominids (Benito-Kwiecinski et al., 2021; Mora-Bermúdez et al., 2016). This set the stage to investigate if there could also be differences between modern humans and our closest known relatives, the extinct archaic humans known as Neanderthals and Denisovans, thereby giving rise to experimental paleoneurobiology (Mora-Bermúdez et al., 2022).

In this context, a striking finding was that during mitosis of early APs—presumably mostly neuroepithelial cells and early aRG—the duration of metaphase is longer in humans than in other animals. Most interestingly, it was shown to be around 50% longer than in other great apes, namely, chimpanzees and orangutans (Mora-Bermúdez et al., 2016). Upon further examination, human APs also showed a higher abundance of spindle assembly checkpoint (SAC) markers than in chimpanzee APs (Mora-Bermúdez et al., 2022), which suggested a higher activation of the SAC. This could explain the longer metaphase, since a core function of the SAC is to delay anaphase onset until all kinetochores have been properly attached to the spindle poles via K-fibers (Foley & Kapoor, 2013; Musacchio, 2015).

Involvement of the SAC suggested that chromosome segregation might differ between the APs of humans and other great apes. This was indeed found to be the case when analysis of cerebral organoids revealed that human APs show around half the incidence of lagging chromosomes in anaphase and telophase than chimpanzee APs (Mora-Bermúdez et al., 2022). This suggests that humans have a higher fidelity of AP chromosome segregation than other great apes, which may have consequences for the genomic integrity and composition of the neurons derived from them.

Could these differences also be present between modern and archaic humans? The full sequencing of the Neanderthal genome has made it possible to compare it to the modern human genome. This has revealed nucleotide changes in exons that lead to around a hundred fixed, or nearly fixed, amino acid differences between the proteomes of modern humans versus Neanderthals and Denisovans (Kuhlwilm & Boeckx, 2019; Prüfer et al., 2014). Intriguingly, six of these modern human-specific differences occur in three proteins that have been linked to SAC and/or kinetochore function—KIF18a (Kinesin 8) with one difference, KNL1 (Casc5) with two differences, and SPAG5 (Astrin) with three differences (Cheeseman & Desai, 2008; Cheeseman et al., 2008; Häfner et al., 2014; Manning et al., 2010; Stumpff et al., 2012; Thein et al., 2007; Zhang et al., 2017) (Figure 2a,b).

The combination of the six amino acid substitutions in these three proteins in modern humans appears to be unique. Therefore, this likely modern human-specific set of six amino acids became a prime candidate to potentially explain the differences in AP metaphase duration and AP chromosome segregation fidelity observed between modern humans and chimpanzees, and potentially many other species, including Neanderthals (Mora-Bermúdez & Huttner, 2022; Mora-Bermúdez et al., 2016; Mora-Bermúdez et al., 2022). Interestingly from an evolutionary point of view, and conveniently from an experimental point of view, all six ancestral amino acids are found not only in Neanderthals and Denisovans, but also in chimpanzees and other great apes, as well as many other species, including other primates, cetaceans, and, notably, rodents.

Since rodents share the same six ancestral amino acids that Neanderthals have in the above three proteins, it was reasonable to generate mouse models where these amino acids were “modern humanized” to study potential consequences for neocortical AP behavior. Just as modern human APs did when compared to chimpanzee APs, modern humanized mouse APs showed a longer lasting metaphase in mitosis compared to the Neanderthal-like wildtype mouse APs (Figure 2c). Interestingly, only the three modern human amino acid substitutions in KIF18a and KNL1 were in fact necessary and sufficient to increase neocortical AP metaphase duration in the modern humanized mice. The neocortical APs in these mice with the three modern humanized amino acids also exhibited a more active SAC and fewer lagging chromosomes in mitosis (Mora-Bermúdez et al., 2022) (Figure 2c).

These findings were corroborated by conducting the converse experiments, where APs in the modern human context of cerebral organoids were compared to human organoids where those three amino acid positions in KIF18a and KNL1 had been “Neanderthalized,” that is, ancestralized, by genome editing of the human ES cells used to grow the cerebral organoids. Neanderthalized organoid APs showed a shorter metaphase, a less active SAC and more lagging chromosomes than modern human organoid APs (Mora-Bermúdez et al., 2022). In this context, it is interesting to note that, so far, modern humans show the longest lasting AP metaphase of all species measured, suggesting it to be a derived characteristic that has undergone positive selection.

Taken together, these results suggest that modern humans have likely had fewer chromosome segregation errors upon AP mitoses in the neocortex than were present in Neanderthals. If these lower proportions of chromosome segregation errors were maintained in the cells derived from those APs, for example, in bRG, basal intermediate progenitors, neurons, and perhaps also macroglia, this would lead to adult modern human radial units (Rakic, 1988, 2000, 2009) composed of cells with fewer aneuploidies than in Neanderthals. Given the thousands of cortical and subcortical connections that cortical neurons establish, even a relatively small decrease in chromosome segregation errors could have consequences for the optimal functioning of cortical columns, and in the brain circuits and networks they participate. It is not inconceivable that such differences could have played a part in the different fates that Neanderthals and modern humans experienced.

3.2 | Transketolase like-1

Another case where a minimal number of modern human-specific amino acid substitutions has profound impact for neocortical

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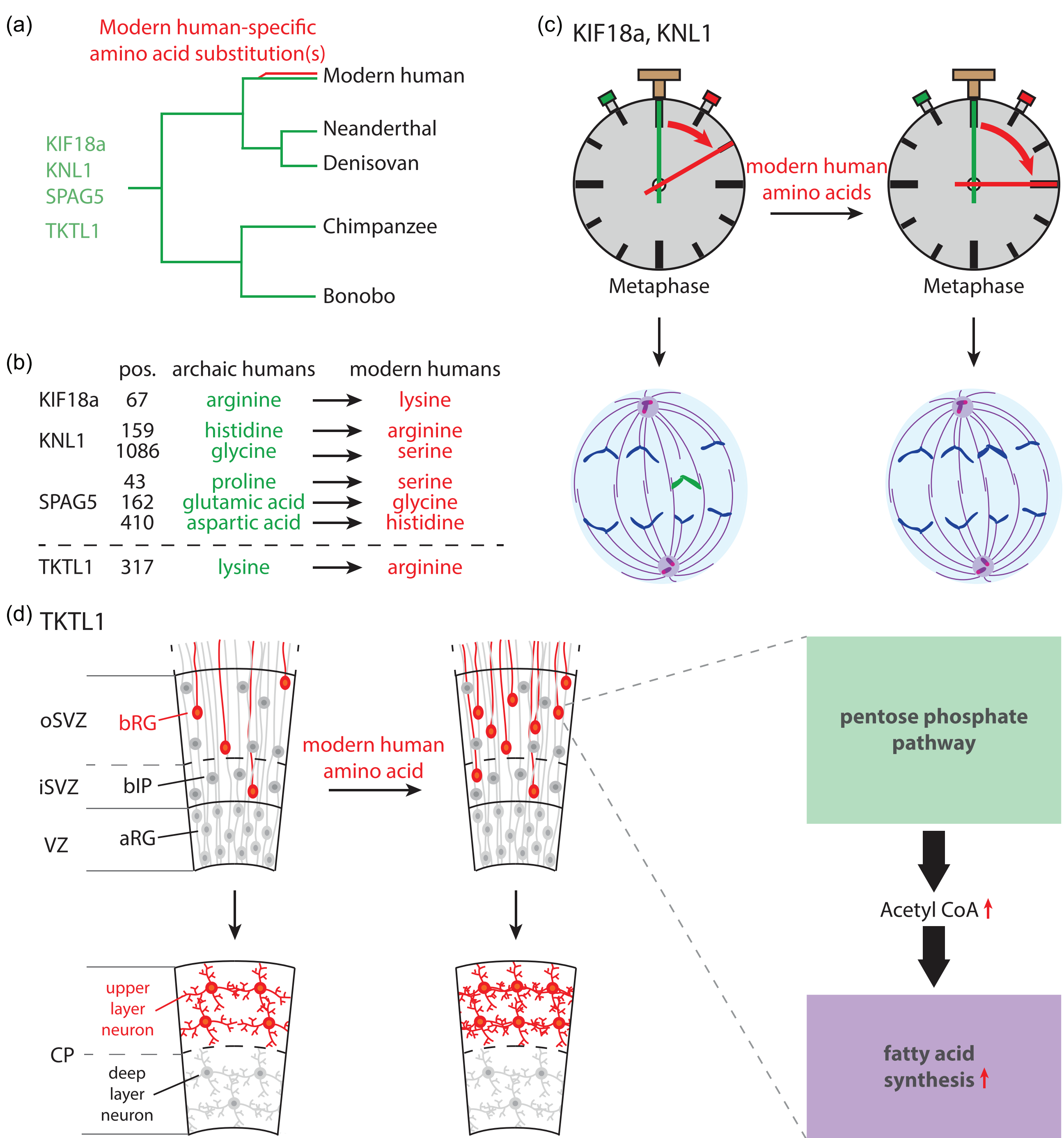

FIGURE 2 Summary of modern human versus Neanderthal differences in neocortical neurogenesis due to modern human-specific amino acid substitutions in key proteins. (a) Selective occurrence in the modern human lineage of amino acid substitution in KIF18a, KNL1, SPAG5, and TKTL1. (b) Type of amino acid substitutions in KIF18a, KNL1, SPAG5, and TKTL1; pos., position in the protein sequence. (c) Metaphase lengthening during AP mitosis in mice expressing "modern humanized" KIF18a and KNL1 is associated with a reduction in lagging chromosomes (green). The increase in metaphase duration as illustrated (10→15 min) is meant to indicate the 8- versus 12-min difference in metaphase length observed in chimpanzee versus human cerebral organoids (Mora-Bermúdez et al., 2016), which is thought to reflect the modern human-specific amino acid substitutions in KIF18a and KNL1. (d) Modern human TKTL1 (arginine317) increases bRG abundance and, consequently, upper-layer neuron number; this action of modern human TKTL1 in bRG involves the pentose phosphate pathway, increases the level of acetyl-CoA, and requires fatty acid synthesis.

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neurogenesis is transketolase like-1 (TKTL1). Modern human TKTL1 differs in just one amino acid from Neanderthal TKTL1, containing an arginine (modern human, hTKTL1) rather than the ancestral lysine (Neanderthal, aTKTL1) (Figure 2a,b). Examining the significance of this single amino acid substitution by expressing either hTKTL1 or aTKTL1 in mouse embryonic neocortex revealed that hTKTL1, but not aTKTL1, increases, among all CSPCs analyzed, the abundance specifically of the key type of BP, the bRG (Pinson et al., 2022) (Figure 2d). Because of their predominantly asymmetric, self-renewing mode of cell division, bRG are known to generate more cortical neurons over time than neurogenic basal intermediate progenitors (Fietz et al., 2010). Accordingly, hTKTL1 expression in embryonic mouse neocortex resulted in an approximately 50% increase in upper-most-layer neurons (Pinson et al., 2022) (Figure 2d).

Like its related protein transketolase (TKT), hTKTL1 is thought to operate in the PPP. Indeed, inhibitor experiments using hemisphere cultures of embryonic mouse brain demonstrated that hTKTL1 action in the PPP eventually results in an increase in acetyl-CoA (Pinson et al., 2022) (Figure 2d). One of the metabolic pathways that acetyl-CoA is critical for is fatty acid synthesis. In fact, further inhibitor experiments demonstrated that the hTKTL1-mediated increase in bRG abundance requires fatty acid synthesis (Figure 2d). In light of this finding, an appealing scenario is that hTKTL1 increases specifically bRG abundance because it allows the synthesis of a special fatty acid. This fatty acid as part of a membrane lipid would be required for the formation and maintenance of the basal process that distinguishes mitotic bRG from mitotic basal intermediate progenitors and promotes bRG proliferation.

The relevance of these effects of hTKTL1 in the embryonic mouse neocortex test system for human neocortical development was demonstrated in two ways. First, CRISPR/Cas9-mediated disruption of *hTKTL1* in fetal human neocortical tissue ex vivo significantly reduced bRG abundance (Pinson et al., 2022). Second, "Neanderthalization" of the single relevant amino acid in TKTL1 (R→K) in human ES cells followed by analysis of cerebral organoids grown from these cells revealed that this Neanderthalization reduced bRG abundance to almost half and substantially decreased neuron numbers (Pinson et al., 2022).

Finally, analysis of the temporal and spatial patterns of *hTKTL1* mRNA expression in fetal human neocortical tissue yielded the exciting results that *hTKTL1* expression increases concomitant with the increase in cortical neurogenesis and is particularly high in the frontal lobe (Pinson et al., 2022). When taking all these results together, it appears that a single modern human-specific amino acid substitution in TKTL1 has endowed modern humans with greater neurogenesis in the frontal lobe during cortical development than Neanderthals.

4 | CONCLUSIONS AND PERSPECTIVES

The findings reviewed and discussed here raise a number of issues and questions that should—and probably could—be addressed in future investigations. Regarding *ARHGAP11B*, it would be desirable to know when exactly the C→G point mutation (Florio et al., 2016) occurred that is so crucial for ARHGAP11B's function. Did it occur prior to the emergence of *Homo habilis*, that is, prior to the beginning of the surge of brain size expansion during human evolution? Or did this mutation in the *ARHGAP11B* gene occur later, only prior to the emergence of *Homo erectus* or even only after the emergence of *Homo erectus* but prior to the emergence of the common ancestor of modern humans and Neanderthals? Current sequencing technologies of ancient DNA do not yet allow to answer these questions. However, with the progression of the man-made climate change and the increasing availability of permafrost-embedded specimen, it is not inconceivable that we may approach answering these questions in the future. From a medical perspective, it would be crucial to determine whether any malformations during human cortical development are associated with mutations in the *ARHGAP11B* gene. Similarly, its potential role in glioblastoma multiforme deserves further investigation. As to the emerging concept that metabolism plays a pivotal role in neocortical development (Namba et al., 2021), an interesting question to explore appears to be a possible interaction of ARHGAP11B with other regulators of metabolism, notably those affecting the flux from αKG to aspartate via OAA using a three-quarter TCA cycle (Namba et al., 2021). Finally, in light of the increase in cognitive performance of *ARHGAP11B*-transgenic mice (Xing et al., 2021), a connectomic analysis of their neuronal circuits in the neocortex seems worthwhile.

Regarding the presumptive differences in neocortical development between modern humans and Neanderthals, a crucial issue of future work appears to be to determine the consequences of the increased fidelity of chromosome segregation to the AP progeny (Mora-Bermúdez et al., 2022) for the formation of the radial units (Rakic, 2000). Also, in light of the existence of mice "modern humanized" for the three modern human-specific amino acid substitutions in Kif18a and KNL1 (Mora-Bermúdez et al., 2022), a thorough analysis of their behavior seems indicated. As to TKTL1, two aspects should be addressed. First, is the requirement of fatty acid synthesis for bRG amplification by TKTL1 (Pinson et al., 2022) really related to the synthesis of a specific fatty acid, and if so, which one? Second, given the implication of our findings that neurogenesis during cortical development is greater in the frontal lobe of modern humans than Neanderthals (Pinson et al., 2022), what are the potential consequences, if any, for the minority of adult humans carrying only the ancestral *TKTL1* allele? Are there compensatory mechanisms that "correct" reduced cortical neurogenesis due to ancestral *TKTL1*? Finally, and perhaps most importantly, as there are about 100 proteins with a minimal number of amino acid substitutions between modern humans and Neanderthals (Pääbo, 2014), is it possible to identify the protein(s) that may have an impact for potentially differential cognitive abilities between these two hominins?

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DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no data sets were generated or analyzed during the current study.

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PEER REVIEW

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