A role for the male germline in the expansion of the mammalian brain

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Abstract

The brain and testis share a surprisingly high number of molecular and cellular similarities. We have previously hypothesised that, throughout evolution, many genetic variants contributing to brain size expansion first arose in spermatogonia where they conferred a selective advantage to the male germline stem cells via a process analogous to oncogenesis – known as 'selfish spermatogonial selection'. Upon transmission of the mutant sperm to the next generation, these selfish variants became constitutive, disproportionately accumulating in signalling pathways active in both spermatogenesis and neurogenesis and which regulate stem cell proliferation. While we believe these observations supporting a close association between germline and brain development are compelling, research in this area is stymied by the relative scarcity of spermatogonia and the inherent stochasticity of the single-cell transcriptomic methods used to profile them. Accordingly, the molecular signatures of spermatogonia are incompletely understood, and their similarity with those of the brain difficult to assess. To support further work on the testis-brain relationship, we combine re-analyses of 34 adult human single-cell testis datasets with data from the Human Protein Atlas to assess the extent to which genes functionally associated with brain growth and development are expressed within testicular cell types. Consistent with our hypothesis, we find that of thousands of proteins functionally associated with the brain, the majority are not only expressed in male germ cells, but show particular enrichment in spermatogonia. We contextualise these results with an extensive literature survey and conclude that further enquiry into the testis-brain connection may yield novel insight into the evolutionary processes that shaped the human condition.

Introduction

Prominent hallmarks of primate evolution include an increased brain volume and disproportionate expansion of its executive and integrative centre, the cortex (1,2). After correcting for allometric scaling with body mass, primates (and humans in particular) have larger brains than almost any other mammal (3), due to an increased number of neurons supporting more elaborate neural architectures and cognitive abilities (4). A recent review of human brain evolution offers a striking perspective on the extent to which neuron numbers have increased in our species: compared to other great apes, developing humans generate around three million more neurons per hour (5). How this came about is an active subject of debate and likely the result of a combination of different factors. In general, the mechanisms producing interspecies differences in neuron number and specialisation implicate the duration, timing, and relative rate of neural stem and progenitor cell (NSPC) divisions (6) during development and in what proportion and when mitoses are symmetrical (proliferative, or

self-renewing) or asymmetrical (producing differentiation-inclined progeny) (7). Consequently, even subtle species-specific differences in NSPC activity can have pronounced effect upon the size and composition of both the brain as a whole, or its regions (8). Human-specific variants in, for instance, *ARHGAP11B* (9), *NOVA1* (10) and *TKTL1* (11) influence NSPC proliferation and by extension the overall size of the progenitor pool, and thereby brain. Hence, by modulating the rate of cell division, or by changing developmental decisions, genetic variants in a specific set of genes can alter the number of neurons produced over time and therefore play causal roles in neocortical expansion.

We have previously drawn attention to selfish spermatogonial selection – a mechanism by which *de novo* mutations that affect stem and progenitor cell regulation in the human testis are preferentially introduced into the genome (12) – as we believe this may offer a fresh perspective on the molecular and developmental basis of brain expansion and its close connection to other phenotypes, in particular fertility. We have outlined evidence for this hypothesis in brief elsewhere (13) and illustrate the core principles in **Figure 1**. As our hypothesis approaches the topic of brain expansion from a different area of biology entirely, we refer readers to recent reviews of human brain evolution and development for subject-specific detail (see, for example, (5,14) and many references therein), and devote the remainder of this introduction to reviewing its supporting evidence. As our focus is selfish spermatogonial selection, a full treatment of other evolutionary forces shaping the brain-testis relationship is beyond the scope of this work, although we introduce some aspects in the **Supplementary Text**.

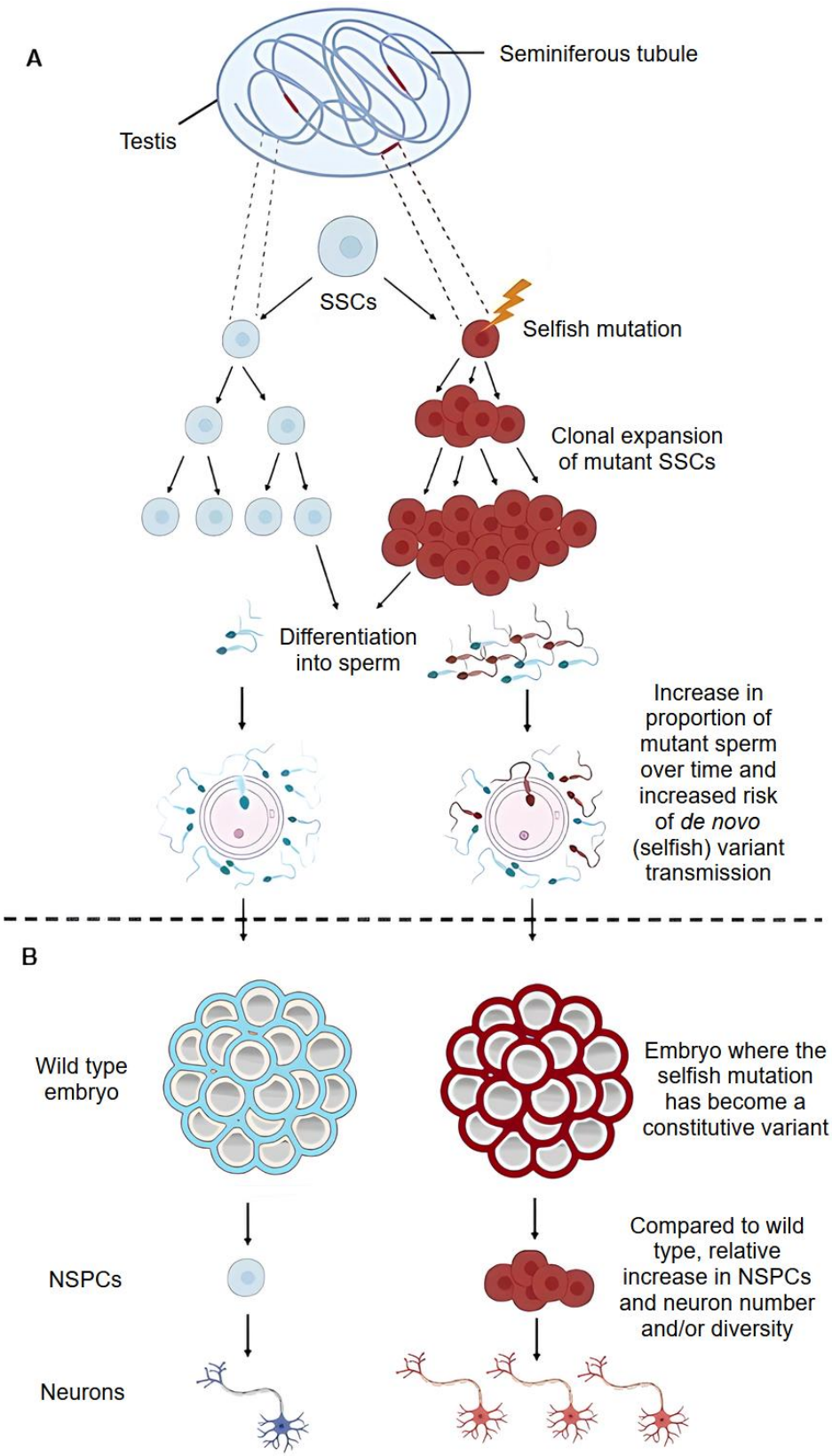

Figure 1. Variants underlying relative increases in brain size (through expansion of the neural progenitor pool) can be introduced into the genome by a male germline-specific evolutionary force, selfish spermatogonial selection.

(A) *In selfish spermatogonial selection, spontaneous oncogenic-like mutations occur in genes functionally involved in spermatogonial stem cell (SSC) regulation. These functional mutations confer a selective advantage to SSCs over their wild-type counterparts (blue), resulting in the*

clonal expansion of mutant SSCs (red) within the seminiferous tubules, increasing the relative proportion of mutant sperm over time. Sperm carrying selfish pathogenic mutations often give rise to offspring with neurodevelopmental disorders, but not all selfish mutations are inherently deleterious. This panel is adapted from that originally published in (12). ***(B)*** *As humans start development from a single zygotic cell, this acts as a bottleneck to fix selfish variants in the offspring. As such, 'mild' selfish mutations (ones selectively beneficial to SSCs and fertility, but not overly detrimental as a constitutive variant in the offspring) may become a source of heritable material across generations and fixed in the population. As selfish selection enriches the genome for mutations affecting SSC regulation, and given that many of the same genes are also expressed in neural stem and progenitor cells (NSPCs), we propose that this process may have introduced functional variants that also promote expansion of the NSPC pool, providing a substrate for both brain size expansion, regional diversification and/or increased complexity*.

Selfish spermatogonial selection is a process taking place during adult spermatogenesis whereby a subset of spontaneously occurring oncogenic-like ('selfish') mutations confer a selective advantage to mutant spermatogonial stem cells (SSCs) which then clonally expand along the length of the seminiferous tubules as men age (see reviews (12,15,16)). As a consequence, selfish mutations disproportionately accrue in the sperm of older men and, compared to classical spontaneous mutations, have a higher likelihood of being inherited as a constitutive mutation in the next generation. To date, the best characterised examples of selfish mutations are pathogenic gain-of-function single nucleotide variants (SNVs), typically (but not exclusively (17)) occurring within components of the RTK/RAS/MAPK signalling pathway, a central regulator of testicular homeostasis (18,19) (specifically, in the genes *BRAF*, *CBL*, *FGFR2*, *FGFR3*, *HRAS*, *KRAS*, *MAP2K1*, *MAP2K2*, *NRAS*, *PTPN11*, *RAF1*, *RET*, *SMAD4* and *SOS1*; see (12,17) and references therein, plus (20) for additional candidates). These selfish mutations are overwhelmingly associated with severe conditions in the offspring including skeletal dysplasia and neurodevelopmental disorders, such as RASopathies (15). While these pathogenic phenotypes are complex and heterogenous (individuals carrying identical RTK/RAS/MAPK mutations can exhibit different clinical presentations (21)), they frequently include features of cranial overgrowth or macrocephaly, a disproportionately large head (22). This is likely because many genes with selfish mutations play functional roles in regulating cell fate decisions between symmetric and asymmetric divisions during neurogenesis (discussed further in the **Supplementary Text**).

These observations provide the empirical grounds for our hypothesis – namely, that the clonal dynamics of SSCs have broader implications beyond fertility, and that changes in mammalian brain volume may, at least in part, have been shaped by the selfish properties of the testis. Despite vast differences in structure and function, the testis and brain share many cellular and molecular similarities, including substantial overlap of both their proteomes and transcriptomes (23), larger repertoires of splice variants than any other organ (24), and – more specifically, and importantly for our hypothesis – coordinated cell fates, with the neural lineage and the germline co-regulated during early development (25). Accordingly, many characteristic markers of spermatogonia can be functionally associated with neurogenesis and brain expansion (13) (see also **Supplementary Text**).

As an evolutionary force, selfish selection favours the introduction into our genome of functional variants altering specific molecular pathways which modulate the self-renewing activity of SSCs (13). As such, when mutant cells in the SSC pool differentiate into sperm, they disproportionately propagate the selfish mutation. Provided that the impact of the selfish mutation, when transmitted as a heterozygous variant to the offspring, is not overly deleterious to the somatic health of the individual, it will become a source of heritable material in the generations to follow. Importantly, tight control of the self-renewal of stem cells at the onset of neurogenesis is essential for generating a large pool of progenitors (see review (26)), the size of which predicts both mature adult brain volume and the relative size of brain regions (27). Consistent with this, mathematical modelling suggests that an additional round of symmetric rather than asymmetric (neurogenic) divisions by

neuroepithelial stem cells would double the number of neurons in the cortex (28). Further supporting a link between male reproduction and neurogenesis, it has also been demonstrated in human brain organoids that androgens (regulators of spermatogenesis) can increase the number of proliferative divisions of cortical progenitors (29).

Female reproductive tissues (which have a similar proteome to the testis (30)) may also be related to brain expansion as they are enriched in genes associated with human endocranial globularity, a phenotype unique to our species and reflecting the timing and pattern of brain growth (31). Similarly, a recent study which performed *in silico* modelling of hominin brain expansion argued that increased brain size was not primarily driven by direct selection but by developmental constraints on fertility (specifically, for an increased number of ovarian follicles, although the study did not have data for males) (32); these constraints may have diverted evolutionary change in a direction that led to brain expansion, such that this phenotype evolved as a spandrel (33).

While we believe these examples of a close association between the germline and brain development are compelling, here we take a more systematic approach to develop our hypothesis. The outstanding challenge with characterising molecular similarities between the brain and either spermatogonia or SSCs (a subset of undifferentiated spermatogonia (34)) is relative paucity of data: not only are spermatogonia a rare cellular population (35) but that compared to other human tissues, relatively few single-cell transcriptomic datasets are available for testis (36). Accordingly, the molecular identity of spermatogonia, and their subsets, remains incompletely understood (37). To address this, we had previously created an integrated single-cell atlas of 34 publicly-available adult human testis samples, reasoning that this would increase the resolution at which we could view spermatogonia and follow their developmental trajectory (38).

Here we combine data from multiple single-cell testis atlases (our own (38), plus that of a previous study (39) and the HumanTestisDB (40)) and the Human Protein Atlas (41–43) to assess the extent to which genes functionally associated with brain expansion and development are also expressed in the testis. We find that of thousands of proteins functionally associated with the brain, the majority are not only expressed in male germ cells, but show particular enrichment in spermatogonia. Furthermore, we also find the converse to be true: genes enriched in spermatogonia, or with human-specific roles in spermatogenesis, are widely expressed throughout the brain. We contextualise these results with an extensive discussion of the literature and conclude that, taken together, further enquiry into the testis-brain connection may provide novel insight into the evolutionary processes that shaped the human condition. Accordingly, we provide extensive datasets (**Supplementary Tables 1 to 7**) as a resource to further this purpose.

Materials and Methods

Sources of raw data

Our analyses draw on several publicly accessible proteomic and transcriptomic datasets. Firstly, from the Human Protein Atlas (HPA) v23 (41–43), we obtained the files ‘normal_tissue.tsv’ and ‘rna_tissue_hpa.tsv’ which represent, respectively, protein-level expression for 56 tissues and 122 distinct cell types (of which 8 cell types were characterised for the testis) and transcript-level expression for 40 tissue types (https://v23.proteinatlas.org/download/normal_tissue.tsv.zip and https://v23.proteinatlas.org/download/rna_tissue_hpa.tsv.zip, downloaded 17th June 2023).

Secondly, we obtained an integrated single-cell atlas of 60,427 adult human testicular cells (representing data from 29 individuals across 9 different studies (44–52) and an age range of 14-66 years), the bioinformatic methods for which we described in previous work (38) and briefly recapitulate here. To create this atlas, we generated cell x gene count matrices using the kallisto/bustools v0.26.3 ‘count’ workflow (53), which were then processed using Seurat v4.0.3 (54) with SCTransform normalisation (55) and integrated using Seurat’s rPCA ‘anchor’ technique. Using an unsupervised clustering approach (the Smart Local Moving algorithm (56)), we then identified 10 distinct cell clusters, which were annotated using both a global (all-against-all) differential gene expression analysis and established biomarkers for the major somatic and germ

cell types (sourced from (44,46,51,57–60) and as previously described (38)). These comprised 3 somatic cell clusters ('myoid and Leydig cells', 'Sertoli cells', and 'endothelia and macrophages', collectively 10,788 cells) and 7 germ cell clusters ('undifferentiated spermatogonia' (undiff SPG), 'differentiating spermatogonia' (diff SPG), 'spermatocyte', 'early spermatid 1', 'early spermatid 2', 'late spermatid 1' and 'late spermatid 2', collectively 49,639 germ cells). The differentially expressed genes in each cluster (that is, cell type) were identified using Seurat's FindAllMarkers function with parameters min.pct = 0.25, logfc.threshold = 0.25 and only.pos = TRUE which, respectively, require that a gene is expressed in >25% of the cells in a given cluster, that it has a $\log_2$ fold-change difference in expression relative to all other clusters of > 0.25 (that is, > 2-fold average expression) and that it is on average more highly expressed in that cluster compared to all others.

Protein-coding genes functionally associated with the human brain

To assess the degree of overlap between genes expressed in the testis and functionally associated with the brain, we first compiled an inclusive list of 'brain genes' on the basis of either an explicit or suggestive phenotype (**Supplementary Table 1**). This comprised 7193 human brain-associated protein-coding genes (36% of the 19,871 in total in the hg38 primary assembly), collated from previous studies of genes associated with macrocephaly (61), megalencephaly (i.e. abnormal brain size) (62), postmortem brain weight (63,64), cognitive function (using intelligence quotient (65) and educational attainment (66) as provisional proxies), and human-accelerated regions (67) (otherwise conserved genomic loci with human-specific substitutions, the expression of which spatially correlates with patterns of cortical expansion (68)). We also collated genes from previous reviews of the molecular factors governing brain development (14), human cognition (69), neocortical evolution (70,71), autism spectrum disorder (72–76), schizophrenia (77–79), genetic epilepsies (80–83), and indirect neurogenesis, a process thought to underlie the evolution of the gyrencephalic cortex (84). The overall list is presented in **Supplementary Table 1**, alongside notes on the sources, summary statistics, and the classification of each gene by the nature of its association. As a resource to support further enquiry, genes are assigned, where possible, to one or more of the seven categories of macrocephaly/megalencephaly (n=379 genes), brain weight (n=879), autism (n=2562), schizophrenia (n=345), epilepsy (n=3372), intelligence/educational attainment (n=2303), or human-acceleration (n=1608). We consider the first five of these seven categories to be explicit rather than suggestive phenotypes and to collectively constitute a conservative set of 5212 brain-associated genes.

Results and Discussion

Genes associated with brain growth and development are widely expressed in the male germline

Multiple genes have been functionally associated with increased brain mass or found to be positively selected along the primate phylogeny or the lineage leading to humans (see reviews (9,14,70,71)). We hypothesised that as the brain and testis have extensive, overlapping, transcriptomes (23), many of these brain-growth-associated genes may also be active in the male germline. To assess this, we draw upon both the Human Protein Atlas v23 (41–43) and an integrated single-cell transcriptomic atlas of 60,427 human testicular cells (**Figure 2**), which we have previously described (38).

Using the single-cell testis atlas, we first examined the expression levels of a list of 7193 brain-associated protein-coding genes (**Supplementary Table 1**). Although extensive, we did not assemble this list systematically or apply filters beyond those of the original authors (see notes to **Supplementary Table 1**); rather, our initial intention was to determine whether even with a broad and unselected 'brain gene' list, there would be significant overlap with expression in the testis. We summarise the testicular expression of each gene at the protein-level in **Supplementary Table 1** and at the transcript-level in **Supplementary Tables 2 and 3**. Of the 7193 genes in **Supplementary Table 1**, 2392 (33%) had either no available protein expression data or no data marked other than

‘uncertain’, and so we excluded these from subsequent summary statistics. Of the 4801 remaining genes, experimental antibody staining had been documented; for 2467 (51%) genes this included evidence for three somatic (peritubular, Leydig, and Sertoli) and five germ cell types, including spermatogonia (detailed further in the notes to **Supplementary Table 1**). For the other 2334 genes (49%), staining had only been performed for two cell types: Leydig cells and the non-specific set of ‘cells in seminiferous ducts’ (that is, seminiferous tubules, comprising all germ cells and Sertoli cells). The HPA only contains the more specific level of antibody staining data (eight cell types, rather than two) for genes with testis-elevated mRNA expression (detailed at https://www.proteinatlas.org/humanproteome/tissue/testis, accessed 12th May 2024). We considered protein expression in seminiferous ducts to represent highly probable germline expression as Sertoli cells, which are the only non-dividing somatic cells within them, represent only a few percent of the total number of duct cells (85). Even if we could not exclude the possibility that some proteins detected in the seminiferous ducts would be Sertoli cell-specific, we note that, of the 2467 genes stained for eight cell types, only 76 (3.1%) could only be detected in Sertoli cells (**Supplementary Table 1**).

Overall, we found that 73% of brain-associated genes had either experimentally-supported or highly probable germline expression (that is, 3512 of 4801 genes for which data was available, irrespective of whether antibody staining was performed only for seminiferous ducts or for specific germ cells; **Supplementary Table 1**). Furthermore, of the 2467 genes where antibody staining data was available for specific germ cells, 2049 brain-associated genes (83%) had detectable protein-level expression in at least one germ cell type, more of which (n = 1476, 59%) were expressed before meiosis (in spermatogonia) than either during (in preleptotene or pachytene spermatocytes; n = 1258, 51%) or after (in round or elongated spermatids; n = 1328, 54%) (**Supplementary Table 1**). The number and distribution of brain-associated genes expressed in the testis is summarised in **Figure 2**.

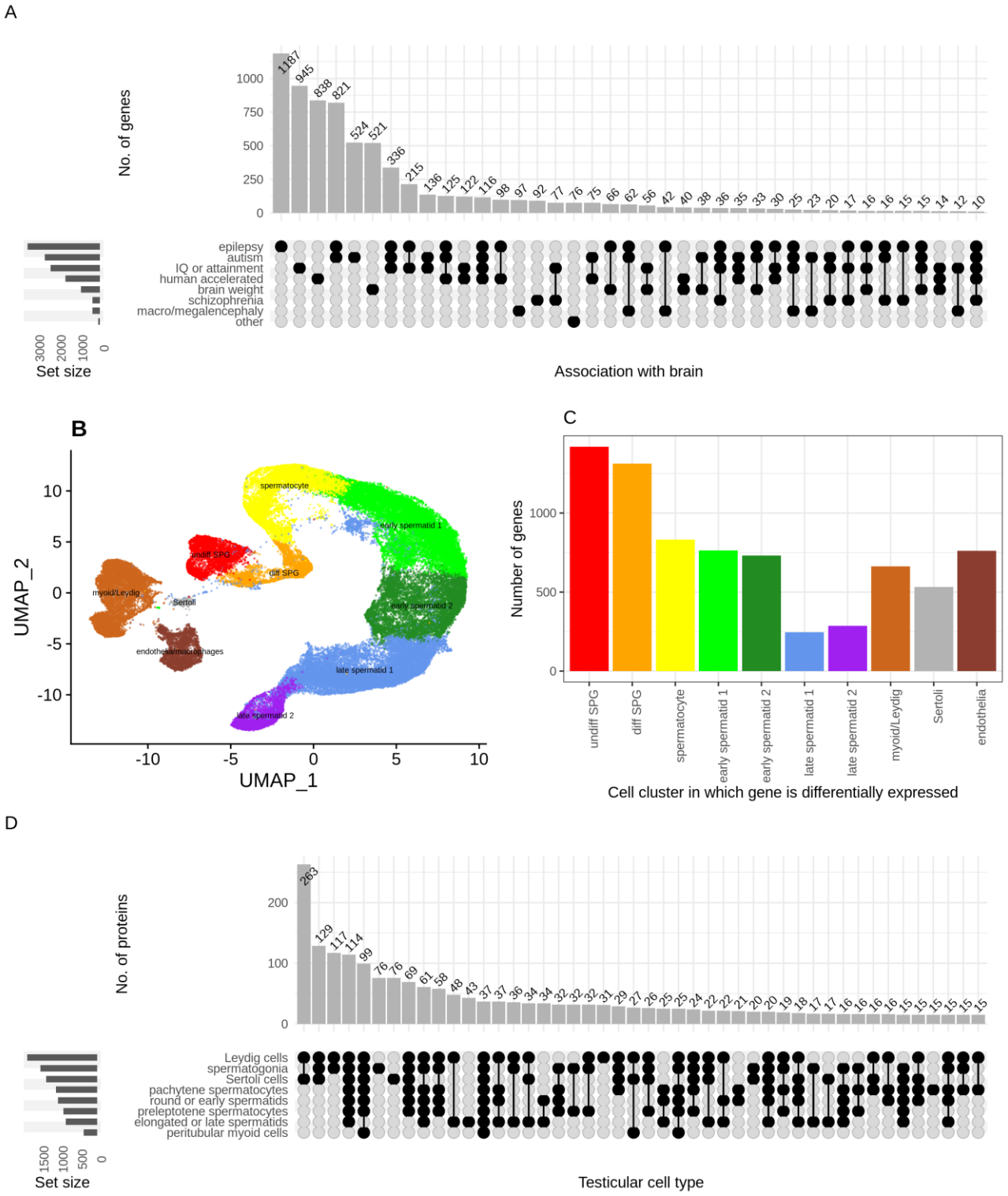

Figure 2. Brain-associated genes and their expression in the human testis.
(A) *Distribution of 7193 genes among an inclusive set of brain-associated phenotypes, both explicit (such as macro- or megalencephaly) and suggestive (such as 'educational attainment', a proxy for cognitive function), detailed further in notes to* ***Supplementary Table 1****. Sets containing < 10 genes are not shown.* ***(B)*** *Single-cell expression atlas of the adult human testis (n = 60,427 cells), annotated into ten cell clusters (3 somatic, 7 germline).* ***(C)*** *Number of genes differentially expressed, at the transcript level, in at least one of these ten clusters (raw data in* ***Supplementary Tables 2 and 3****).* ***(D)*** *Protein-level expression of 2049 brain-associated genes for which antibody staining data was available for each of 8 testicular cell types (3 somatic, 5 germline) and with a*

protein detected in at least one (raw data from https://v23.proteinatlas.org/about/download/normal_tissue.tsv, accessed 17th June 2023). Sets represent detectable protein-level expression (i.e. classification into either of the HPA categories of low, medium or high expression) in each cell type (raw data in ***Supplementary Table 1****). Sets containing < 15 proteins are not shown. A version of this figure restricted to 5212 genes with five explicit brain-associated phenotypes (postmortem brain weight, macrocephaly/megalencephaly, epilepsy, autism, and schizophrenia) is presented as* ***Supplementary Figure 1*** *and shows quantitatively similar results.*

To further assess whether genes associated with brain growth and development have enriched expression in spermatogonia, we next considered transcript-level expression (**Supplementary Tables 2 and 3**). The testicular transcriptome and proteome are surprisingly dissimilar (86): while the testis is abundant in mRNAs, many proteins with high testicular mRNA expression are 'missing' in that they have yet to be identified by antibodies or mass spectrometry, with no obvious technical explanation available (although a compelling suggestion is that because these 'missing' proteins are enriched for spermatogenesis-related processes, they have transient, specialised, functions and are rapidly degraded (86)).

Using data from the testicular single-cell RNA-seq atlas (38), we found that 6293 of the 7193 brain-associated genes (87%) were detectably expressed in the germline (having one or more sequenced reads in >1% of the germ cells; **Supplementary Table 3**). The number of genes differentially expressed in a germ cell cluster declined across the spermatogenic trajectory, being at its highest for 'undiff SPG' (1420 genes, or 20% of the total, including both *NOVA1* (10) and *TKTL1* (11), two genes in which human-specific substitutions have been implicated in neocortical expansion), then decreasing to 1312 ('diff SPG'), 831 ('spermatocyte'), 763 ('early spermatid 1'), 731 ('early spermatid 2'), 245 ('late spermatid 1') and 286 ('late spermatid 2') (**Figure 2** and **Supplementary Table 2**).

Overall, we interpret these findings to mean that many genes functionally associated with brain growth are expressed not only in the testis but show particularly enriched expression in undifferentiated spermatogonia, more so than either meiotic or post-meiotic adult germ cells (**Figure 2C**). We note, however, that many of the genes in **Figure 2A** have suggestive (such as IQ (65) or educational attainment (66), broad proxies for cognitive function) or indirect (such as 'human-accelerated sequence' (67)) associations with the brain, but that these do not represent discreet phenotypes. Nevertheless, if we repeat the analysis after excluding genes found only in these categories, we obtain quantitively similar results (**Supplementary Figure 1**). Moreover, we found brain-associated genes to be significantly more highly expressed in undifferentiated spermatogonia than all other germ cell types, excepting spermatocytes (Mann-Whitney U $p < 0.05$; **Supplementary Figure 2**).

In terms of single cell expression profiles, the male germline resembles a continuum of 'states' (**Figure 2B**) with its division into discrete cell types imposed by a clustering algorithm. As such, it is possible that our conclusions about the relative enrichment of 'brain genes' across the germline (that is, degree to which they are differentially expressed; **Figure 2C**) are dependent on choice of method. To ensure our results were robust to this, we repeated the analysis using comparable Seurat clustering results from four previously published human single-cell whole-testis and male-germline atlases (39,40), each of which annotated their cell types independently and varied in the cells used, age range, granularity, and methodology (described further in **Supplementary Table 4**). Regardless of dataset, we found more brain-associated genes differentially expressed in germ than somatic cells (**Supplementary Figures 3 and 4**). Consistent with the results from our own expression atlas (38), the differentially expressed genes were also particularly abundant towards the start of the spermatogenic trajectory (**Supplementary Figures 3 and 4**). However, unlike our own atlas, each of these alternative datasets included foetal, pre-, and peri-pubertal cells (**Supplementary Table 4**), and accordingly the cell type with the highest proportion of differentially expressed genes was often 'adjacent' to spermatogonia in the germ cell trajectory, namely leptotene spermatocytes and

primordial germ cells (PGCs; **Supplementary Figures 3 and 4**). Notably, PGCs differentiate into fetal spermatogonia (87) which, transcriptomically, closely resemble 'state 0' adult spermatogonia, considered the most undifferentiated state (88).

To test whether our results were driven by genes associated with a specific brain phenotype, we repeated our analysis for each of the seven phenotypic categories independently. We consistently found differentially expressed genes enriched in the spermatogonia of our own atlas (**Supplementary Figure 5**) and, with few exceptions, around the start of the spermatogenic trajectory in four others (**Supplementary Figures 6, 7, 8 and 9**). We next assessed whether our observed overlap of testis and brain expression reflected a broader overlap of developmental or housekeeping genes (given their disproportionate association with cell proliferation). To do so, we plotted, per brain phenotype, the number of proteins detected in each of the 56 tissues comprising the HPA. As expected, we found that the greatest proportion of each set of genes (proteins) were expressed in cerebral cortex, cerebellum, and testis (**Supplementary Figure 10**).

Further supporting a bidirectional link between genes functional in the brain and male germline, we also found evidence for the converse: genes enriched in spermatogonia, including those with human-specific roles in spermatogenesis, are widely expressed throughout the brain (discussed further in the **Supplementary Text**; see also **Supplementary Tables 5 and 6**). Moreover, given the breadth of the HPA antibody staining data, it also offers an additional line of evidence for a functional connection between the male germline and brain: proteins whose expression is restricted to the two tissues. Although we found only 16 genes out of 5379 (0.3%) whose proteins were only detectable in both a male germ and brain cell, and no other cell type in any other (healthy) tissue (**Supplementary Table 6**), these represent potential entry points for further exploring the molecular parallels between the human brain and the male germline. Notably, three of these genes – *ERMN* (which promotes oligodendroglial differentiation (89)), *HTR2A* (ectopic expression of which increases the number of basal progenitor cells in embryonic mice (90)), and *OPALIN* (which promotes oligodendrocyte terminal differentiation (91)) – have been associated with increased postmortem brain weight (63), one (*HYLS1*, a conserved centriole protein (92)) with macrocephaly (61), and one (*OMG*, an oligodendrocyte glycoprotein which inhibits cell proliferation (93)) with a co-evolutionary relationship with primate brain mass (64) (**Supplementary Table 1**). Nevertheless, we could not exclude the possibility that some may also be expressed in unsampled tissues or at levels below the detection thresholds of the HPA dataset.

Having demonstrated that many genes expressed in spermatogonia are functionally associated with the brain, including the brain-expansion phenotypes of macrocephaly and megalencephaly, we next considered whether the germline was also enriched for genes harbouring human-specific variants, as these are disproportionately associated with neurodevelopment (68).

Proteins with human-specific amino acid changes are simultaneously enriched for neurodevelopmental functions and widely expressed in male germ cells

Advances in the scope and quality of genome sequencing have facilitated study of the genetic basis of the human condition, identifying a number of variants which distinguish modern humans from archaic hominins (see review (94)). Although the set of mutations underpinning human-specific traits are dependent on archaic sample availability, reference genome annotation and coverage, as well as genotype calling and filtering methods, there is nevertheless a broad consensus on the strongest candidates – protein-coding changes in cell cycle-related genes to which the brain is especially sensitive (95). A study which compared human variants to that of Denisovan and Neanderthal genomes identified 571 genes containing non-synonymous amino acid changes (all at high variant allele frequencies), and another 36 genes in which 42 amino acid changes were fixed in modern humans (95). Enrichment analyses suggested that these genes mostly affected cell division and the brain growth trajectory, with noticeable expression in the frontal cortex during infancy (95). However, this gene set could also be associated with the testis, as it is significantly enriched for the GO term 'spermatoproteasome complex'. Furthermore, 3 of the 42 fixed amino acid changes were

found in one gene, *SPAG5* (sperm-associated antigen 5), involved both in meiosis and spermatid morphogenesis (96) (yet dispensable for fertility (97)) but also expressed in the ventricular zone of the brain during fetal development, when cortical neurons are formed (98). *SPAG5* encodes a mitotic spindle protein, with mutant phenotypes consistent with a critical role in whether a cell divides symmetrically or asymmetrically (94). This is particularly relevant to NSPCs as the orientation of the mitotic cleavage plane can impact cell fate decisions, and thereby the composition of the progenitor pool (99).

On the basis of these observations, we hypothesised that many of the genes containing human-specific amino acid changes would also be detectably expressed (and putatively functional) in the male germline, and spermatogonia in particular. To assess this, we provide the protein-level expression in the male germline of 309 of this total set of 607 genes in **Supplementary Table 7** (genes with no available protein data, or no data marked other than 'uncertain', are excluded), of which 70% had either experimentally-supported or highly probable (that is, in seminiferous ducts) male germline expression. Furthermore, of the 152 genes where protein expression data was available for five germ cell types, 22 were only detectable in spermatogonia (compared to 4 in spermatocytes and 15 in spermatids). These include *ASCC1*, mutations in which downregulate genes associated with neurogenesis and neuronal migration (100), *LRRTM1*, an imprinted gene expressed throughout forebrain development and associated paternally with cerebral asymmetry (101), and *TCF3*, which represses neuronal differentiation and increases the self-renewal of NSPCs during neocortical development (102).

General discussion: a role for the male germline in the expansion of the mammalian brain

We had previously hypothesised that the dysregulation of male germline homeostasis provides a novel source of insight into the mechanisms underlying mammalian brain expansion (13). In the present work we develop this hypothesis by assessing the extent to which genes functionally involved in brain growth, development or evolution were also expressed in the male germline. In conjunction with numerous observations from the literature, we summarise our results, alongside other evidence supporting our hypothesis, in **Table 1**.

Evidence	Source
Strong (pathogenic) selfish spermatogonial mutations produce neurodevelopmental disorders widely associated with macrocephaly, establishing an empirical link between cell proliferation in the testis and brain.	Reviewed in (12,15,16).
Macrocephaly occurs above chance level in individuals with *de novo* variants in mTOR-related genes, key mediators of spermatogonial stem cell maintenance and differentiation (103).	Results from (104), using 101 mTOR-related genes defined by (105).
Non-penetrant (or mildly deleterious) selfish mutations may be inherited and maintained across generations. Thus, they may progressively accumulate such that their impact on particular signalling pathways is additive.	Discussed in the present work, and also (106).
Selfish spermatogonial selection affects numerous genes involved in testicular homeostasis – and which are shared with the brain – and is a prevalent natural phenomenon in the ageing testis.	14 genes are already known to harbour selfish spermatogonial mutations (12,17), with recent large-scale trio sequencing proposing 27 more candidates (20).
Many genes under positive selection in the human male germline (ostensibly for	Of 40 genes positively selected in the human male germline (107), 29 can be associated with

reproductive phenotypes) can be associated with macrocephaly.	brain growth, function, development, or evolution, listed in **Supplementary Table 1** (*ARHGAP35, BMPR2, BRAF, CBL, CTNNB1, CUL3, DDX3X, EP300, FGFR2, FGFR3, HRAS, KDM5B, KDM5C, KMT2D, KMT2E, KRAS, MIB1, NF1, NSD1, PTEN, PTPN11, RAF1, RASA2, RBM12, RIT1, ROBO1, SCAF4, SMAD4, TCF12*). Three others (*CSNK2B*, *FAM222B*, *PRRC2A*) are family members of those in **Supplementary Table 1** (*CSNK2A1*, *FAM222A*, *PRRC2C*). Underlined genes are associated with either macrocephaly or megalencephaly.
An *in silico* model of hominin brain expansion suggests that human brain size evolves as a by-product of selection for fertility-determinant traits.	Results from (32,33).
Of thousands of proteins functionally associated with the brain, many are also expressed in male germ cells, particularly spermatogonia. The converse is also true: genes enriched in spermatogonia, or with human-specific roles in spermatogenesis, are widely expressed throughout the brain.	**Figure 2**, **Supplementary Figures 2 to 9**, **Supplementary Table 1**, and **Supplementary Text**.
Many genes have been functionally characterised with dual roles in both the male germline and neural stem and progenitor cells, with specific mutations associated with human brain expansion (for example, in *TKTL1* (11)).	Evidence for a functional role in both human brain expansion and spermatogonia is described for ten genes in (13). Other genes expressed in spermatogonia and with roles in cell proliferation in the brain are described in the **Supplementary Text**.
In the Human Protein Atlas, very few proteins are only detected in both a male germ cell and brain cell (and no other cell type), but those that are can be associated with increased postmortem brain weight (ERMN, HTRA2A, OPALIN) and macrocephaly (HYLS1).	**Supplementary Table 6**.

Table 1. Overview of the lines of evidence implicating a link between the male germline, and spermatogonia in particular, and human brain expansion.

Our entryway into this topic was the detection of spontaneous oncogenic-like mutations in spermatogonial stem cells, in genes that are functionally critical to brain development. The underlying mechanism provides a causal link between the otherwise disparate fields of spermatogenesis, neurogenesis, and oncogenesis. This link may offer a novel perspective on a number of observations. For instance, a genome-wide association study of the genetic variants associated with human head size (108) (a highly heritable trait and strong correlate of brain size) identified 67 candidate loci. These loci were disproportionately located near genes preferentially expressed in intermediate neural progenitors, whose increased proliferation has been linked to human brain expansion (109), as well as being enriched for pathways involved in both macrocephaly and cancer. Consistent with our hypothesis, many of the proteins encoded by these genes are abundant in spermatogonia, including ALAS1, BNIP3L, FOXO3, HMGA2, IGF2BP1, NCK1, NFIX, PPP6R3, and STAG1 (**Supplementary Table 1**).

In addition, a genome-wide association study of human brain magnetic resonance imaging (MRI) data (110) identified 199 loci significantly associated with either cortical surface area or thickness, implicating effects upon NSPC proliferation rate and their number of neurogenic divisions, respectively. Of note, many of these loci occur in regions functionally linked to genes involved in Wnt signalling, which among other developmental processes regulates spermatogenic cell fate (111). Also consistent with our hypothesis, a number of these loci occur within genes functionally associated with the establishment and maintenance of the male germline, including rs1934057 in *PAX7* (a marker of spermatogonial stem cells in mice (112)), rs2999158 in *MOV10* (knockdown of which affects spermatogonial cell fate decisions in mice (113)), three SNPs (rs17834032, rs75341124, rs78445564) in *DAAM1* (involved in the cytoskeletal dynamics underpinning post-natal development of the rat testis (114)), and four SNPs (rs1178101, rs12536836, rs6461386, rs10237280) in *HDAC9* which, in chickens, promotes the differentiation of embryonic stem cells into male germ cells (115).

As discussed above, these commonalities reflect the shared developmental and regulatory origins of the neural lineage and the germline. In humans, this coordination centres on the early specification of primordial germ cells (PGCs), mediated by the core transcriptional network of *OCT4* (*POU5F1*), *PAX5*, and *PRDM1* (*BLIMP1*). This regulatory 'master switch' simultaneously activates germline and represses somatic differentiation programmes (the default for which in mice is neuronal (116)), thereby committing cells to a germline fate (reviewed in (117,118)). The establishment and maintenance of this fate requires extensive chromatin reconfiguration (119); disruption of this process can lead to reprogramming, with PGCs aberrantly expressing somatic genes. Tellingly, when germ cells reprogram as soma in *C. elegans*, they often express pan-neuronal markers, extrude neurite-like projections, and differentiate into neurons (120).

Although our focus here has been on the conceptual links between SSCs and NSPCs (as the primary stem cells responsible for sperm and neuron production), the genetic architecture of brain size is multifaceted and complex (121). It includes, among others, gene duplication (122,123), gene family size variation (124–126), and changes to coding (127,128), non-coding (129), and regulatory (130) sequences, these mechanisms collectively giving rise to relatively large brains through multiple independent evolutionary trajectories (131). Moreover, larger brains are not only defined by a greater number of neurons: they also differ in neuronal size, regional specialisation, and packing density, as well as their number and proportion of somatic support cells (glia) (132). Importantly, not all genes implicated in brain size expansion are functionally active in the testis as the overlap between testis and brain proteomes, although substantial, is not exhaustive. For example, human-specific *NOTCH2NL* genes, which arose from recent gene duplication events to play critical roles in cortical neurogenesis (133), have no known association with spermatogenesis.

To conclude, we have drawn upon the biology of the testis to offer a fresh perspective on an old question: how did the human brain become so large? We believe new insight into this problem can arise from selfish spermatogonial selection, a male germline-specific evolutionary force that enriches genomes for oncogenic-like mutations in pathways involved in the regulation of spermatogonial stem cells – and which are also functional in neural stem and progenitor cells. In this respect, we can conceive of the male germline as both a generator of mutations and a filter, disproportionately passing to the next generation those of a particular ('selfish') nature – ones benefiting reproductive success and robust sperm production whilst ageing. It follows that if selfish mutations disproportionately accumulate within pathways essential for germline proliferation and fertility, they may also influence the phenotype of other (somatic) cells in which the same genes are expressed. Consistent with this, protein-coding changes in genes involved in cell cycling rank among the strongest candidates for human-specific sequence variation (95).

Scaled up to the population level, the long-term effect of this additive, non-random, germline mutagenesis may have been to expose to selection variants that modulate baseline rates of cell division, potentially exerting second-order effects in related lineages, such as neural progenitors. This relates to current theories of human brain evolution through the concept of bradychrony

('slowed time'), which refers to the relative slowing of equivalent developmental processes between species (134). Notably, differences in developmental tempo correlate with differences in brain size. In humans, brain maturation is markedly protracted (bradychronic) compared to other primates, taking a longer proportion of the lifespan to reach adult brain weight (134). Early human neurodevelopment is particularly prolonged, with the time window for tangential cortical expansion (driven by symmetrically-dividing radial glial cells) lasting approximately a month in humans but a fortnight in macaques (134). The mechanisms driving differences in developmental tempo are an area of active enquiry with, for example, calcium regulation playing a regulatory role in neuronal development (135). We suggest that selfish selection may also contribute by modulating the relative rate of stem cell turnaround, thereby influencing the duration and extent of neurogenic output.

Conclusion

We have highlighted a pronounced, yet largely unexplored, role for the male germline – and spermatogonia in particular – in shaping both the development and evolution of the brain. Alongside identifying a substantial overlap between genes expressed in spermatogonia and functionally associated with brain development, we draw conceptual parallels between the neural and spermatogonial stem cell lineages noting that the timing and balance of self-renewing and differentiating cell divisions are not only critical to both but especially so for longer-lived species (such as humans), who reproduce over many decades. We suggest that variants selfishly selected in testes (12,15,16) may bias this balance towards increasing cellular output (i.e. more sperm and/or neurons), thereby influencing both fertility and cognitive potential. We hope our results will encourage further enquiry into this topic as we find it particularly compelling to consider how mutations selectively advantageous to human spermatogonia may have been co-opted by other tissues, contributing in part to the genomic innovations necessary for our larger brains.

Supplementary Figures

Supplementary Figure 1. A conservative set of brain-associated genes and their expression in the human testis.
(A) Distribution of 5212 genes among an explicit set of brain-associated phenotypes, detailed further in notes to **Supplementary Table 1**. Sets containing < 10 genes are not shown. **(B)** Single-cell expression atlas of the adult human testis (n = 60,427 cells), annotated into ten cell clusters (3 somatic, 7 germline). **(C)** Number of genes differentially expressed, at the transcript level, in at least one of these ten clusters (raw data in **Supplementary Tables 2 and 3**). **(D)** Protein-level expression of 1573 brain-associated genes for which antibody staining data was available for each of 8 testicular cell types (3 somatic, 5 germline) and with a protein detected in at least one (raw data from https://v23.proteinatlas.org/about/download/normal_tissue.tsv, accessed 17th June 2023). Sets represent detectable protein-level expression (i.e. classification into either of the HPA categories of low, medium or high expression) in each cell type (raw data in **Supplementary Table 1**). Sets containing < 15 proteins are not shown.

Supplementary Figure 2. Average expression across, respectively, an (A) inclusive and (B) conservative set of 7193 and 5212 brain-associated genes for each of ten cell clusters in an adult human testis expression atlas.
The brain-associated gene sets are detailed in **Supplementary Table 1** with expression level per testicular cell type in **Supplementary Table 2**. Statistical comparisons are Mann-Whitney U tests, implemented using the R package 'ggpubr.'

Supplementary Figure 3. Number of differentially expressed brain-associated genes per cell cluster, for each of four testis and male germline single-cell atlases, and using an inclusive set of brain-associated genes.

For each of the four single-cell atlases, barplots show the number of genes significantly differentially expressed within an inclusive set of brain-associated genes (n=7193). The cell atlases and their respective annotations are described in **Supplementary Table 4**, alongside the differential expression analysis results, which were filtered according to a common set of criteria. The brain-associated gene sets are detailed in **Supplementary Table 1**.

Supplementary Figure 4. Number of differentially expressed brain-associated genes per cell cluster, for each of four testis and male germline single-cell atlases, and using a conservative set of brain-associated genes.
For each of the four single-cell atlases, barplots show the number of genes significantly differentially expressed within an inclusive set of brain-associated genes (n=5212). The cell atlases and their respective annotations are described in **Supplementary Table 4**, alongside the differential expression analysis results, which were filtered according to a common set of criteria. The brain-associated gene sets are detailed in **Supplementary Table 1**.

Supplementary Figure 5. Number of brain-associated genes differentially expressed, at the transcript level, in one or more of ten testicular cell clusters.
The list of brain-associated genes is given in **Supplementary Table 1**, with raw data for this figure in **Supplementary Tables 2 and 3**. Panels A to G show the distribution of differentially expressed genes for seven different categories of phenotype, whereas panels H and I require that each gene is associated with at least 2 or 3 different categories, respectively, not including the two 'indirect' phenotypes of IQ/attainment and human-acceleration.

Supplementary Figure 6. Number of brain-associated genes differentially expressed, at the transcript level, in one or more of cell clusters from the 'Salehi 2023' single-cell expression atlas.
The 'Salehi 2023' expression atlas, its respective annotations, and the differential expression analysis results used for this figure, are detailed in **Supplementary Table 4**. The brain-associated gene sets are detailed in **Supplementary Table 1**. Panels A to G show the distribution of differentially expressed genes for seven different categories of phenotype, whereas panels H and I require that each gene is associated with at least 2 or 3 different categories, respectively, not including the two 'indirect' phenotypes of IQ/attainment and human-acceleration.

Supplementary Figure 7. Number of brain-associated genes differentially expressed, at the transcript level, in one or more of cell clusters from the 'Wang 2025 (all cells)' single-cell expression atlas.
The 'Wang 2025 (all cells)' expression atlas, its respective annotations, and the differential expression analysis results used for this figure, are detailed in **Supplementary Table 4**. The brain-associated gene sets are detailed in **Supplementary Table 1**. Panels A to G show the distribution of differentially expressed genes for seven different categories of phenotype, whereas panels H and I require that each gene is associated with at least 2 or 3 different categories, respectively, not including the two 'indirect' phenotypes of IQ/attainment and human-acceleration.

Supplementary Figure 8. Number of brain-associated genes differentially expressed, at the transcript level, in one or more of cell clusters from the 'Wang 2025 (germ cells)' single-cell expression atlas.
The 'Wang 2025 (germ cells)' expression atlas, its respective annotations, and the differential expression analysis results used for this figure, are detailed in **Supplementary Table 4**. The brain-associated gene sets are detailed in **Supplementary Table 1**. Panels A to G show the distribution of differentially expressed genes for seven different categories of phenotype, whereas panels H and I require that each gene is associated with at least 2 or 3 different categories, respectively, not including the two 'indirect' phenotypes of IQ/attainment and human-acceleration.

Supplementary Figure 9. Number of brain-associated genes differentially expressed, at the transcript level, in one or more of cell clusters from the 'Wang 2025 (germ cells – part 1)' single-cell expression atlas.
The 'Wang 2025 (germ cells – part 1)' expression atlas, its respective annotations, and the differential expression analysis results used for this figure, are detailed in **Supplementary Table 4**. The brain-associated gene sets are detailed in **Supplementary Table 1**. Panels A to G show the distribution of differentially expressed genes for seven different categories of phenotype, whereas panels H and I require that each gene is associated with at least 2 or 3 different categories, respectively, not including the two 'indirect' phenotypes of IQ/attainment and human-acceleration.

Supplementary Figure 10. Number of brain-associated genes whose proteins are detectably expressed in human tissues.
Panels A to G show the distribution of brain-associated genes for seven different categories of phenotype, whereas panels H and I require that each gene is associated with at least 2 or 3 different categories, respectively, not including the two 'indirect' phenotypes of IQ/attainment and human-acceleration. Raw data for this figure, representing protein expression level per cell type per tissue, was obtained from the Human Protein Atlas v23.0 (https://v23.proteinatlas.org/about/download/normal_tissue.tsv, downloaded 17th June 2023). A protein was considered expressed in a given tissue if, in any cell type of that tissue, 'level' was either 'low', 'medium' or 'high' and 'reliability' was not 'uncertain'. Cell types per tissue are detailed in **Supplementary Table 6**. For ease of comparison, brain tissues are coloured blue, testis red, and all others grey.

Supplementary Tables

Supplementary Table 1. Summary of the testicular expression, at the protein-level, of 7193 genes associated with brain growth, function, development, or evolution.

Supplementary Table 2. Transcript-level expression of genes associated with brain growth, function, development, or evolution, using an adult human testis single-cell expression atlas.

Supplementary Table 3. Summary of the testicular expression, at the transcript-level, of genes associated with brain growth, function, development, or evolution.

Supplementary Table 4. Sources of cluster (cell type) annotations, and Seurat differential gene expression analysis results, for four previously published single-cell human testis atlases.

Supplementary Table 5. Transcript-level expression in the brain of spermatogonia-enriched genes and human-specific germline-associated genes.

Supplementary Table 6. Proportion of male germline-expressed proteins detectably expressed in other tissues.

Supplementary Table 7. Summary of the testicular expression, at the protein-level, of protein-coding genes containing human-specific high-frequency missense changes.

Authors' contributions

Stephen J. Bush: conceptualisation, methodology, formal analysis, data curation, investigation, validation, visualisation, software, writing – original draft, writing – review & editing.
Anne Goriely: conceptualisation, validation, funding acquisition, writing – review & editing.

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Ethics statement

This work did not involve animal or human subjects and so no ethical approval was required.

Data availability

The data supporting this work is available either in the article itself or in its electronic supplementary material.

Code availability

All scripts used to perform the analyses and create both figures and tables are available at www.github.com/sjbush/testis_brain.

Competing interests statement

The authors declare that there are no conflicts of interest.

References

1. Nomura T, Gotoh H, Ono K. Changes in the regulation of cortical neurogenesis contribute to encephalization during amniote brain evolution. Nat Commun. 2013;4:2206.

2. Kaas JH. The evolution of brains from early mammals to humans. Wiley Interdiscip Rev Cogn Sci. 2013 Jan;4(1):33–45.

3. Barton RA. Primate brain evolution: Integrating comparative, neurophysiological, and ethological data. Evolutionary Anthropology: Issues, News, and Reviews. 2006 Nov 1;15(6):224–36.

4. Geschwind DH, Rakic P. Cortical Evolution: Judge the Brain by Its Cover. Neuron. 2013 Oct 30;80(3):633–47.

5. Lancaster MA. Unraveling mechanisms of human brain evolution. Cell. 2024 Oct 17;187(21):5838–57.

6. Otani T, Marchetto MC, Gage FH, Simons BD, Livesey FJ. 2D and 3D Stem Cell Models of Primate Cortical Development Identify Species-Specific Differences in Progenitor Behavior Contributing to Brain Size. Cell Stem Cell. 2016 Apr 7;18(4):467–80.

7. Homem CCF, Repic M, Knoblich JA. Proliferation control in neural stem and progenitor cells. Nature Reviews Neuroscience. 2015 Nov 1;16(11):647–59.

8. Dyer MA. Stem Cells Expand Insights into Human Brain Evolution. Cell Stem Cell. 2016 Apr 7;18(4):425–6.

9. Huttner WB, Heide M, Mora-Bermúdez F, Namba T. Neocortical neurogenesis in development and evolution-Human-specific features. J Comp Neurol. 2024 Feb;532(2):e25576.

10. Trujillo CA, Rice ES, Schaefer NK, Chaim IA, Wheeler EC, Madrigal AA, et al. Reintroduction of the archaic variant of NOVA1 in cortical organoids alters neurodevelopment. Science. 2021 Feb 12;371(6530):eaax2537.

11. Pinson A, Xing L, Namba T, Kalebic N, Peters J, Oegema CE, et al. Human TKTL1 implies greater neurogenesis in frontal neocortex of modern humans than Neanderthals. Science. 377(6611):eabl6422.

12. Wood KA, Goriely A. The impact of paternal age on new mutations and disease in the next generation. Fertility and Sterility. 2022 Dec 1;118(6):1001–12.

13. Bush SJ, Goriely A. Can the male germline offer insight into mammalian brain size expansion? Andrology [Internet]. 2024 Sep 18 [cited 2024 Sep 18];n/a(n/a). Available from: https://doi.org/10.1111/andr.13766

14. Zhou Y, Song H, Ming G li. Genetics of human brain development. Nature Reviews Genetics. 2024 Jan 1;25(1):26–45.

15. Goriely A, Wilkie AOM. Paternal age effect mutations and selfish spermatogonial selection: causes and consequences for human disease. Am J Hum Genet. 2012 Feb 10;90(2):175–200.

16. Goriely A, McGrath JJ, Hultman CM, Wilkie AOM, Malaspina D. 'Selfish spermatogonial selection': a novel mechanism for the association between advanced paternal age and neurodevelopmental disorders. Am J Psychiatry. 2013 Jun;170(6):599–608.

17. Wood KA, Tong RS, Motta M, Cordeddu V, Scimone ER, Bush SJ, et al. SMAD4 mutations causing Myhre syndrome are under positive selection in the male germline. Am J Hum Genet. 2024 Sep 5;111(9):1953–69.

18. Li MWM, Mruk DD, Cheng CY. Mitogen-activated protein kinases in male reproductive function. Trends Mol Med. 2009 Apr;15(4):159–68.

19. Wei BH, Hao SL, Yang WX. Regulation of spermatogonial stem cell self-renewal and proliferation in mammals. Histol Histopathol. 2022 Sep;37(9):825–38.

20. Seplyarskiy V, Moldovan MA, Koch EM, Kar P, Neville MD, Rahbari R, et al. Cohort-level analysis of human de novo mutations points to drivers of clonal expansion in spermatogonia. medRxiv. 2025 Jan 1;2025.01.03.25319979.

21. Krab LC, Goorden SMI, Elgersma Y. Oncogenes on my mind: ERK and MTOR signaling in cognitive diseases. Trends Genet. 2008 Oct;24(10):498–510.

22. Williams CA, Dagli A, Battaglia A. Genetic disorders associated with macrocephaly. American Journal of Medical Genetics Part A. 2008 Aug 1;146A(15):2023–37.

23. Matos B, Publicover SJ, Castro LFC, Esteves PJ, Fardilha M. Brain and testis: more alike than previously thought? Open Biology. 2021 Jun 2;11(6):200322.

24. Naro C, Cesari E, Sette C. Splicing regulation in brain and testis: common themes for highly specialized organs. Cell Cycle. 2021 Mar;20(5–6):480–9.

25. Schulz M, Teissandier A, De La Mata Santaella E, Armand M, Iranzo J, El Marjou F, et al. DNA methylation restricts coordinated germline and neural fates in embryonic stem cell differentiation. Nature Structural & Molecular Biology. 2024 Jan 1;31(1):102–14.

26. Casas Gimeno G, Paridaen JTML. The Symmetry of Neural Stem Cell and Progenitor Divisions in the Vertebrate Brain. Front Cell Dev Biol. 2022;10:885269.

27. Jean F, Stuart A, Tarailo-Graovac M. Dissecting the Genetic and Etiological Causes of Primary Microcephaly. Front Neurol. 2020;11:570830.

28. Montgomery SH, Capellini I, Venditti C, Barton RA, Mundy NI. Adaptive evolution of four microcephaly genes and the evolution of brain size in anthropoid primates. Mol Biol Evol. 2011 Jan;28(1):625–38.

29. Kelava I, Chiaradia I, Pellegrini L, Kalinka AT, Lancaster MA. Androgens increase excitatory neurogenic potential in human brain organoids. Nature. 2022 Feb;602(7895):112–6.

30. Kosti I, Jain N, Aran D, Butte AJ, Sirota M. Cross-tissue Analysis of Gene and Protein Expression in Normal and Cancer Tissues. Sci Rep. 2016 May 4;6:24799.

31. Molz B, Eising E, Alagöz G, Schijven D, Francks C, Gunz P, et al. Imaging genomics reveals genetic architecture of the globular human braincase. bioRxiv. 2024 Jan 1;2024.03.20.585712.

32. González-Forero M. Evolutionary–developmental (evo-devo) dynamics of hominin brain size. Nature Human Behaviour. 2024 Jul 1;8(7):1321–33.

33. González-Forero M, Gómez-Robles A. Why did the human brain size evolve? A way forward. Philos Trans R Soc Lond B Biol Sci. 2025 Jun 26;380(1929):20240114.

34. Tan K, Wilkinson MF. Human Spermatogonial Stem Cells Scrutinized under the Single-Cell Magnifying Glass. Cell Stem Cell. 2019 Feb 7;24(2):201–3.

35. Fayomi AP, Orwig KE. Spermatogonial stem cells and spermatogenesis in mice, monkeys and men. Stem Cell Res. 2018 May;29:207–14.

36. Tirumalasetty MB, Bhattacharya I, Mohiuddin MS, Baki VB, Choubey M. Understanding testicular single cell transcriptional atlas: from developmental complications to male infertility. Front Endocrinol (Lausanne). 2024;15:1394812.

37. Di Persio S, Neuhaus N. Human spermatogonial stem cells and their niche in male (in)fertility: novel concepts from single-cell RNA-sequencing. Hum Reprod. 2023 Jan 5;38(1):1–13.

38. Bush SJ, Nikola R, Han S, Suzuki S, Yoshida S, Simons BD, et al. Adult Human, but Not Rodent, Spermatogonial Stem Cells Retain States with a Foetal-like Signature. Cells. 2024;13(9).

39. Salehi N, Totonchi M. The construction of a testis transcriptional cell atlas from embryo to adult reveals various somatic cells and their molecular roles. Journal of Translational Medicine. 2023 Nov 27;21(1):859.

40. Wang M(汪梦杰), Li L(李来花), Cheng Q(程青), Zhang H(张浩), Liu Z(刘昭德), Cui Y(崔益强), et al. HumanTestisDB: A Comprehensive Atlas of Testicular Transcriptomes and Cellular Interactions. Genomics Proteomics Bioinformatics. 2025 May 10;23(1).

41. Djureinovic D, Fagerberg L, Hallström B, Danielsson A, Lindskog C, Uhlén M, et al. The human testis-specific proteome defined by transcriptomics and antibody-based profiling. Mol Hum Reprod. 2014 Jun;20(6):476–88.

42. Pineau C, Hikmet F, Zhang C, Oksvold P, Chen S, Fagerberg L, et al. Cell Type-Specific Expression of Testis Elevated Genes Based on Transcriptomics and Antibody-Based Proteomics. J Proteome Res. 2019 Dec 6;18(12):4215–30.

43. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Tissue-based map of the human proteome. Science. 2015 Jan 23;347(6220):1260419.

44. Guo J, Grow EJ, Mlcochova H, Maher GJ, Lindskog C, Nie X, et al. The adult human testis transcriptional cell atlas. Cell Res. 2018 Dec;28(12):1141–57.

45. Di Persio S, Tekath T, Siebert-Kuss LM, Cremers JF, Wistuba J, Li X, et al. Single-cell RNA-seq unravels alterations of the human spermatogonial stem cell compartment in patients with impaired spermatogenesis. Cell Rep Med. 2021 Sep 21;2(9):100395.

46. Sohni A, Tan K, Song HW, Burow D, de Rooij DG, Laurent L, et al. The Neonatal and Adult Human Testis Defined at the Single-Cell Level. Cell Rep. 2019 Feb 5;26(6):1501-1517.e4.

47. Shami AN, Zheng X, Munyoki SK, Ma Q, Manske GL, Green CD, et al. Single-Cell RNA Sequencing of Human, Macaque, and Mouse Testes Uncovers Conserved and Divergent Features of Mammalian Spermatogenesis. Dev Cell. 2020 Aug 24;54(4):529-547.e12.

48. Chen S, An G, Wang H, Wu X, Ping P, Hu L, et al. Human obstructive (postvasectomy) and nonobstructive azoospermia – Insights from scRNA-Seq and transcriptome analysis. Genes & Diseases. 2022 May 1;9(3):766–76.

49. Zhao L, Yao C, Xing X, Jing T, Li P, Zhu Z, et al. Single-cell analysis of developing and azoospermia human testicles reveals central role of Sertoli cells. Nat Commun. 2020 Nov 10;11(1):5683.

50. Hermann BP, Cheng K, Singh A, Roa-De La Cruz L, Mutoji KN, Chen IC, et al. The Mammalian Spermatogenesis Single-Cell Transcriptome, from Spermatogonial Stem Cells to Spermatids. Cell Rep. 2018 Nov 6;25(6):1650-1667.e8.

51. Guo J, Nie X, Giebler M, Mlcochova H, Wang Y, Grow EJ, et al. The Dynamic Transcriptional Cell Atlas of Testis Development during Human Puberty. Cell Stem Cell. 2020 Feb 6;26(2):262-276.e4.

52. Nie X, Munyoki SK, Sukhwani M, Schmid N, Missel A, Emery BR, et al. Single-cell analysis of human testis aging and correlation with elevated body mass index. Developmental Cell. 2022 May 9;57(9):1160-1176.e5.

53. Melsted P, Booeshaghi AS, Liu L, Gao F, Lu L, Min KH (Joseph), et al. Modular, efficient and constant-memory single-cell RNA-seq preprocessing. Nature Biotechnology. 2021 Jul 1;39(7):813–8.

54. Hao Y, Hao S, Andersen-Nissen E, Mauck WM, Zheng S, Butler A, et al. Integrated analysis of multimodal single-cell data. Cell. 2021 Jun 24;184(13):3573-3587.e29.

55. Hafemeister C, Satija R. Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression. Genome Biol. 2019 Dec 23;20(1):296.

56. Waltman L, van Eck NJ. A smart local moving algorithm for large-scale modularity-based community detection. The European Physical Journal B. 2013 Nov 13;86(11):471.

57. Laurentino S, Heckmann L, Di Persio S, Li X, Meyer zu Hörste G, Wistuba J, et al. High-resolution analysis of germ cells from men with sex chromosomal aneuploidies reveals normal transcriptome but impaired imprinting. Clinical Epigenetics. 2019 Aug 28;11(1):127.

58. Li L, Dong J, Yan L, Yong J, Liu X, Hu Y, et al. Single-Cell RNA-Seq Analysis Maps Development of Human Germline Cells and Gonadal Niche Interactions. Cell Stem Cell. 2017 Jun 1;20(6):858-873.e4.

59. Tan K, Song HW, Thompson M, Munyoki S, Sukhwani M, Hsieh TC, et al. Transcriptome profiling reveals signaling conditions dictating human spermatogonia fate in vitro. Proc Natl Acad Sci U S A. 2020 Jul 28;117(30):17832–41.

60. Wang M, Liu X, Chang G, Chen Y, An G, Yan L, et al. Single-Cell RNA Sequencing Analysis Reveals Sequential Cell Fate Transition during Human Spermatogenesis. Cell Stem Cell. 2018 Oct 4;23(4):599-614.e4.

61. Bastos GC, Tolezano GC, Krepischi ACV. Rare CNVs and Known Genes Linked to Macrocephaly: Review of Genomic Loci and Promising Candidate Genes. Genes (Basel). 2022 Dec 4;13(12).

62. DeCasien AR, Trujillo AE, Janiak MC, Harshaw EP, Caes ZN, Galindo GA, et al. Equivocal evidence for a link between megalencephaly-related genes and primate brain size evolution. Sci Rep. 2022 Jun 28;12(1):10902.

63. Seidlitz J, Mallard TT, Vogel JW, Lee YH, Warrier V, Ball G, et al. The molecular genetic landscape of human brain size variation. Cell Rep. 2023 Nov 28;42(11):113439.

64. Boddy AM, Harrison PW, Montgomery SH, Caravas JA, Raghanti MA, Phillips KA, et al. Evidence of a Conserved Molecular Response to Selection for Increased Brain Size in Primates. Genome Biol Evol. 2017 Mar 1;9(3):700–13.

65. Savage JE, Jansen PR, Stringer S, Watanabe K, Bryois J, de Leeuw CA, et al. Genome-wide association meta-analysis in 269,867 individuals identifies new genetic and functional links to intelligence. Nat Genet. 2018 Jul;50(7):912–9.

66. Lee JJ, Wedow R, Okbay A, Kong E, Maghzian O, Zacher M, et al. Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. Nature Genetics. 2018 Aug 1;50(8):1112–21.

67. Wei Y, de Lange SC, Scholtens LH, Watanabe K, Ardesch DJ, Jansen PR, et al. Genetic mapping and evolutionary analysis of human-expanded cognitive networks. Nat Commun. 2019 Oct 24;10(1):4839.

68. Guardiola-Ripoll M, Fatjó-Vilas M. A Systematic Review of the Human Accelerated Regions in Schizophrenia and Related Disorders: Where the Evolutionary and Neurodevelopmental Hypotheses Converge. Int J Mol Sci. 2023 Feb 10;24(4).

69. Somel M, Liu X, Khaitovich P. Human brain evolution: transcripts, metabolites and their regulators. Nat Rev Neurosci. 2013 Feb;14(2):112–27.

70. Wilsch-Bräuninger M, Florio M, Huttner WB. Neocortex expansion in development and evolution - from cell biology to single genes. Curr Opin Neurobiol. 2016 Aug;39:122–32.

71. Lui JH, Hansen DV, Kriegstein AR. Development and Evolution of the Human Neocortex. Cell. 2011 Jul 8;146(1):18–36.

72. Banerjee-Basu S, Packer A. SFARI Gene: an evolving database for the autism research community. Dis Model Mech. 2010 Apr;3(3–4):133–5.

73. Qiu S, Qiu Y, Li Y, Cong X. Genetics of autism spectrum disorder: an umbrella review of systematic reviews and meta-analyses. Translational Psychiatry. 2022 Jun 15;12(1):249.

74. Rylaarsdam L, Guemez-Gamboa A. Genetic Causes and Modifiers of Autism Spectrum Disorder. Front Cell Neurosci. 2019;13:385.

75. Sanders SJ, He X, Willsey AJ, Ercan-Sencicek AG, Samocha KE, Cicek AE, et al. Insights into Autism Spectrum Disorder Genomic Architecture and Biology from 71 Risk Loci. Neuron. 2015 Sep 23;87(6):1215–33.

76. Satterstrom FK, Kosmicki JA, Wang J, Breen MS, De Rubeis S, An JY, et al. Large-Scale Exome Sequencing Study Implicates Both Developmental and Functional Changes in the Neurobiology of Autism. Cell. 2020 Feb 6;180(3):568-584.e23.

77. Owen MJ, Legge SE, Rees E, Walters JTR, O'Donovan MC. Genomic findings in schizophrenia and their implications. Mol Psychiatry. 2023 Sep;28(9):3638–47.

78. Singh T, Poterba T, Curtis D, Akil H, Al Eissa M, Barchas JD, et al. Rare coding variants in ten genes confer substantial risk for schizophrenia. Nature. 2022 Apr 1;604(7906):509–16.

79. Ripke S, Neale BM, Corvin A, Walters JTR, Farh KH, Holmans PA, et al. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014 Jul 1;511(7510):421–7.

80. Perucca P, Bahlo M, Berkovic SF. The Genetics of Epilepsy. Annu Rev Genomics Hum Genet. 2020 Aug 31;21:205–30.

81. Rastin C, Schenkel LC, Sadikovic B. Complexity in Genetic Epilepsies: A Comprehensive Review. International Journal of Molecular Sciences. 2023;24(19).

82. Thakran S, Guin D, Singh P, Singh P, Kukal S, Rawat C, et al. Genetic Landscape of Common Epilepsies: Advancing towards Precision in Treatment. International Journal of Molecular Sciences. 2020;21(20).

83. Dhindsa RS, Weido BA, Dhindsa JS, Shetty AJ, Sands CF, Petrovski S, et al. Genome-wide prediction of dominant and recessive neurodevelopmental disorder-associated genes. The American Journal of Human Genetics. 2025 Mar 6;112(3):693–708.

84. Thor S. Indirect neurogenesis in space and time. Nature Reviews Neuroscience. 2024 Aug 1;25(8):519–34.

85. Thumfart KM, Mansuy IM. What are Sertoli cells? Historical, methodological, and functional aspects. Andrology. 2023 Jul 1;11(5):849–59.

86. Wang D, Eraslan B, Wieland T, Hallström B, Hopf T, Zolg DP, et al. A deep proteome and transcriptome abundance atlas of 29 healthy human tissues. Mol Syst Biol. 2019 Feb 18;15(2):e8503.

87. Nikolic A, Volarevic V, Armstrong L, Lako M, Stojkovic M. Primordial Germ Cells: Current Knowledge and Perspectives. Stem Cells Int. 2016;2016:1741072.

88. Guo J, Sosa E, Chitiashvili T, Nie X, Rojas EJ, Oliver E, et al. Single-cell analysis of the developing human testis reveals somatic niche cell specification and fetal germline stem cell establishment. Cell Stem Cell. 2021 Apr 1;28(4):764-778.e4.

89. Wang S, Wang T, Liu T, Xie RG, Zhao XH, Wang L, et al. Ermin is a p116(RIP) -interacting protein promoting oligodendroglial differentiation and myelin maintenance. Glia. 2020 Nov;68(11):2264–76.

90. Xing L, Kalebic N, Namba T, Vaid S, Wimberger P, Huttner WB. Serotonin Receptor 2A Activation Promotes Evolutionarily Relevant Basal Progenitor Proliferation in the Developing Neocortex. Neuron. 2020 Dec 23;108(6):1113-1129.e6.

91. de Faria O, Dhaunchak AS, Kamen Y, Roth AD, Kuhlmann T, Colman DR, et al. TMEM10 Promotes Oligodendrocyte Differentiation and is Expressed by Oligodendrocytes in Human Remyelinating Multiple Sclerosis Plaques. Scientific Reports. 2019 Mar 5;9(1):3606.

92. Hou Y, Wu Z, Zhang Y, Chen H, Hu J, Guo Y, et al. Functional Analysis of Hydrolethalus Syndrome Protein HYLS1 in Ciliogenesis and Spermatogenesis in Drosophila. Front Cell Dev Biol. 2020;8:301.

93. Vourc'h P, Andres C. Oligodendrocyte myelin glycoprotein (OMgp): evolution, structure and function. Brain Res Brain Res Rev. 2004 May;45(2):115–24.

94. Pääbo S. The human condition-a molecular approach. Cell. 2014 Mar 27;157(1):216–26.

95. Kuhlwilm M, Boeckx C. A catalog of single nucleotide changes distinguishing modern humans from archaic hominins. Scientific Reports. 2019 Jun 11;9(1):8463.

96. Salemi M, Longo GA, La Vignera S, Romano C, Condorelli RA, Romano C, et al. SPAG5 mRNA is over-expressed in peripheral blood leukocytes of patients with Down's syndrome and cryptorchidism. Neurological Sciences. 2013 Apr 1;34(4):549–51.

97. Xue J, Tarnasky HA, Rancourt DE, van Der Hoorn FA. Targeted disruption of the testicular SPAG5/deepest protein does not affect spermatogenesis or fertility. Mol Cell Biol. 2002 Apr;22(7):1993–7.

98. Chen M, Wang D, Xu Y, Yang C. Upregulation of sperm-associated antigen 5 expression in endometrial carcinoma was associated with poor prognosis and immune dysregulation, and promoted cell migration and invasion. Scientific Reports. 2024 Jun 11;14(1):13415.

99. Fietz SA, Huttner WB. Cortical progenitor expansion, self-renewal and neurogenesis-a polarized perspective. Curr Opin Neurobiol. 2011 Feb;21(1):23–35.

100. Knierim E, Hirata H, Wolf NI, Morales-Gonzalez S, Schottmann G, Tanaka Y, et al. Mutations in Subunits of the Activating Signal Cointegrator 1 Complex Are Associated with Prenatal Spinal Muscular Atrophy and Congenital Bone Fractures. The American Journal of Human Genetics. 2016 Mar 3;98(3):473–89.

101. Francks C, Maegawa S, Laurén J, Abrahams BS, Velayos-Baeza A, Medland SE, et al. LRRTM1 on chromosome 2p12 is a maternally suppressed gene that is associated paternally with handedness and schizophrenia. Molecular Psychiatry. 2007 Dec 1;12(12):1129–39.

102. Kuwahara A, Sakai H, Xu Y, Itoh Y, Hirabayashi Y, Gotoh Y. Tcf3 represses Wnt-β-catenin signaling and maintains neural stem cell population during neocortical development. PLoS One. 2014;9(5):e94408.

103. Moreira BP, Oliveira PF, Alves MG. Molecular Mechanisms Controlled by mTOR in Male Reproductive System. Int J Mol Sci. 2019 Apr 2;20(7).

104. Arenella M, Mota NR, Teunissen MWA, Brunner HG, Bralten J. Autism spectrum disorder and brain volume link through a set of mTOR-related genes. J Child Psychol Psychiatry. 2023 Jul;64(7):1007–14.

105. Reijnders MRF, Kousi M, van Woerden GM, Klein M, Bralten J, Mancini GMS, et al. Variation in a range of mTOR-related genes associates with intracranial volume and intellectual disability. Nat Commun. 2017 Oct 20;8(1):1052.

106. Yavuz BR, Arici MK, Demirel HC, Tsai CJ, Jang H, Nussinov R, et al. Neurodevelopmental disorders and cancer networks share pathways, but differ in mechanisms, signaling strength, and outcome. npj Genomic Medicine. 2023 Nov 4;8(1):37.

107. Neville MD, Lawson AR, Sanghvi R, Abascal F, Pham MH, Cagan A, et al. Sperm sequencing reveals extensive positive selection in the male germline. medRxiv. 2024 Jan 1;2024.10.30.24316414.

108. Knol MJ, Poot RA, Evans TE, Satizabal CL, Mishra A, Sargurupremraj M, et al. Genetic variants for head size share genes and pathways with cancer. Cell Reports Medicine. 2024 May 21;5(5):101529.

109. Hansen DV, Lui JH, Parker PRL, Kriegstein AR. Neurogenic radial glia in the outer subventricular zone of human neocortex. Nature. 2010 Mar 1;464(7288):554–61.

110. Grasby KL, Jahanshad N, Painter JN, Colodro-Conde L, Bralten J, Hibar DP, et al. The genetic architecture of the human cerebral cortex. Science. 2020 Mar 20;367(6484).

111. Young JC, Kerr G, Micati D, Nielsen JE, Rajpert-De Meyts E, Abud HE, et al. WNT signalling in the normal human adult testis and in male germ cell neoplasms. Hum Reprod. 2020 Sep 1;35(9):1991–2003.

112. Aloisio GM, Nakada Y, Saatcioglu HD, Peña CG, Baker MD, Tarnawa ED, et al. PAX7 expression defines germline stem cells in the adult testis. J Clin Invest. 2014 Sep;124(9):3929–44.

113. Fu K, Tian S, Tan H, Wang C, Wang H, Wang M, et al. Biological and RNA regulatory function of MOV10 in mammalian germ cells. BMC Biology. 2019 May 14;17(1):39.

114. Pariante P, Dotolo R, Venditti M, Ferrara D, Donizetti A, Aniello F, et al. First Evidence of DAAM1 Localization During the Post-Natal Development of Rat Testis and in Mammalian Sperm. Journal of Cellular Physiology. 2016 Oct 1;231(10):2172–84.

115. Li X, Yu Y, Zhang Q, Luo X, Yu L, Zhao Z. Effect of HDAC9 on the differentiation of chicken embryonic stem cells into male germ cells. Anim Reprod. 2024;21(2):e20240011.

116. Sasaki K, Yokobayashi S, Nakamura T, Okamoto I, Yabuta Y, Kurimoto K, et al. Robust In Vitro Induction of Human Germ Cell Fate from Pluripotent Stem Cells. Cell Stem Cell. 2015 Aug 6;17(2):178–94.

117. Fang F, Iaquinta PJ, Xia N, Liu L, Diao L, Reijo Pera RA. Transcriptional control of human gametogenesis. Human Reproduction Update. 2022 Jun 1;28(3):313–45.

118. Yao C, Yao R, Luo H, Shuai L. Germline specification from pluripotent stem cells. Stem Cell Res Ther. 2022 Feb 21;13(1):74.

119. Strome S, Updike D. Specifying and protecting germ cell fate. Nature Reviews Molecular Cell Biology. 2015 Jul 1;16(7):406–16.

120. Updike DL, Knutson AK, Egelhofer TA, Campbell AC, Strome S. Germ-granule components prevent somatic development in the C. elegans germline. Curr Biol. 2014 May 5;24(9):970–5.

121. Barton RA, Harvey PH. Mosaic evolution of brain structure in mammals. Nature. 2000 Jun 29;405(6790):1055–8.

122. Marques-Bonet T, Kidd JM, Ventura M, Graves TA, Cheng Z, Hillier LW, et al. A burst of segmental duplications in the genome of the African great ape ancestor. Nature. 2009 Feb 1;457(7231):877–81.

123. Holland LZ, Short S. Gene duplication, co-option and recruitment during the origin of the vertebrate brain from the invertebrate chordate brain. Brain Behav Evol. 2008;72(2):91–105.

124. Castillo-Morales A, Monzón-Sandoval J, de Sousa AA, Urrutia AO, Gutierrez H. Neocortex expansion is linked to size variations in gene families with chemotaxis, cell-cell signalling and immune response functions in mammals. Open Biol. 2016 Oct;6(10).

125. Castillo-Morales A, Monzón-Sandoval J, Urrutia AO, Gutiérrez H. Increased brain size in mammals is associated with size variations in gene families with cell signalling, chemotaxis and immune-related functions. Proc Biol Sci. 2014 Jan 22;281(1775):20132428.

126. Kilili H, Padilla-Morales B, Castillo-Morales A, Monzón-Sandoval J, Díaz-Barba K, Cornejo-Paramo P, et al. Maximum lifespan and brain size in mammals are associated with gene family size expansion related to immune system functions. Scientific Reports. 2025 Apr 29;15(1):15087.

127. Uddin M, Opazo JC, Wildman DE, Sherwood CC, Hof PR, Goodman M, et al. Molecular evolution of the cytochrome c oxidase subunit 5A gene in primates. BMC Evol Biol. 2008 Jan 15;8:8.

128. Vallender EJ, Lahn BT. A primate-specific acceleration in the evolution of the caspase-dependent apoptosis pathway. Hum Mol Genet. 2006 Oct 15;15(20):3034–40.

129. Zhang R, Wang YQ, Su B. Molecular evolution of a primate-specific microRNA family. Mol Biol Evol. 2008 Jul;25(7):1493–502.

130. Haygood R, Fedrigo O, Hanson B, Yokoyama KD, Wray GA. Promoter regions of many neural- and nutrition-related genes have experienced positive selection during human evolution. Nat Genet. 2007 Sep;39(9):1140–4.

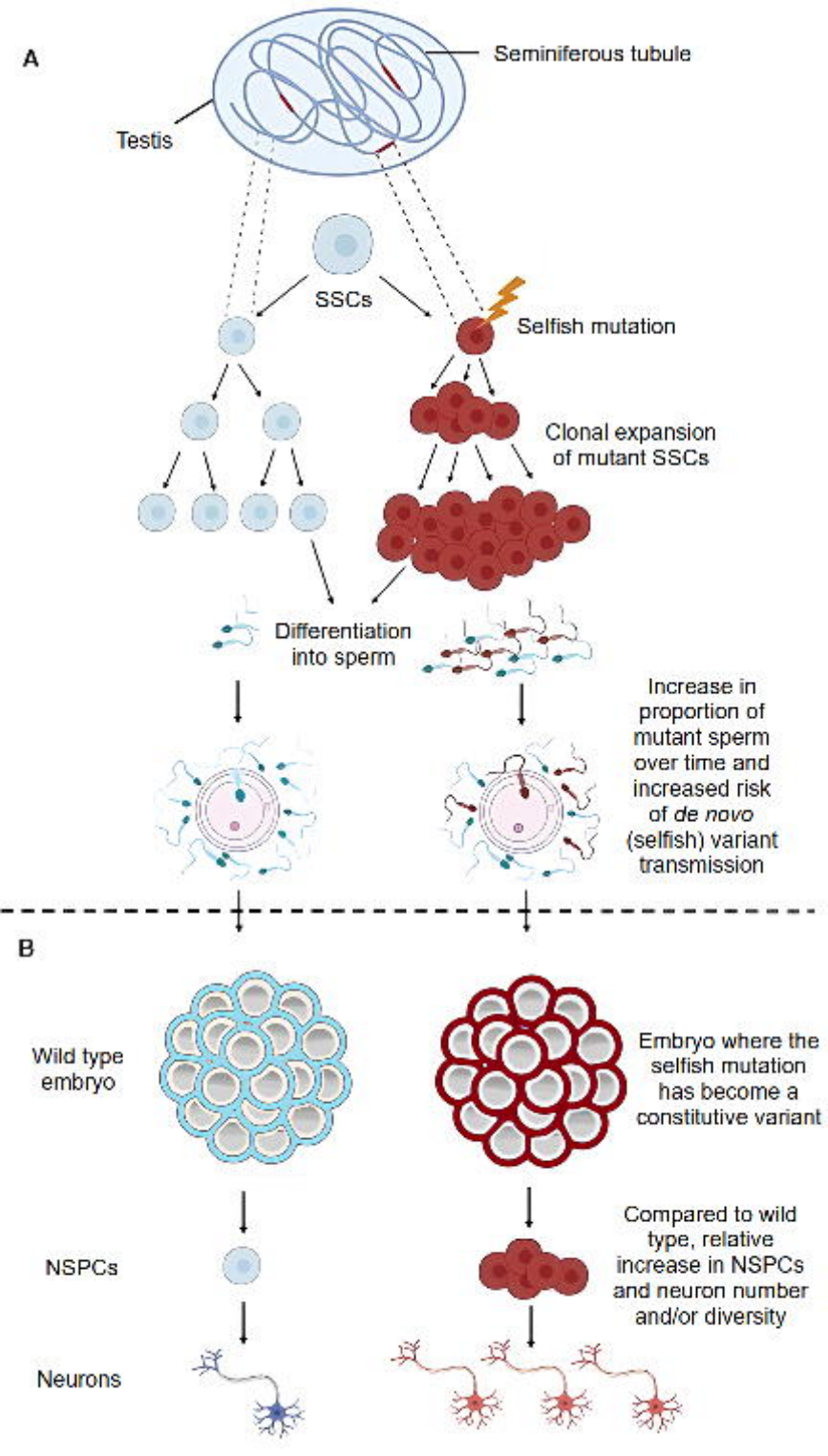
131. Smaers JB, Rothman RS, Hudson DR, Balanoff AM, Beatty B, Dechmann DKN, et al. The evolution of mammalian brain size. Sci Adv. 2021 Apr;7(18).

132. Herculano-Houzel S. The glia/neuron ratio: how it varies uniformly across brain structures and species and what that means for brain physiology and evolution. Glia. 2014 Sep;62(9):1377–91.

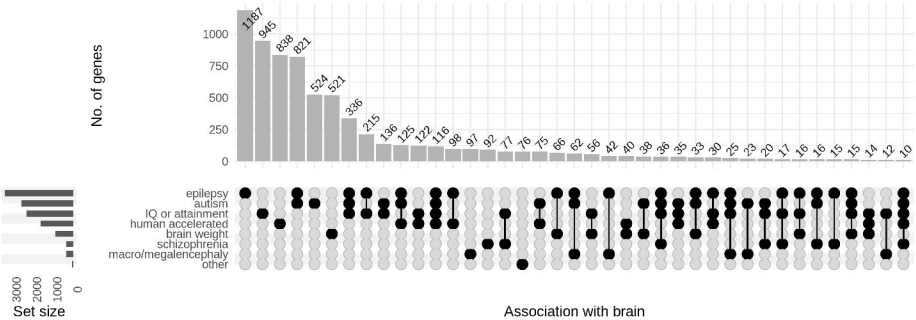
133. Fiddes IT, Lodewijk GA, Mooring M, Bosworth CM, Ewing AD, Mantalas GL, et al. Human-Specific NOTCH2NL Genes Affect Notch Signaling and Cortical Neurogenesis. Cell. 2018 May 31;173(6):1356-1369.e22.

134. Lindhout FW, Krienen FM, Pollard KS, Lancaster MA. A molecular and cellular perspective on human brain evolution and tempo. Nature. 2024 Jun;630(8017):596–608.

135. Lindhout FW, Szafranska HM, Imaz-Rosshandler I, Guglielmi L, Moarefian M, Voitiuk K, et al. Calcium dynamics tune developmental tempo to generate evolutionarily divergent axon tract lengths. bioRxiv. 2025 Jan 1;2024.12.28.630576.


A
Seminiferous tubule
Testis
SSCs
Selfish mutation
Clonal expansion of mutant SSCs
Differentiation into sperm
Increase in proportion of mutant sperm over time and increased risk of *de novo* (selfish) variant transmission
B
Wild type embryo
Embryo where the selfish mutation has become a constitutive variant
NSPCs
Compared to wild type, relative increase in NSPCs and neuron number and/or diversity
Neurons

A


No. of genes
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500
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epilepsy
autism
IQ or attainment
human accelerated
brain weight
schizophrenia
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other
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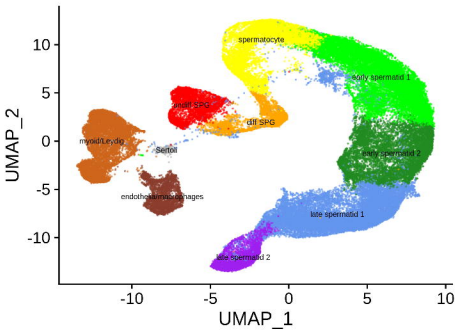
Set size
Association with brain

B


UMAP_2
UMAP_1
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spermatocyte
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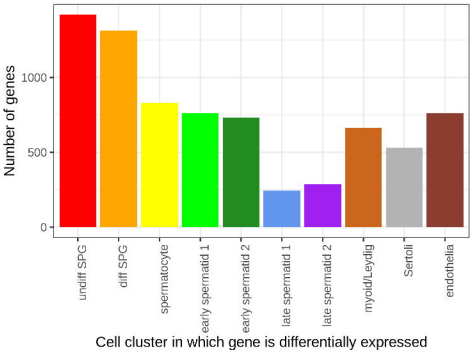
myoid/Leydig
Sertoli
early spermatid 2
endothelial/macrophages
late spermatid 1
late spermatid 2

C


Number of genes
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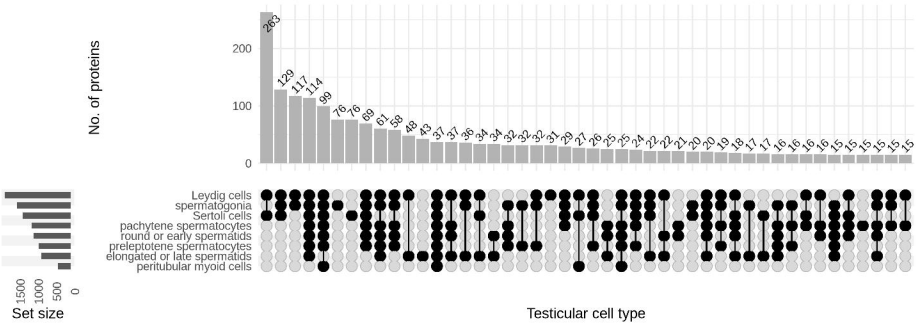
Sertoli
endothelia
Cell cluster in which gene is differentially expressed

D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preleptotene spermatocytes
elongated or late spermatids
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Set size
Testicular cell type