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New genes contribute to genetic and phenotypic novelties in human evolution

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New genes in human genomes have been found relevant in evolution and biology of humans. It was conservatively estimated that the human genome encodes more than 300 human-specific genes and 1000 primate-specific genes. These new arrivals appear to be implicated in brain function and male reproduction. Surprisingly, increasing evidence indicates that they may also bring negative pleiotropic effects, while assuming various possible biological functions as sources of phenotypic novelties, suggesting a non-progressive route for functional evolution. Similar to these fixed new genes, polymorphic new genes were found to contribute to functional evolution within species, for example, with respect to digestion or disease resistance, revealing that new genes can acquire new or diverged functions in its initial stage as prototypic genes. These progresses have provided new opportunities to explore the genetic basis of human biology and human evolutionary history in a new dimension.

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Introduction

Evolutionarily new genes, referred to genes emerged in recent evolution [1], have attracted a broad interest, since the first mechanistic model was proposed in the 1930s [2]. Thanks to extensive studies of molecular evolution and genomic biology in the last decade, a dozen of distinct molecular mechanisms to generate new genes were found, including the most frequently investigated DNA-based or RNA-based duplication mechanisms and a recent additional hot topic of *de novo* origination [1,3]. These mechanisms lead to pervasive new gene origination, which in turn participated in lineage-specific or species-specific phenotypic evolution [4]. For human biology, tremendous efforts have been dedicated to study human-specific genes absent in other primates or polymorphic genes within human species, which leads to a significant progress in understanding how often these new genes contributed to phenotypic evolution and how they are implicated in disease [5,6•,7].

To discuss the progress in technical and conceptual investigation of new human genes, we provide here a concise and updated overview. We first focus on the rate and describe a few efforts in identifying primate-specific or even human-specific new genes encoded by the human genome. We describe the emerging themes in the functionality of these recently evolved genes and highlight their significance for brain and testis evolution. Then, we discuss a new hypothesis regarding the phenotypic evolution by new genes in the light of recent functional data indicating that new genes can promote tumorigenesis, while evolving advantageous functions. We further discuss the initial stage of new gene evolution when a new gene is polymorphic in a species population and discuss how these genes contribute to phenotypic difference between individuals or populations. We end the review with a summary of potentially important directions.

The human genome gains a high flux of new genes

The pioneering effort via cDNA array-based comparative genomic hybridization (aCGH) identified 134 genes showing copy number expansion after the split of human and great apes [8]. Further genomic analysis including three additional mammalian species identified 689 human-specific genes, that is, the ones not shared by chimpanzees, and 870 hominoid genes shared by human and chimpanzee but absent in mouse and dog [9]. A third analysis of 18 vertebrate genomes detected 389 human-specific genes and 1828 primate-specific genes [10]. Besides different identification strategies, the changing number could also result from ever-changing annotation. For new genes, this issue became more serious due to their poor conservation and narrow expression [11••]. For example, out of 1828 primate-specific genes, more than half were revised by later Ensembl updates as pseudogenes or noncoding transcripts, or unduly removed from the annotation [11••]. In other words, the annotation database is getting more conservative when including entries of new gene. Such an issue should be cautioned when studying new gene evolution.

The difficulty that the unstable and insufficient annotation brought to the study of new gene evolution was demonstrated by the contrasting number of human-specific *de novo* genes across different studies. Comparative analyses across multiple primate genomes in Ensembl v47 revealed three human-specific *de novo* genes supported by both transcription and proteomics data [12]. Pooling of multiple Ensembl versions (v40–v56) led to an exciting discovery of 60 human-specific *de novo* genes [13]. A third analysis based on Ensembl v51 pooled out 11 human-specific *de novo* genes [14]. All these efforts are similar technically: (1) to call proteins with the corresponding orthologous region in outgroups incapable of coding the open reading frame in the genomes of recent human ancestors; (2) to ensure that candidate *de novo* genes are supported by peptide databases. However, as pointed out in [15], the difficulty roots in the lability of human annotation of new genes and the arbitrariness of bioinformatic parameters. Nevertheless, combining complementary efforts on both duplicated new genes and *de novo* new genes, it seems prudent to conclude that substantial changes occurred in the human gene reservoir with about 300 human-specific genes and 1000 primate-specific genes added.

Primate-specific or human-specific new genes are often implicated in brain development and male reproduction

Whether or not a new gene contributes a crucial phenotypic effect in evolution is an interesting problem. As one of the early reported primate-specific gene families, *morpheus* was found to encode nuclear pore complex interacting protein (NPIP) with wide transcription in numerous tissues and organs [16] and its specific function has been known more for its activity involved in the HIV replication [17]. Recently, numerous cases of new genes were reported related to various molecular functions or phenotypic effects (more examples can be seen in Table 1). Quite a few cases appear to be related with brain functions such as the glutamate dehydrogenase 2 (*GLUD2*) [18,19] or the neuroblastoma breakpoint (DUF1220) family [20,21]. A recently well characterized case in support of the significance of new gene emergence for human brain evolution is Slit-Robo Rho GTPase-activating protein 2c or *SRGAP2C*, which is a DNA-level duplicate originated around 2 million years ago [22••]. As a partial copy, *SRGAP2C* inhibits the function of its parental gene *SRGAP2A* and induces neoteny during dendric spine maturation [23].

The enriched recruitment of new genes into brain expression is not only detected by these case studies, but also strongly supported by genome-wide studies. Comparative transcriptome profiling across major organs revealed that the proportion of brain transcriptome contributed by primate-specific genes in human is significantly higher than that contributed by rodent-specific genes in mouse [31].

Analogously, transcriptome profiling of hominoid-specific and human-specific *de novo* genes also showed that these genes tend to be transcribed in brain and testis [13,14]. Primate-specific genes transcribed in brain is enriched for zinc finger (ZNF) genes [31], which appear to be mainly contributed by the *Kruppel*-type or KRAB family [32]. Interestingly, about 40% of primate-specific KRAB-ZNF genes are differentially transcribed between human and chimpanzee prefrontal cortex, which may lead to extensive gene expression difference between the two species [33]. Why brain acts like an evolutionary hotbed in recruiting new genes likely roots in the complexity of its molecular network. Before the genomic era, it was

Table 1

Examples of new genes evolved after the split of primate from other mammals. Human-specific new genes refer to those genes absent in the other primates including chimpanzee. Homininae-specific genes refer to those shared by human, chimpanzee and gorilla. Hominoid-specific genes refer to those evolved recently in the lineages of apes but absent in rhesus monkey and other primates. Primate-specific genes refer to those absent in non-primate mammals.

Gene	Origination mechanism	Age	Function	Citation
Brain-related new genes				
GLUD2	RNA-based duplication (retroposition)	Hominoid-specific	Glutamate metabolism in brain	[18,19]
DUF1220 family	DNA-based duplication	Primate-specific	Transcribed in brain	[20,21]
SRGAP2C	DNA-based duplication	Human-specific	Dendric spine maturation	[22••,23]
Testis related				
SPANXA/D	DNA-based duplication	Homininae-specific	Spermatid morphogenesis	[24]
Cancer related				
CT45A1	DNA-based duplication	Primate-specific	Upregulate oncogenic and metastatic genes	[25]
TBC1D3	DNA-based duplication	Hominoid-specific	Modulator of epidermal growth factor receptor signaling pathway	[26,27]
NCYM	*De novo*	Homininae-specific	Stabilize the oncogene *MYCN*	[28••,29]
PBOV1	*De novo*	Human-specific	Possibly repress tumorigenesis	[30]
Other				
Morpheus family	DNA-based duplication	Primate-specific	Broadly transcribed	[16,17]

already known that the amount of RNAs transcribed in brain was two- or three-fold higher than that in tissues such as liver or kidney [34]. The updated transcriptome data via RNA-sequencing confirmed that there were more genes with highest expression in brain compared to liver and kidney [35]. Thus, the complex nature of the brain provides more interaction partners for a preexisting old gene of interest that might compromise its evolvability [36]. By contrast, a new gene has much less pleotropic constraint and it can be integrated into this network under positive natural selection [37].

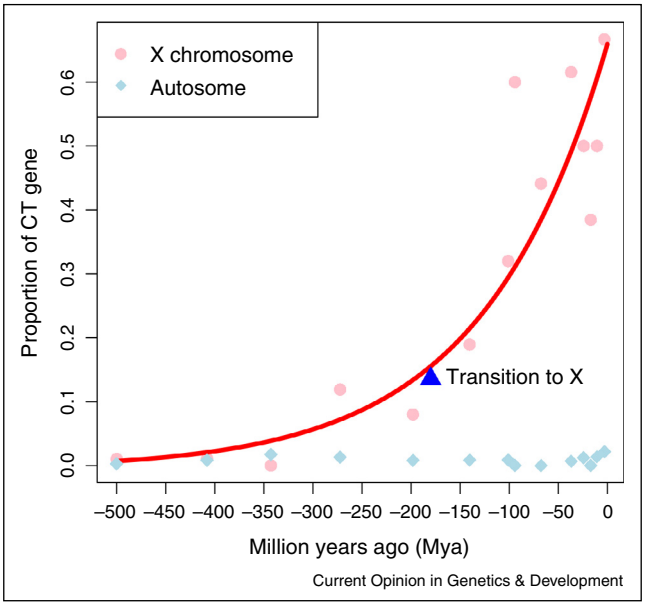
However, it is important to note that the transcription of new genes is often not limited to brain. Actually, the out-of-testis hypothesis stated that new genes tend to gain their functionality initially in testis possibly due to its permissive transcriptional regulation and then extend expression into other tissues [38,39•], which was supported by two separate genome-wide analyses showing that primate-specific genes, especially X-linked ones, are often predominantly or specifically transcribed in testis [10,38]. A recent case study provided the valuable insight into the biological role of testis-biased expressed new genes. A better-characterized case is *SPANXA/D* family, which consists of three X-linked sperm proteins associated with nucleus, SPANXA1, SPANXA2 and SPANXD present only in human and great apes [24]. It was found that during spermatid morphogenesis, the SPANXA/D protein migrated into the base of the sperm head [24].

New genes appear to promote tumorigenesis while evolving new advantageous functions, which supports a mode of non-progressive functional evolution

It was not expected that the newly evolved testis genes could be implicated in tumorigenesis until the *SPANX* actually as a Cancer/Testis (CT) antigen was found [40]. Despite the poorly characterized function, most CT genes display a characteristic transcription pattern only in testis and somatic cancer tissues possibly due to functional similarity between gametogenesis and tumorigenesis [41]. Although CTs were known for decades, recent evidence began to indicate that these proteins can facilitate tumorigenesis [42]. For example, primate-specific X-linked *CT45A1* upregulated various oncogenic and metastatic genes in breast tumor [25] (Table 1). For some CTs, hypomethylation appears to be correlated with their misexpression in tumors, but whether or not copy number increase occurs is barely known [25].

Like *SPANX* or *CT45A1*, CT genes overall tend to arise during or after the origin of placental mammals as found in recent comparative genomic studies [43]. Furthermore, there is a significant correlation between the age of X-linked CTs (or CT-Xs) and their proportion out of corresponding age groups (Figure 1): the proportion increases when CT-Xs become younger and about 50%

Figure 1



Distribution of new genes with CT expression with respect to their evolutionary ages. For the X chromosome, the proportion is defined as the number of CT-X genes divided by the number of all X-linked genes in the same age group. It was analogously defined for autosomes. The age class was computationally generated on Ensembl v69 by using the pipelines developed in [10] with the time information in TimeTree [44], while the CT gene list was downloaded from CTpedia database in July 2014 [40]. We fitted the observed frequencies of CT-Xs in various stages of human genome evolution to an exponential decay formula ($f(t) \sim e^{rt}$). We mark the species split time with a blue triangle when the mammalian X chromosome was originated, that is, before the split of human and opossum [10,45].

of X-linked primate-specific genes are CTs. By contrast, the proportion of autosomal CTs remains almost constant across various periods. This pattern indicates that the previously reported increase of X-linked testis-biased genes in human [10] is largely contributed by CT-Xs.

More than that, the young ages of the CT genes show the intriguing connection between new gene origination and tumorigenesis. As a matter of fact, new genes could drive tumorigenesis even they are not categorized as CTs as shown in following cases. One of the earliest cases is the hominoid-specific oncogene TBC1 domain family, member 3 (*TBC1D3*), which modulated epidermal growth factor receptor signaling pathway [26]. In breast cancer, *TBC1D3* was found in recurrent amplicons which were associated with lower survival span [46]. This line of facts suggests that *TBC1D3* situates in a genomic region prone to duplication. In other words, such a mutagenic nature not only increases the duplicability of *TBC1D3* in evolution, but creates more copies in tumor and supports tumorigenesis. Compared to *TBC1D3*, *NCYM* emerged *de novo* in the ancestor of human and chimpanzee as an antisense transcript of the well characterized oncogene,

MYCN [28••]. Given such a topology, *NCYM* is always co-amplified with *MYCN* in neuroblastomas [28••]. More than that, the NYCM protein stabilized MYCN, by repressing GSK3β, which promoted the degradation of MYCN [28••]. Meanwhile, it was found that new genes could also repress tumorigenesis. For instance, prostate and breast cancer overexpressed gene 1 (*PBOV1*) evolved as a human specific *de novo* gene, whose expression is associated positively with the survival possibility of patients [30].

Different from new genes categorized as CTs (e.g. *CT45A1*), *TBC1D3* is widely transcribed [27] and *NCYM* is expressed in fetal development [29]. Supposedly, CT type new genes emerged in the ancestral genomes of humans to aid the male functions in testis while non-CT type new genes play some other or general functionality. However, *CT45A1*, *TBC1D3* and *NCYM* act like oncogenes while they may assume various biological functions as their expression patterns suggested, which bear a previously unexpected theoretical significance in understanding evolutionary process of new gene functions. Specifically, while new genes evolved advantageous functions as expected, they might also bring negative effects for the survival of organisms, which may be viewed as a pleiotropic consequence. This has been predicted by recently proposed the selection, pleiotropy and compensation hypothesis (SPC) for adaptive evolution [47••]. Based on the SPC hypothesis, widely observed adaptive evolution of new genes (e.g. [1,48,49]) might be a consequence of further evolution to 'solve' a new negative problem(s) brought by the fixation of new genes as compensatory changes. This is a derivation from the SPC hypothesis to new gene evolution, different from the notion of progressively adaptive improvement of new gene function, awaiting further test in the future.

Evolutionarily underexplored polymorphic new genes contribute to within-species phenotypic variation

Almost all the above studies are performed to understand how human differs from other primates or other mammals under the conventional framework of comparative genomics. The revolution of the 2nd generation sequencing techniques rapidly promotes the field from between-species level to within-species level. From this angle, we now have the opportunity to understand the early picture on new gene origination. For DNA-level or RNA-level duplicates, they should initially arise as copy number variation (CNV). Since CNVs tend to be deleterious [50], human CNVs have revealed the important phenotypic consequence in the context of disease [51]. Progress has also been made in understanding the evolutionary consequence of CNVs, as revealed in numerous case studies that detect their adaptive functional evolution [6•]. Among these cases, salivary amylase gene (*AMY1*) and CC chemokine ligand 3-like 1 (*CCL3L1*) gene copy number gains have been relatively better characterized, which enables adaptation to a high-starch diet and is linked with lower susceptibility to HIV infection, respectively [52,53]. Genome-wide association studies further revealed that low copy number of *AMY1* predisposes the carrier with high possibility of obesity [54•] suggesting that a single polymorphic duplicated gene locus may induce multifold phenotypic difference between individuals.

Regardless of the prevalence of CNVs [55], their evolutionary consequence is less known, except for a handful cases such as *AMY1* or *CCL3L1*. The difficulty partially roots in that the exact structure and sequence of CNVs could not be readily inferred based on the short reads (~100 bp) provided by the 2nd generation sequencing [56] because a significant proportion of CNVs in human is much bigger than 1 kb [57]. Clearly, such information is helpful or even essential for studying the function of these loci. Fortunately, technical advancement based on the 3rd generation sequencing (e.g. PacBio) enables the full assembly of complex duplicate [56]. As shown in [58], a 100 kb region enriched with repeats was fully assembled with high accuracy (>99.9%) based on targeted sequencing on the PacBio platform.

Compared to polymorphic new genes generated through duplication-based mechanisms, polymorphic *de novo* genes just began to be appreciated for its scale in the standing genetic variation. Limited evidence indicates that there may be more pervasive *de novo* gene origination than currently appreciated. As shown by a *Drosophila* survey, 144 testis-expressed *de novo* genes emerged recently, which were subject to adaptive selection as shown by the valley of the nucleotide diversity [3]. By contrast, the *de novo* genes in human genomes may be even much more abundant, as implicated by a recent test that detected 5737 polymorphic open reading frames [59].

Conclusion and the prospective

Tremendous efforts in recent years have revealed a high rate of new gene origination in the human genome and their significant roles in evolution toward versatile functionality in human biology. The sequencing data accumulated rapidly in astronomical scale have unveiled the evolutionary processes in which new genes emerged and evolved; previous studies also raised new and interesting conceptual and technical problems to solve. These progresses have provided an unprecedented opportunity to explore the genetic basis of human biology and human evolutionary history. The literatures we reviewed above can be taken as starting points to further detect underlying mechanistic processes and evolutionary forces. Deciphering the new genes-related gene–gene interaction networks would help understand how a new gene gets integrated into an ancestral gene network and examining the effects of new genes on the human phenotypes

would help reconstruct the phenotypic evolution that our ancestors might have experienced.

The role of new genes in functional evolution will continue to be an enthusiastic topic for research. It should be noted here that the new gene studies have been almost all focused on protein-coding genes with a few exceptions (e.g. [60]). However, non-coding genes may represent an underexplored but potentially valuable field, especially considering their rapid turnover rates [61•]. Actually, primate-specific miRNAs may account for 19% of the whole annotated miRNA pool in human, which is much higher than the proportion (9%) of protein-coding genes [10]. More strikingly, a transcriptome survey identified 14,682 long noncoding RNAs in human with 70% (10,359) of them being primate-specific [35]. These short or long noncoding genes present an exciting challenge to understanding their roles in evolution of the human genome.

Finally, the improvement in gene annotation can be expected if the technical endeavor accounting for the observed serious bias against young genes [11••] is made. The integration of rapidly developing functional genomics techniques such as ribosome-profiling or proteogenomics and computational methods to detect evolutionary constraint [62,63•] can increase reliability in detecting *de novo* genes from genome sequences and in discerning pseudogenes from functional genes.

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