Population-specific, recent positive directional selection suggests adaptation of human male reproductive genes to different environmental conditions

Helmut Schaschl and Bernard Wallner

Abstract

Background: Recent human transcriptomic analyses revealed a very large number of testis-enriched genes, many of which are involved in spermatogenesis. This comprehensive transcriptomic data lead us to the question whether positive selection was a decisive force influencing the evolution and variability of testis-enriched genes in humans. We used two methodological approaches to detect different levels of positive selection, namely episodic positive diversifying selection (i.e., past selection) in the human lineage within primate phylogeny, potentially driven by sperm competition, and recent positive directional selection in contemporary human populations, which would indicate adaptation to different environments.

Results: In the human lineage (after correction for multiple testing) we found that only the gene TULP2, for which no functional data are yet available, is subject to episodic positive diversifying selection. Using less stringent statistical criteria (uncorrected p-values), also the gene SPATA16, which has a pivotal role in male fertility and for which episodes of adaptive evolution have been suggested, also displays a putative signal of diversifying selection in the human branch. At the same time, we found evidence for recent positive directional selection acting on several human testis-enriched genes (MORC1, SLC9B1, ROPN1L, DMRT1, PLCZ1, RNF17, FAM71D and WBP2NL) that play important roles in human spermatogenesis and fertilization. Most of these genes are population-specifically under positive selection.

Conclusion: Episodic diversifying selection, possibly driven by sperm competition, was not an important force driving the evolution of testis-enriched genes in the human lineage. Population-specific, recent positive directional selection suggests an adaptation of male reproductive genes to different environmental conditions. Positive selection acts on eQTLs and sQTLs, indicating selective effects on important gene regulatory functions. In particular, the transcriptional diversity regulated by sQTLs in testis-enriched genes may be important for spermatocytes to respond to environmental and physiological stress.

Keywords: Human testis-enriched genes, Male reproductive genes, Genetic adaptation, Positive selection
Background

The remarkable diversity of life histories is inevitably linked to the optimisation of the reproductive system in species. In evolutionary biology, the important question is therefore what role natural selection has played in the evolution of the reproductive systems in different species. The key male reproductive organ in humans is the testes. They have two main functions: the efficient production of sperm (spermatogenesis) over a male’s reproductive life span and the synthesis of hormones necessary to develop male sex characteristics. Spermatogenesis takes place in the testis within the seminiferous tubules, supported by Sertoli cells. This process comprises highly complex cellular events in which the proliferation and maturation of germ cells, derived from self-renewing stem cells, produces about 200 million sperm daily from puberty through the entire male adulthood [1]. Human spermatogenesis requires about 70 days for a complete cycle. Due to the very high number of mitotic replications of spermatogonia and the subsequent critical reduction of chromosome number in spermatocytes to the haploid state, the male reproductive system needs to maintain and protect the genomic integrity in the spermatocytes against the accumulation of DNA replication errors and exposure to environmental mutagens. The second important function of the testes is steroidogenesis within the Leydig cells, where cholesterol is converted to testosterone. Testosterone, together with the two gonadotropic hormones follicle stimulating-hormone (FSH) and luteinizing hormone (LH) form the testicular endocrinal system that controls spermatogenesis and the development of sexual characteristics. The mature and ejaculated spermatoozoa are carried to the female tract in seminal plasma, which supports key sperm functions such as interactions with the various environments of the tubular genital tract, with the oocyte and with the female immune system and potentially helps modulate sperm rejection or tolerance [2].

Recent tissue-specific transcriptomic analyses of humans revealed a very large number of expressed genes in the testis [3–5]. The Human Protein Atlas database (www.proteinatlas.org) reports that about 84% (n = 16, 598) of all human proteins are expressed in this tissue, and about 950 of these genes show testis-enriched expression when compared with all other analysed human tissues. Testes therefore belong to the tissues (like the brain) with the largest number of tissue-enriched genes. Many of the testis-enriched genes are related to testis-specific functions and spermatogenesis [3, 4].

These comprehensive transcriptomic data raise the question whether episodic positive diversifying selection was a decisive force influencing the evolution and variability of the testis-enriched genes in the human lineage. The selective pressures on the amino-acid level can be quantified by models of molecular evolution that incorporate the ratio (ω) of nonsynonymous (dN) to synonymous (dS) substitutions within and among species [6]. The ratio (ω) can vary over sites (site-to-site) and time (branch-site). Branch-site models enable studying the history of natural selection under particular phylogenetic hypotheses by measuring ω in different lineages along the phylogeny. If changes in amino acids offer selective advantages, leading to accelerated fixation of the nonsynonymous mutations, then the nonsynonymous substitution rate will be higher than the synonymous rate (dN/dS > 1). This would indicate positive diversifying selection. If ω < 1, then negative selection can be inferred, while ω = 1 suggests that the protein is evolving neutrally [7–9].

We should expect, however, that the coding sequences of important reproductive genes are mostly under purifying selection. This is because nonsynonymous substitutions may alter the structure of a protein and therefore harm its function and consequently fitness. Accordingly, phenotypic differences between closely related species or populations should be driven rather by gene regulatory changes, such as cis-regulatory elements (e.g., promoters, enhancers etc.), than by changes in the coding sequences. Nonetheless, a significant number of male fertilization genes show accelerated evolution in the coding sequences in different species (reviewed by [10]). This has led to the question why the rapid evolution of reproductive proteins is a widespread phenomenon. Several mechanisms such as sperm competition, pathogen resistance, cryptic female choice, sexual conflict, reinforcement, and avoidance of heterospecific fertilization have been forwarded [10, 11]. In particular, sperm competition, in which ejaculates from more than one male compete for the fertilization of a female’s eggs, is thought to be a powerful mechanism of (post-copulatory) sexual selection. This is because it can potentially generate selective pressure to increase testis size and sperm numbers, to change sperm phenotype to increase swimming speed, and to alter male physiology [12–18]. In primates, the expressed proteins of protamine P1 (PRM1) and protamine P2 (PRM2) are the most abundant sperm nuclear proteins and play a crucial role in correctly packaging the paternal DNA. PRM1 and PRM2 are two of the most rapidly diverging proteins in some primate species [19]. Subsequent studies found that the rapid evolution of protamine genes in humans and chimpanzees is due the action of positive selection, which is possibly linked to sperm competition [18, 20]. Furthermore, several studies report accelerated evolution of different male reproductive genes in human and non-human primates, including the genes spermatogenesis associated 16 (SPATA16) [21], ESX homeobox 1 (ESX1) [22], zonadhesin (ZAN) [23], polycystin family receptor for egg jelly (PKDREJ) [24], and semenogelin 2 (SEMG2) [25, 26]. These genes are functionally involved in spermatogenesis,
and positive selection at these genes is thought to be driven mainly by sperm competition.

In contrast to the codon-substitution model, which detects past selection, population genetics models of natural selection detect ongoing selection in populations. Modern humans spread from Africa within about the last 80,000 years to different parts of the world and populated a remarkably broad range of environments. Moreover, during the Neolithic demographic transition about 9000 to 13,000 years ago most humans switched from being hunter-gatherers to agriculturists, which included substantial changes in lifestyles associated with plant and animal domestication. Contemporary humans not only inhabit diverse environments but also display a wide phenotypic diversity across geographically distributed populations; much of this diversity undoubtedly reflects genetic adaptation to the different environmental conditions [27]. Whether any of the human testis-enriched expressed genes show a signature of recent positive directional selection, which would indicate adaptation to different environments, has not yet been studied comprehensively incorporating the recently available extensive transcriptomic data.

In the present study, we used two methodological approaches to detect different levels of positive selection, namely episodic positive diversifying selection (i.e., past selection) in the human lineage within the primate phyllogeny and recent positive directional selection in contemporary human populations. Specifically, we used the recently published method by Smith and co-workers [28], the adaptive branch-site random effects likelihood method (aBSREL), to test the hypothesis that episodic positive diversifying selection in the human lineage acted on testis-enriched genes, in particular on genes involved in spermatogenesis, possibly driven by sperm competition. Furthermore, we applied the integrated haplotype score method (iHS) [29] to identify human testis-enriched genes that are under recent positive directional selection in diverse human populations, which would indicate local genetic adaptation to different environments.

Methods

Human testis-specific transcriptome data

We obtained the testis-specific transcriptome/proteome data from the Human Protein Atlas database (https://www.proteinatlas.org/humanproteome/tissue/testis) [5]. In total, 950 genes are testis-enriched expressed, showing an at least four-fold higher mRNA level in the testes compared to any other tissues. The data were accessed and downloaded between April and September 2019.

Human 1000 genomes project phase 3 SNP data

We used the phased genetic data of the 1000 Genomes project phase 3 data (FTP server: http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/). We included from this database single nucleotide polymorphism (SNP) data from 12 human populations with the following genetic ancestries (as defined by the 1000 Genome Project) and number of subjects (n): East Asian ancestry: Han Chinese in Beijing, China (CHB, n = 103)), Japanese in Tokyo, Japan (JPT, n = 104), and Kin in Ho Chi Minh City, Vietnam (KHV, n = 99); South Asian ancestry: Bengali in Bangladesh (BEB, n = 86), Indian Telugu in the United Kingdom (ITU, n = 102) and Punjabi in Lahore, Pakistan (PIL, n = 96)); African ancestry: Gambians in Western Division, The Gambia (GWD, n = 113), Luhy in Webuye, Kenya (LWK, n = 99), and Esan in Nigeria (ESN, n = 99)); European ancestry: British in England and Scotland, United Kingdom (GBR, n = 91), Finnish in Finland (FIN, n = 99), and Toscani in Italy (TSI, n = 99). Because of the underlying population genetics models of natural selection, we excluded recently admixed populations and populations that are in close geographic proximity. We used the software programmes PLINK 1.9 [30] (https://www.sphrase.com.plink/1.9/) and VCFtool v0.1.14 [31] (https://vcftools.github.io/index.html) to process variant call format (VCF) files from the 1000 Genomes database for all chromosomes. We also excluded all structural variants and restricted our analysis to bi-allelic SNPs with minor allele frequency (MAF) > 0.05. The UCSC Genome Browser (http://genome.ucsc.edu/) was used to retrieve the genomic position of the testis-specific genes (including 5kb up- and downstream of the gene) in accordance to the reference genome GRCh37/hg19.

Phylogeny selection for lineage-specific analysis

We used the software BioMart [32], which is integrated in the Ensembl database [33] (http://wwwensembl.org), to obtain the human DNA gene sequences of the human testis-enriched genes as well as the corresponding orthologous genes of chimpanzee (Pan troglodytes), gorilla (Gorilla gorilla), orang-utan (Pongo abelii), macaque (Macaca mulatta), olive baboon (Papio anubis), and common marmoset (Callithrix jacchus). The primate species studied also present different mating systems and testis sizes [34]. We used the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi), bioMaRt version 2.40.0 within the R version 3.5/Bioconductor programme [35], as well as a python script to obtain the DNA sequences from orthologous genes from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) [36]. We included in the evolutionary analysis only testis-enriched genes that showed $d_S/d_N \geq 2.0$ on the Ensembl database, i.e., human sequences vs. the other orthologous primate genes, and genes known to be under positive selection in primate branches. In total, we analysed 87 human testis-specific genes for episodic positive diversifying selection in the subsequent evolutionary
analysis. The software programme AliView version 1.26 [37] with the integrated alignment programme MUSCLE version 3.8.425 [38] was used to generate codon-based alignments of the gene sequences. The few cases where no homologous gene sequences were available or could not be properly aligned were excluded from the analysis.

Evolutionary analysis: detection of episodic positive diversifying selection in the human lineage

We used the adaptive branch-site random effects likelihood (aBSREL) method to identify human testis-enriched genes that show signs of episodic positive diversifying selection [28]. The method models both the site-level and branch-level $\omega$ distribution over sites, and tests for each branch in the phylogeny whether a proportion of sites have evolved under positive selection. The method acknowledges that different branches may feature more or less complex evolutionary patterns and hence may be better modelled by more or fewer $\omega$ classes. Significance was assessed by the likelihood ratio test (LRT) at a threshold of $p \leq 0.05$. The aBSREL method uses the implemented Holm–Bonferroni sequential rejection procedure to control the family-wise error rate [28]. In this study, however, we report both the corrected test $p$-values and the uncorrected $p$-values. The aBSREL is implemented and available from the Datamonkey.org webserver (http://www.datamonkey.org/abssrel) [39].

Population genetic analysis: detection of positive selection and $F_{ST}$ analysis

We used the integrated haplotype score test (iHS) to detect genome-wide positive selection [29]. The iHS approach compares integrated EHH (Extended Haplotype Homozygosity) values between alleles at a given SNP; the method is based on the decay of haplotype homozygosity as a function of recombination distance. The underlying rationale is that selected alleles will have an unusually long-range linkage disequilibrium (LD) given their frequency in the population. Significant negative iHS values (absolute iHS score $< -2.0$) indicate unusually long haplotypes carrying the derived allele, and significant positive values (absolute iHS score $> 2.0$) are associated with long haplotypes carrying the ancestral allele [29]. We used the software programme selscan version 1.2.0a (https://github.com/szpiech/selscan), which has implemented the iHS/EHH approaches [40], to analyze the genomic data for sites under positive selection. All scans were run on phased whole chromosome data with the default model parameters of the selscan programme. The unstandardized iHS scores were normalized in frequency bins across the entire genome using the script norm, provided with the selscan programme. We considered a SNP to have a candidate selection signal if it was within a ‘cluster’ of $\geq 20$ SNPs that also had elevated iHS scores. We used a bash script to identify, among the 950 testis-enriched genes, those that showed evidence for positive directional selection in at least three populations per genetic ancestry, i.e., in Africans (AFR), Europeans (EUR), South Asians (SAS), and East Asians (EAS). In addition, we used the R package REHH to analyse the data and to generate outputs of the EHH decay plots [41]. Pairwise $F_{ST}$ were calculated for each SNP under positive selection using the Weir & Cockerham $F_{ST}$ calculation [42], which is implemented in VCFtool v0.1.14 programme [31].

Gene ontology (GO) analysis and genotype-tissue expression (GTEx) data

The GO molecular function and biological process of the studied genes were obtained from neXtProt release 2019-01-11 [43, 44]. Furthermore, we used the open-source GOnet web-application (available at http://tools.dice-database.org/GOnet/) to perform GO term annotation analysis and graphical presentation of the human genes found to be under positive selection [45]. The GTEx Portal V8 Release (https://www.gtexportal.org/home/) was used to obtain data (dbGaP Accession phs000424.v8.p2) on expression quantitative trait loci (eQTLs) and splicing quantitative trait loci (sQTLs) [46].

Results

Positive diversifying selection of testis-enriched genes in the human lineage

Previous studies found that the genes PRM1, PRM2, ESX1, SPATA16, CATSPER1, ZAN, and PKDREJ evolve rapidly in the human lineage [18, 20–26]. We first used the branch-site aBSREL method to reanalyse these genes to find evidence of positive diversifying selection in the human branch. The original hypothesis that these genes in the human lineage are under positive selection was not supported by the aBSREL analysis because the human branches had, after correction for multiple testing, test $p$-values $> 0.05$. Accordingly, the null hypothesis of neutral or negative selection is not rejected for these genes (Additional file 1). Among the other analysed testis-enriched genes, after correction for multiple testing, only the gene tubby like protein 2 (TULP2) remains significantly (test $p$-value = 0.027) associated with positive diversifying selection in the human branch (Table 1). However, if we consider the uncorrected $p$-values (at the threshold $\leq 0.05$), then aBSREL also identifies the genes C9orf43, C9orf131, C12orf40, FAM209A, MAGEB16, NACA2, POTED, SPATA16, TMCOS5A, and ZFAND4 as potential candidates for such selection (Table 1). Few biological data are available for most of these genes. The GO analysis and the literature suggest that the proteins of the SPATA16 and possibly of TMCOS5A and MAGEB16 are involved in spermatogenesis [47–51]. Furthermore, the
Table 1 Results of the aBSREL analysis with the ω distribution over the sites of the human testis-enriched genes with corrected and uncorrected p-values (in bold, the significant test p-value). The gene ontology terms (GO) are also given.

| Gene  | Chr | Gene description                          | GO molecular/GO biological                                      | Test p-value | Uncorrected p-value | ω distribution over sites |
|-------|-----|-------------------------------------------|----------------------------------------------------------------|--------------|---------------------|--------------------------|
| SPATA16 | 3   | Spermatogenesis associated 16             | Spermatogenesis GO:0007283                                      | 0.21         | 0.019               | ω₁ > 1000 (100%)         |
| C9orf43 | 9   | Chromosome 9 open reading frame 43        | Protein binding GO:0005515                                      | 0.21         | 0.026               | ω₁ > 1000 (100%)         |
| C9orf131 | 9   | Chromosome 9 open reading frame 131       | No data available                                              | 0.11         | 0.012               | ω₁ = 7.19 (100%)         |
| ZFAND4 | 10  | Zinc finger AN1-type containing 4         | Zinc ion binding GO:0008270                                     | 0.55         | 0.05                | ω₁ = 1000 (100%)         |
| C12orf40 | 12  | Chromosome 12 open reading frame 40       | Protein binding GO:0005515                                      | 0.35         | 0.032               | ω₁ > 1000 (100%)         |
| TMCO5A | 15  | Transmembrane and coiled-coil domains 5A  | No data available                                              | 0.4          | 0.036               | ω₁ > 1000 (100%)         |
| NACA2 | 17  | Nascent polypeptide associated complex alpha subunit 2 | Protein transport GO:0015031                                  | 0.4          | 0.044               | ω₁ > 1000 (100%)         |
| TULP2 | 19  | Tubby like protein 2                     | Phosphatidylinositol binding GO:0035091                          | **0.027**    | **0.002**           | ω₁ = 0.00 (98%)          |
|        |     |                                           | Protein-containing complex binding GO:0044877                  | **0.027**    | **0.002**           | ω₂ = 1290 (1.5%)         |
|        |     |                                           | Protein localization to cilium GO:0061512                       | **0.027**    | **0.002**           | ω₁ > 1000 (100%)         |
|        |     |                                           | Protein localization to photoreceptor outer segment GO:1903546 | **0.027**    | **0.002**           | ω₁ > 1000 (100%)         |
|        |     |                                           | Visual perception GO:0007601                                   | **0.027**    | **0.002**           | ω₁ > 1000 (100%)         |
| FAM209A | 20  | Family with sequence similarity 209 member A | No data available                                             | 0.07         | 0.008               | ω₁ = 0.00 (96%)          |
|        |     |                                           | ω₂ = 1350 (4.0%)                                              |              |                     |                          |
| POTED  | 21  | POTE ankyrin domain family member D       | Actin binding GO:0003779                                        | 0.098        | 0.011               | ω₁ > 1000 (100%)         |
|        |     |                                           | Actin cytoskeleton organization GO:0030036                    | 0.098        | 0.011               | ω₁ > 1000 (100%)         |
| MAGEB16 | X   | MAGE family member B16                    | No data available                                             | 0.4          | 0.044               | ω₁ = 0.00 (97%)          |
|        |     |                                           | ω₂ = 42.3 (3.3%)                                              |              |                     |                          |

POTED gene belongs to the primate-specific POTE gene family. The genes of this family are expressed in spermatids and the expressed proteins potentially play a role in cell apoptosis [52].

Positive diversifying selection of testis-specific genes in non-human primate lineages
The branch-site method (aBSREL) found evidence (test p-value ≤0.05) of positive diversifying selection in 12 out of 87 analysed orthologous testis-specific genes in the non-human primate lineages (Additional file 1). Most genes show a species-specific signature of diversifying selection (Additional file 2). The GO analysis did not yield any significantly enriched pathways. Other, functional studies, however, suggest that some of these genes are involved in spermatogenesis and fertilization. The expressed proteins of SEMG2 are involved in the formation of the semen coagulum [25, 53]. This gene has already been found to be subjected to positive diversifying selection in the chimpanzee lineage and in the white-cheeked gibbon lineage [25, 26]. We determined here that this gene in the marmoset lineage is subjected to positive diversifying selection. In this species, the gene AKAP4 also shows a signature of such selection. For this gene, a recent functional genetic study on mice showed its indispensable role in the integrity of the sperm flagellum and in spermatozoa maturation [54]. Furthermore, we identified the gene INHA, which is functionally involved in regulating follicle-stimulating hormone secretion [55], to be subjected to diversifying selection in the Rhesus macaque and olive baboon.

Positive selection of testis-enriched genes in different human populations
The LD-based test statistics iHS detected several testis-enriched genes under recent positive directional selection (Table 2). In the populations with African genetic ancestry, the genes MORCI, RNF17, and WBP2NL are under positive selection. In Europeans, this also appears to be the case for FAM71D as well as DMRT1 and PLCZ1; the latter two are also positively selected in South Asians. In East Asians, only the gene ROPNIL is under positive selection. The solute carrier SLC9B1 is positively selected in all studied human populations. However, this selection acts on this gene in Africans on ancestral alleles, whereas in the non-African populations the derived alleles show a signature of positive selection (Additional file 3). The gene enrichment analysis shows that the genes under selection are involved in spermatogenesis (DMRT1, MORCI, RNF17, ROPNIL), in egg activation (PLCZ1 and WBP2NL) and single fertilization (zygote formation) (SLC9B1) (Fig. 1). We obtained no GO terms for...
**Table 2** Human testis-enriched genes under positive selection detected in different human populations and genetic ancestries. Given are the SNPs with the highest iHS values, gene ontology (GO) terms and available QTL information (from the Genotype-Tissue Expression (GTX) database)

| Gene     | Chr | Gene description                  | GO molecular function/GO biological process                                         | Genetic ancestry | iHS   | GTEx testis tissue |
|----------|-----|-----------------------------------|---------------------------------------------------------------------------------------|------------------|-------|-------------------|
| MORC1    | 3   | MORC family CW-type zinc finger 1 | DNA methylation involved in gamete generation GO:0043046                               | AFR              | rs12695191: 3.7  | –                 |
|          |     |                                   | Zinc ion binding GO:0008270                                                           |                  |       |                   |
|          |     |                                   | Spermatogenesis GO:0007283                                                            |                  |       |                   |
|          |     |                                   | DNA hypermethylation GO:0044026                                                       |                  |       |                   |
| SLC9B1   | 4   | Solute carrier family 9 member B1 | Flagellated sperm motility GO:0030317                                               | AFR              | rs3974604: 4.1  | sQTL              |
|          |     |                                   | Proton transmembrane transport GO:1902600                                             | EUR              | rs11722779: −4.0|                   |
|          |     |                                   | Regulation of intracellular pH GO:0051453                                             | SAS              | rs11722779: −3.9| sQTL              |
|          |     |                                   | Single fertilization GO:0007338                                                      | EAS              | rs11722779: −3.5|                   |
|          |     |                                   | Sodium ion transmembrane transport GO:0035725                                         |                  |       |                   |
| RPN1L    | 5   | Rhophilin associated tail protein 1 like | cilia movement GO:0003341, flagellated sperm motility GO:0030317, sperm capacitation GO:0048240 | EAS              | rs2673855: −2.8 | –                 |
| DMRT1    | 9   | Doublesex and mab-3 related transcription factor 1 | Transcription regulator activity GO:0140110, Sex determination GO:0007530, Developmental process involved in reproduction GO:0003006, Gamete generation GO:0007276 | EUR              | rs166790: −3.4  | –                 |
|          |     |                                   |                                                                                     | SAS              | rs166790: −3.1  | –                 |
| PLCZ1    | 12  | Phospholipase C zeta 1            | Phosphatidylinositol phospholipase C activity GO:0004435                             | EUR              | rs10459068: −2.7| eQTL              |
|          |     |                                   | Phosphatidylinositol-3-phosphate binding GO:0032266                                 | SAS              | rs10459068: −2.7|                   |
|          |     |                                   | Egg activation GO:0007343, Positive regulation of cytosolic calcium ion concentration involved in egg activation GO:0060470 |                  |       |                   |
| RNF17    | 13  | Ring finger protein 17            | Metal ion binding GO:0046872, Spermatid development GO:0007286                       | AFR              | rs71431709: 2.7 | sQTL              |
| FAM71D   | 14  | Family with sequence similarity 71 member D | No data available                                                                | EUR              | rs10431714: −3.4| –                 |
| WBP2NL   | 22  | WBP2 N-terminal like              | Chromatin DNA binding GO:0031490, WW domain binding GO:0050699, Transcription coactivator activity GO:00003713, Meiotic cell cycle GO:0051321, female pronucleus assembly GO:0035038, male pronucleus assembly GO:0035039 | AFR              | rs57796605: 3.0 | –                 |

**FAM71D**, but a recent functional genetic study revealed that **FAM71D** is expressed in the flagellum of mature sperm in both mice and humans [56]. The two SNPs rs3974604 and rs11722779 of the gene **SLC9B1** that are under positive selection are associated with variation in isoform usage (splicing quantitative trait loci – sQTL) (Additional file 4). These SNPs also show relative high pairwise $F_{ST}$ (> 0.28) between the African populations and the other continental groups (Additional file 5). Finally, the SNP rs71431709 of **RNF17**, which is under positive selection only in Africans, also presents a sQTL (Additional file 4). The SNP rs10459068 of the **PLCZ1** gene, which is under positive selection in Europeans and South Asians, functions as an expression quantitative trait locus (eQTL), and the derived-T allele of this SNP is associated with increased gene expression (Additional file 6).

**Discussion**

**Episodic positive diversifying selection in the human lineage**

Our study found little evidence for widespread episodic positive diversifying selection in the human lineage. After correction for false discovery rates, only the gene **TULP2** remained statistically significantly (test $p$-value = 0.027) associated with diversifying selection. The exact function of this gene is not known yet. It does, however, appear to also be expressed in the human retina [57]. It is therefore unclear whether this form of selection acting on **TULP2** is linked to its function in the retina or in the testis.

Furthermore, aBSREL found evidence of positive diversifying selection for 12 testis-enriched, orthologous genes in non-human primates. The GO analysis revealed an
association with reproduction only for SEMG2 (flagellated sperm motility and sperm capacitation), AKAP4 (spermatogenesis) and INHA (positive regulation of follicle-stimulating hormone secretion). In addition, our study provides evidence that RHOXF2, an X-linked homeobox gene, exhibits diversifying selection in the chimpanzee lineage, confirming a previous study that showed strong positive selection for the lineages leading to humans and chimpanzees [58]. We found SEMG2 to be subjected to positive diversifying selection in the common marmoset lineage, as previously reported for the chimpanzee and white-cheeked gibbon lineages [25, 26]. The chimpanzee has a multi-male mating system, and the common marmoset breeding system is flexible, ranging from monogamous and polygynous to polyandrous [59]. It is therefore currently not possible to draw conclusions about the impact of different mating systems and thus potential sperm competition on this gene in these species.

If we accept less stringent statistical criteria, i.e., using the uncorrected \( p \)-values at the threshold \( \leq 0.05 \), then for the human lineage several other human testis-enriched genes show a potential signature of diversifying selection (see Table 1). For most of these genes, however, no comprehensive biological data are available. For example, the gene SPATA16 – for which episodes of adaptive evolution in both the human and the chimpanzee lineage have been suggested [21] – displays a putative signal of diversifying selection (albeit only in the human branch in our study). Functional genetic studies suggest that the SPATA16 molecules play important roles in human sperm formation and male fertility [51, 60]. Recent studies suggest that at least MAGEB16 is potentially involved in spermatogenesis [48, 50], and possibly TMCO5A, as shown in the rat model [49]. Furthermore, POTED belongs to the primate-specific POSE gene family. The POTE proteins have a pro-apoptotic function, and these proteins are highly expressed in human round spermatids that are undergoing apoptosis [52]. Nonetheless, these genes are not statistically substantiated (after correction for multiple testing), so that it remains speculative whether they have actually evolved under diversifying selection in the human lineage.

Why have not we found the same human testis-specific genes to be under positive diversifying selection as previous studies? Most of those earlier studies used the branch-site models implemented in the PAML method (Phylogenetic Analysis by Maximum Likelihood) [61], which differs from the method used here. The adaptive branch-site model aBSREL analyses the data...

**Fig. 1** Graphical presentation of the significant \( (p < 4.12 \times 10^{-5}) \) GO terms for testis-enriched genes under positive selection in hierarchical layout (less specific GO terms are placed at the top of the network, more specific GO terms at the bottom).
under a model whose complexity is inferred from the data together with continuous model parameters [28]. Smith et al. [28] showed that most branches in gene phylogenies can be adequately modelled with a single \( \omega \) ratio model. This greatly reduces model complexity, thereby increasing the sensitivity to detect episodic positive diversifying selection in the phylogenies. Furthermore, most studies that tested more than one branch did not control for the family-wise error rate. In the present study, we therefore applied the implemented Holm–Bonferroni sequential rejection procedure to correct for multiple testing. Apart from the methodological differences, there is also the possibility that the role of diversifying selection in driving male reproductive genes is overestimated. In fact, several studies discussed and suggested that relaxation of purifying selection rather than positive selection is responsible for the fast evolutionary rates found in certain reproductive genes [62–65]. Moreover, because of the stochastic nature of mutation, it is expected that \( d_N > d_S \) will frequently occur at certain codons merely by chance [62]. Note also that sperm competition has been invoked as an important selective force driving the evolution of some male reproductive genes. Among primates, testis size varies, and several studies suggest an association between relative testis size and mating system in primates and the level of sperm competition. Monogamous or polygynous primates typically have relatively small testes, whereas testis size is relatively large in species with a multi-male system that potentially involves sperm competition (reviewed by [66]). The size of the human testis is intermediate relative to body size, somewhat closer to the monogamous gorilla than the polygamous chimpanzee [13]. This suggests that, in contrast to chimpanzees, humans (like gorillas) may not have been subject to strong positive diversifying selection driven by sperm competition for high levels of ejaculate production [67]. Combining all these results leads us to conclude that this form of selection probably did not play its purportedly important role in the evolution of human male reproductive genes.

**Evidence for positive directional selection in human populations**

We found several testis-enriched genes to be under recent positive directional selection in different human populations. In Africans, the genes MORC1, RNF17 and WBP2NL are under positive selection. MORC1 and RNF17 are involved in spermatogenesis and WBP2NL in egg activation. In Europeans, the genes DMRT1, PLCZ1 and FAM71D show signatures of positive selection. The expressed protein of the PLCZ1 gene (PLCζ) plays an important role at oocyte activation. PLCζ localizes in the acrosome in spermatozoa and elicits Ca (2+) oscillations for oocyte activation during fertilization [68]. Moreover, in this gene the derived-T allele of the SNP rs10459068 functions as an eQTL and is associated with increased expression, suggesting that positive selection drives higher expression of this gene in Europeans and South Asians (Additional file 6). The frequency of the derived-T allele also differs substantially between Europeans/ South Asians and Africans because the derived allele occurs in Africans at less than 9%, whereas in Europeans and South Asians the frequencies are 56 and 63%, respectively. The gene FAM71D, which is under positive selection only in Europeans, is expressed in the flagellum of mature sperm in both mice and humans, suggesting functional involvement in sperm motility [56]. The SNP rs10431714 of this gene shows relative high \( F_{ST} \) values between different continental groups (Additional file 5). For example, Europeans are highly diverged from Africans at this locus, with \( F_{ST} = 0.69 \). In East Asians, ROPN1L is under positive selection in a population-specific manner. This gene plays an important role in spermatozoa capacitation and sperm motility [69]. This gene is, however, embedded in a larger genome region that is under positive selection, which also includes the gene membrane-associated ring finger (C3HC4) 6, E3 ubiquitin protein ligase (MARCH6). It is therefore unclear whether positive selection is acting mainly on ROPN1L or on MARCH6 in East Asians.

The solute carrier gene SLC9B1 is under positive selection in all studied populations. This gene belongs to the SLC9 family of genes that encode Na+/H+ exchangers that play a role in regulating pH, cell volume and ion homeostasis [70–72]. Spermatozoa are exposed in different tissues to different pH levels that increase from a relatively low pH < 7 in the cauda epididymis to pH ~ 7.4 in the female oviduct. Accordingly, intracellular pH regulation is very important for sperm physiology, including motility, maturation and the acrosome reaction [70, 73]. Indeed, experimental studies in animals showed that SLC9B1 is essential not only for male fertility, but also for survival [70, 71]. This male reproductive gene is probably vital for reproduction in many species. In humans, specific methylated sites within this gene are associated with foetal distress [74]. Finally, this gene and for RNF17 the positively selected SNPs present splicing QTLs (sQTLs), which are associated with changes in the splicing ratios of the transcripts (Additional file 4). Alternative splicing contributes to transcript diversity, enabling a gene to express different mRNAs and thus encode different proteins. Positive selection acting on the SNP sQTLs of these two genes may be an important molecular mechanism to generate a broader repertoire of functional isoforms of testis-enriched genes. The functional diversity of testis-enriched transcripts may be particularly important in enabling spermatocytes to respond to environmental and perhaps also to physiological stress such as the above-mentioned exposure to different pH levels.
Conclusion
We conclude that episodic diversifying selection, possibly driven by sperm competition, was not an important force driving the evolution of testis-enriched genes in the human lineage. However, recent positive directional selection plays an important role for various testis-enriched genes that have vital functions in human reproduction. Almost all genes are population-specifically under positive selection, suggesting genetic adaptation to different environmental conditions. The gene SLC9B1 is under positive selection in all studied populations, possibly linked to its important function in male fertility. Moreover, positive selection acts on eQTLs and sQTLs, suggesting selective effects on important gene regulatory functions. Functional transcript diversity regulated by sQTLs may be important for spermatocytes to respond to environmental and physiological stress.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12862-019-1575-0.

Additional file 1: Table S1. Results of the adaptive branch-site random effects likelihood (aBSREL) analysis.

Additional file 2: Table S2. Results of the aBSREL analysis with the ω distribution over sites of testis-specific genes in non-human primate branches with test p-value ≤0.05. Given are gene known ontology (GO) terms for the genes.

Additional file 3: Figure S1. EHH plot of the SNP rs11722779 of gene SLC9B1 in the European (TSI) vs. African (LWK) vs population.

Additional file 4: Figure S2. Violin plots showing the SNP splicing QTLs (sQTLs) of the testis-enriched genes SLC9B1 and RNF17 that are under positive selection. The normalised intron excision ratio and graphical presentations were obtained from the GTEx Portal. The ancestral alleles of these SNPs (rs3974604 ancestral-C; rs11722779 ancestral-G; rs71431709 ancestral-A) are associated with higher intron splicing ratios.

Additional file 5: Table S3. Pairwise FST analyse of SNPs under positive selection in selected populations (for abbreviation see details in Methods) with different genetic ancestry. A) FST for SNPs under positive selection in all studied populations. B) FST for SNPs under positive selection in African and South Asia populations. C) FST for SNPs under positive selection in East Asia populations.

Additional file 6: Figure S3. Violin plot of the eQTL SNP rs10459068 (T/C) of the PLCZ1 gene. The derived-T allele is associated with increased expression of that gene in the testis tissue. Expression data and the violin plot were obtained from the GTEx Portal.

Abbreviations
aBSREL: Adaptive Branch-site Random Effects Likelihood; dbGaP: Database of Genotypes and Phenotypes; EHH: Extended Haplotype Homozygosity; eQTLs: Expression Quantitative Trait Loci; GO: Gene Ontology; iHS: Integrated Haplotype Score; LD: Linkage Disequilibrium; LRT: Likelihood Ratio Test; sQTLs: Splicing Quantitative Trait Loci

Acknowledgements
We thank the Department of Evolutionary Anthropology for supporting this work. Furthermore, we thank Michael Stachowitsch for valuable comments on the manuscript.

Authors’ contributions
HS conceived the study; HS and BW wrote the paper. All authors have read and approved the manuscript.

Funding
Not applicable.

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
Helmut Schaschl is an Editorial Board Member.

Author details
1Department of Evolutionary Anthropology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria. 2Department of Behavioural Biology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria.

Received: 17 October 2019 Accepted: 30 December 2019

Published online: 13 February 2020

References
1. de Kretser DM, Loveland KAL, O’Byran MK. Spermatogenesis. In: Endocrinology. Edited by Jameson JL, De Groot LJ, de Kretser DM, Giudice LC, Grossman AB, Melmed S, Potts Jr JT, Weir GC, vol. 1 and 2. Philadelphia PA USA: Elsevier; 2016: 2325–2353.
2. Rodriguez-Martinez H, Kvist U, Ermerudt J, Sanz L, Calvette JJ. Seminal plasma proteins: what role do they play? Am J Reprod Immunol. 2011;66:11–22.
3. Djurcinovic D, Fagerberg L, Hallstrom B, Danielson A, Lindskog C, Uhlen M, Ponten F. The human testis-specific proteome defined by transcriptomics and antibody-based profiling. Mol Hum Reprod. 2014;20(6):476–88.
4. Pineau C, Hikmet F, Zhang C, Oksvold P, Chen S, Fagerberg L, Uhlen M, Lindskog C. Cell Type-Specific Expression of Testis Elevated Genes Based on Transcriptomics and Antibody-Based Proteomics. J Proteome Res. 2019 xxx, xxx-xxx.
5. Uhlen M, Fagerberg L, Hallstrom B, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A, et al. Tissue-based map of the human proteome. Science. 2015;347(6220).
6. Nielsen R, Yang ZH. Likelihood models for detecting positively selected amino acid sites and applications to the HIV-1 envelope gene. Genetics. 1998;148(3):929–36.
7. Yang ZH, Swanson WJ. Codon-substitution models to detect adaptive evolution that account for heterogeneous selective pressures among site classes. Mol Biol Evol. 2002;19(1):49–57.
8. Yang ZH, dos Reis M. Statistical properties of the branch-site test of positive selection. Mol Biol Evol. 2011;28(3):1217–28.
9. Zhang JZ, Nielsen R, Yang ZH. Evaluation of an improved branch-site likelihood method for detecting positive selection at the molecular level. Mol Biol Evol. 2005;22(12):2472–9.
10. Turner LM, Hoekstra HE. Causes and consequences of the evolution of reproductive proteins. Int J Dev Biol. 2008;52(5–6):769–80.
11. Dixon AF. Copulatory and Postcopulatory sexual selection in Primates. Folia Primatol. 2018;89(3–4):258–86.
12. Anderson MJ, Dixson AF. Sperm competition – motility and the midpiece in primates. Nature. 2002;416(6880):496.
13. Harcourt AH, Purvis A, Liles L. Sperm competition: mating system, not breeding season, affects testes size of Primates. Funct Ecol. 1995;9(3):468–78.
14. Montoto LG, Magana C, Tourmente M, Martin-Coeño J, Crespo C, Luque-Lavenna JI, Gomendio M, ERS R. Sperm Competition, Sperm Numbers and Sperm Quality in Muroid Rodents. PLoS One. 2011;6(3).
15. Nascimento JM, Shi LZ, Meyers S, Gagneux P, Loskutoff NM, Botvinick EL, Bems MW. The use of optical tweezers to study sperm competition and motility in primates. J R Soc Interface. 2008;5(20):297–302.
16. Swanson VJ, Vacquier VD. The rapid evolution of reproductive proteins. Nat Rev Genet. 2002;3(2):137–44.

17. Tourmente M, Comendio M, Roldan ERS. Sperm competition and the evolution of sperm design in mammals. BMC Evol Biol. 2011;11.

18. Wyckoff GJ, Wang W, Wu C. Rapid evolution of male reproductive genes in the descent of man. Nature. 2000;403(6763):304–9.

19. Retief JD, Winkfein RJ, Dixon GH, Aadorre R, Quersat R, Ballabriga J, Oliva R. Evolution of protamine P1 genes in primates. J Mol Evol. 1993;37(4):426–34.

20. Rooney AP, Zhang JZ. Rapid evolution of a priamte sperm protein: relaxation of functional constraint or positive Darwinian selection? Mol Biol Evol. 1999;16(5):706–10.

21. Zhang Q, Zhang F, Chen XH, Wang YQ, Wang WQ, Lin AA, Cavalli-Sforza LL, Jin L, Hsu R, Sha JH, et al. Rapid evolution, genetic variations, and functional association of the human spermatogenesis-related gene NYD-SP12. J Mol Evol. 2007;65(2):154–61.

22. Wang XX, Zhang JZ. Rapid evolution of priamte ESX1, an X-linked placenta- and testis-expressed homeobox gene. Hum Mol Genet. 2007;16(17):2053–60.

23. Gasper J, Swanson WJ. Molecular population genetics of the gene encoding the human fertilization protein zonadhesin reveals rapid adaptive evolution. Am J Hum Genet. 2000;67(5):820–30.

24. Hamm D, Mautz BS, Wolfner MF, Aquadro CF, Swanson WJ. Evidence of amino acid diversity-enhancing selection within humans and among primates at the candidate sperm-receptor gene PKDREJ. Am J Hum Genet. 2007;81(1):144–50.

25. Dorus S, Evans FD, Wyckoff GJ, Choi SS, Lahn BT. Rate of molecular evolution of the seminal protein gene SEMG2 correlates with levels of female promiscuity. Nat Genet. 2004;36(2):1326–9.

26. Ishishki M, Ishida T. Molecular evolution of the semenogelin 1 and 2 and mating system in gibbons. Am J Phys Anthrop. 2019;168(2):364–9.

27. Hancock AM, Alkorta-Aranburu G, Witonsky DB, Di Rienzo A. Adaptations to new environments in humans: the role of subtle allele frequency shifts. Hum Genet. 2006;120(1):41–7.

28. Smith MD, Wertheim JO, Weaver S, Murrell B, Scheffler K, Pond SLK. Less is more: an adaptive branch-site random effects model for efficient detection of positive selection at the nucleotide sequence level. Heredity. 2007;98(3):303–10.

29. Katsanis N, Pletcher MD, Zehnder J, Staines D, Derwent P, Kerhornou A, et al. Ensembl BioMarts: a hub for integration of genomic datasets with the R/bioconductor package biomaRt. Nat Protoc. 2009;4(8):1184–9.

30. North MA, Naggert JK, Yan YZ, Noben-Trauth K, Nishina PM. Molecular evolutionary processes. Mol Biol Evol. 2018;35(3):773–86.

31. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, et al. The variant call format and variant representation in the VCF format. Bioinformatics. 2011;27(15):2156–60.

32. Smith ME, Wetheim IQ, Weaver S, Murrell B, Scheffler K, Pond SLK. Less is more: an adaptive branch-site random effects model for efficient detection of episodic diversifying selection. Mol Biol Evol. 2015;32(5):1342–53.

33. Voight BF, Kudaravalli S, Wen XQ, Pritchard JK. A map of recent positive selection in the human genome. Nat Genet. 2004;36(2):1188–91.

34. Zhang CC, Chow CC, Teller L, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK, rising to the challenge of larger and richer datasets. Gigascience. 2015;4.

35. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, et al. The variant call format and variant representation in the VCF format. Bioinformatics. 2011;27(15):1516–8.

36. Kissens RJ, Kahari A, Haider S, Samora J, Proctor G, Spudich G, Almeida-King J, Staines D, Deswarte P, Kenkhomou A, et al. Ensembl Biomarts: a hub for data retrieval across taxonomic space. Database. 2011.

37. Zerboino DR, Achuthan P, Akanni W, Amode MR, Barrell D, Bhai J, Billis K, Cummins G, Caff A, Giron CG, et al. Ensemble 2018. Nucleic Acids Res. 2018;46(D1):D754–61.

38. Harcourt AH, Harvey PH, Larson SG, Short RV. Tests weight, body weight and breeding system in primates. Nature. 1981;293(5827):55–9.

39. Dunrick S, Spellman PT, Birney E, Huber W. Mapping identifiers for the human descent-of-man protein zonadhesin reveals rapid adaptive evolution. Am J Hum Genet. 2000;67(5):820–30.

40. Gaudet P, Michel PA, Zahn-Zabal M, Britan A, Cusin I, Domagaliski M, Durek PD, Gateau A, Gleizes A, Hinard V, et al. The neXtProt knowledgebase on human proteins: 2017 update. Nucleic Acids Res. 2017;45(D1):D1177–82.

41. Neale BM, Naggert JK, Yan YZ, Noben-Trauth K, Nishina PM. Molecular evolutionary processes. Mol Biol Evol. 2018;35(3):773–86.

42. Fujihara Y, Oji A, Laratasi T, Kojima-Kita K, Ikawa M. Human Globozoospermia-Related Gene Spata16 Is Required for Sperm Formation Revealed by CRISPR/Cas9-Mediated Mouse Models. Int J Mol Sci. 2017;18(10).

43. Gaspar JA, Srinivasan SP, Sureshkumar P, Doss MJ, Hescher J, Papadopoulos S, Sachindras A. Depletion of Mageb16 induces differentiation of pluripotent stem cells predominantly into mesodermal derivatives. Sci Rep. 2017;7.

44. Bera TK, Walker DA, Sherins RJ, Pisan I. Positive selection at the nucleotide sequence level. Heredity. 2007;98(3):303–10.

45. North MA, Naggert JK, Yan YZ, Noben-Trauth K, Nishina PM. Molecular characterization of a novel human testis-specific Golgi protein, NYD-SP12. Mol Hum Reprod. 2003(9):19–7.

46. Benf TM, Walker DA, Sherins RJ, Pisan I. Positive selection at the nucleotide sequence level. Heredity. 2007;98(3):303–10.

47. Fujihara Y, Wang Q, Jiang SY, Wang Q, Yu YJ, Tao DC, Yang Y, Ma YX, Zhang SH. Demyelination of CpG islands in the 5' upstream regions mediates the expression of the human testis-specific gene MAGEB16 and its mouse homolog MAGEb16. BMB Rep. 2014;47(7):86–91.

48. Xu M, Xiao JH, Chen J, Li J, Min YL, Zhu H, Zhou ZM, Sha JH. Identification and characterization of a novel human testis-specific Golgi protein, NYD-SP12. Mol Hum Reprod. 2003(9):19–7.

49. Zhang CC, Chow CC, Teller L, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK, rising to the challenge of larger and richer datasets. Gigascience. 2015;4.

50. Dam A, Koscinski I, Kremer JAM, Moutou C, Jaeger AS, Oudakker AR, Schauss M, Yildiz S, van Bokhoven H, et al. The neXtProt knowledgebase on human proteins: 2017 update. Nucleic Acids Res. 2017;45(D1):D177–82.

51. Dahl C, Vetrivel P, Kuprin J, Lehesjoki AE, Tiainen J, et al. The neXtProt knowledgebase on human proteins: 2017 update. Nucleic Acids Res. 2017;45(D1):D177–82.

52. Bera TK, Walker DA, Sherins RJ, Pisan I. Positive selection at the nucleotide sequence level. Heredity. 2007;98(3):303–10.
64. Lüke L, Tourmente M, Dopazo H, Serra F, Roldan ERS. Selective constraints on protamine 2 in primates and rodents. BMC Evol Biol. 2016;16.
65. Walters JR, Harrison RG. Decoupling of rapid adaptive evolution among seminal fluid proteins in heliconius butterflies with divergent mating system. Evolution. 2011;65(10):2855–71.
66. Martin RD. The Evolution of Human Reproduction: A Primatological Perspective. Yearbook of Physical Anthropology, Vol 50. 2007;50:59–84.
67. Simmons LW, Firman RC, Rhodes G, Peters M. Human sperm competition: testis size, sperm production and rates of extrapair copulations. Anim Behav. 2004;68:297–302.
68. Kashir J, Nomikos M, Lai FA. Phospholipase C zeta and calcium oscillations at fertilisation: the evidence, applications, and further questions. Adv Biol Regul. 2018;67:148–62.
69. Fiedler SE, Dudík T, Vijayaraghavan S, Carr DW. Loss of R2D2 Proteins ROPN1 and ROPN1L Causes Defects in Murine Sperm Motility, Phosphorylation, and Fibrous Sheath Integrity. Biol Reprod. 2013;88(2).
70. Chen SR, Chen M, Deng SL, Hao XX, Wang XX, Liu YX. Sodium-hydrogen exchanger NHA1 and NHA2 control sperm motility and male fertility. Cell Death Dis. 2016;7.
71. Chintapalli VR, Kato A, Henderson L, Hirata T, Woods DJ, Overend G, Davies SA, Romero MF, Dow JAT. Transport proteins NHA1 and NHA2 are essential for survival, but have distinct transport modalities. Proc Natl Acad Sci U S A. 2015;112(37):11720–5.
72. Donowitz M, Tse CM, Fuster D. SLC9/NHE gene family, a plasma membrane and organelle family of Na+/H+ exchangers. Mol Asp Med. 2013;34(2–3):236–51.
73. Florman HM, Jungnickel MK, Sutton KA. Shedding light on sperm pHertility. Cell. 2010;140(3):310–2.
74. Knight AK, Conneely KN, Kilaru V, Cobb D, Payne JL, Meilman S, Corwin EJ, Kaminsky ZA, Dunlop AL, Smith AK. SLC9B1 methylation predicts fetal intolerance of labor. Epigenetics. 2018;13(1):33–9.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.