Null mutations in human and mouse orthologs frequently result in different phenotypes

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One-to-one orthologous genes of relatively closely related species are widely assumed to have similar functions and cause similar phenotypes when deleted from the genome. Although this assumption is the foundation of comparative genomics and the basis for the use of model organisms to study human biology and disease, its validity is known only from anecdotes rather than from systematic examination. Comparing documented phenotypes of null mutations in humans and mice, we find that >20% of human essential genes have nonessential mouse orthologs. These changes of gene essentiality appear to be associated with adaptive evolution at the protein-sequence, but not gene-expression, level. Proteins localized to the vacuole, a cellular compartment for waste management, are highly enriched among essentiality-changing genes. It is probable that the evolution of the prolonged life history in humans required enhanced waste management for proper cellular function until the time of reproduction, which rendered these vacuole proteins essential and generated selective pressures for their improvement. If our gene sample represents the entire genome, our results would mean frequent changes of phenotypic effects of one-to-one orthologous genes even between relatively closely related species, a possibility that should be considered in comparative genomic studies and in making cross-species inferences of gene function and phenotypic effect.

evolution | mammals | gene essentiality | vacuole

When a species diverges into two separate species, the divergent copies of a single gene in the resulting species are said to be orthologous (1, 2). Although genome-wide patterns of conservation between orthologous genes have been extensively studied at the DNA and protein-sequence levels (3–5) and have started to be investigated at the gene-expression level (6–9), little is known about the evolutionary conservation at the levels of gene function and phenotypic effect on gene deletion. This lack of knowledge is in part due to the widely held presumption that orthologous genes from different species are similar in function and phenotypic effect (2), which probably originated from a few reports that orthologous genes from distantly related species can be swapped without causing apparent phenotypic defects (10–13). Because this presumption is fundamental to comparative genomics (5, 14) and is the basis for using model organisms such as mice to study human biology and disease (15, 16), it deserves a systematic verification.

Two model organisms, the bacterium *Escherichia coli* (17) and the yeast *Saccharomyces cerevisiae* (18), have been subject to genome-wide gene-deletion experiments with available information on the fitness of each gene-deletion strain and thus could be compared in terms of the phenotypes of orthologous deletions at the genomic scale. However, these two organisms belong to prokaryotes and eukaryotes, respectively, and are so different even in basic cellular processes that the comparison is neither feasible nor meaningful. We thus choose to compare human (*Homo sapiens*) and mouse (*Mus musculus*), which are both placental mammals and have overall similar biology. Our comparison also has practical value because of the common use of mouse as a model organism for studying human biology and disease. In fact, to facilitate the use of mouse models in human biomedical research, the international genetics community recently initiated the Knockout Mouse Project (KOMP) to individually knock out every gene in the mouse genome and acquire phenotypic data (16). Our analysis will be valuable in guiding the proper use of the KOMP data.

In the present study, we focus on one of the most dramatic types of change in a gene's phenotypic effect, namely, a change in gene essentiality. A gene is said to be essential to an organism if the loss of its function renders the fitness of the organism zero; otherwise, the gene is said to be nonessential. We show that >20% of human essential genes have nonessential mouse orthologs and elucidate the mechanisms underlying the changes of gene essentiality in evolution.

Results and Discussion

Many Human Essential Genes Have Nonessential Mouse Orthologs. From Online Mendelian Inheritance in Man (OMIM) (19), we identified 1,716 human genes with clear gene–disease associations, in which 1,450 genes have unambiguous one-to-one orthologs in the mouse genome (see *Methods*). This set contains 756 human genes whose mouse orthologs have been experimentally deleted with the resulting phenotypes cataloged in the database of Mouse Genome Informatics (MGI). For the 594 human genes associated with mild diseases, we cannot infer gene essentiality, because mild diseases may be due to mild mutations in essential genes or null mutations in nonessential genes. From the remaining 162 potentially essential genes, we removed 24 immunity-related genes, because the essentiality of their mouse orthologs may not have been adequately assessed in lab. We further removed 18 genes for which there is no evidence that the human disease is due to null mutations. We thus focused on the remaining 120 human genes with clinical features of death before puberty (20) or infertility when null mutations occur, and considered them to be essential in human [supporting information (SI) Dataset S1]. We determined the essentiality of the mouse orthologs of these human genes, based on MGI and relevant literature. Specifically, a mouse gene is considered essential if the knockout mice cannot survive to reproductive age (50 days) or are infertile.

To our surprise, 27 (22.5%) of the 120 mouse orthologs of human essential genes are nonessential (Table 1 and Dataset S1). Furthermore, except for reduced survival or fecundity for *Mthfr*, *Smpd1 Hexb*, and *Neu1*-knockout mice, the other 23 knockout mouse strains (19.2%) are able to breed as successfully as the wild type at least up to the age of 6 months (Table 1). For convenience, we term these 27 human-essential–mouse-nonessential orthologs as H_eM_n orthologs and the other 93 human-essential–mouse-essential or-

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Table 1. One-to-one orthologous genes that are essential in human but nonessential in mouse

Human gene name	Human disease name	Mouse gene knockout phenotypes
Arylsulfatase A (*ARSA*)*	Metachromatic leukodystrophy†	Normal fertility and litter size; impaired balance and spatial learning ability; sulfatide accumulation in the white matter of the brain; reduced myelin sheath thickness in the corpus callosum and optic nerves; a low frequency head tremor develops after 2 years of age.
α-Mannosidase, class 2B, member 1 (*MAN2B1*)	Mannosidosis, α-, types I and II†	Normal development and fertility; no elevated mortality; mild form of human α-mannosidosis
Dystrophia myotonica protein kinase (*DMPK*)*	Myotonic dystrophy-1†	Normal fertility and litter size; abnormal sodium channel gating in cardiac myocytes; cardiac conduction defects; late-onset progressive skeletal myopathy; abnormal muscle intracellular calcium levels
Lysosomal acid lipase (*LIPA*)	Wolman disease; cholesteryl ester storage disease†	Normal development and fertility; accumulation of triglycerides and cholesteryl esters occurs in several organs
Axonemal heavy chain dynein type 11 (*DNAH11*)	Primary ciliary dyskinesia; Kartagener syndrome‡	Normal fertility; abnormal left–right axis patterning
Sialyltransferase 9 (*ST3GAL5*)	Amish infantile epilepsy syndrome†	Normal viability and fertility; hypoglycemia; increased insulin sensitivity; abnormal lipid level
Patched homolog 2 (*PTCH2*)	Medulloblastoma; basal cell carcinoma†	Normal viability and fertility; normal cell proliferation or differentiation in the cerebellum; abnormal dermal morphology in some males
Granulocyte colony-stimulating factor (*CSF3R*)	Kostmann neutropenia†	Normal development and fertility; reduced numbers of peripheral neutrophils; fewer hematopoietic progenitors in bone marrow; impaired expansion and terminal differentiation of progenitors into granulocytes
5,10-methylenetetrahydrofolate reductase (*MTHFR*)	Homocystinuria due to MTHFR deficiency†	Reduced survival rate but fertile; delayed development; elevated plasma levels of homocysteine
Transforming growth factor-β interacting factor (*TGIF1*)	Holoprosencephaly-4†	Normal growth, behavior and fertility
Aacid phosphatase-2 (*ACP2*)*	Acid phosphatase deficiency†	Normal development and fertility; skeletal defects in mutants $>$6 months of age; a small percentage of mutants exhibit tonic–clonic seizures
Cathepsin A (*CTSA*)*	Galactosialidosis†	Normal fertility; death occurs at $\approx$12 months; aberrant lysosomal storage; enlarged spleen and liver; abormally flat face; reduced body size; generalized edema, ataxia, and tremors
N-acetylglucosaminidase (*NAGLU*)*	Sanfilippo syndrome, type B†	Appear normal, healthy, and fertile up to 6 months of age; survive to 8–12 months; reduced open field activity; massive accumulation of heparan sulfate in kidney and liver; elevated gangliosides in brain; and presence of vacuoles in macrophages, epithelial cells, and neurons.
β-Mannosidase (*MANBA*)*	β-Mannosidosis†	Normal appearance, growth, and fertility to 1 year of age; cytoplasmic vacuolation in central nervous system and visceral organs
Ubiquitin–protein ligase e3 component n-recognin 1 (*UBR1*)	Johanson–Blizzard syndrome†	Normal viability and fertility; 20% lower body weight; reduced muscle and adipose tissue; abnormal metabolism; enhanced nonspatial learning; impaired spatial learning

thologs as H_eM_e orthologs, where H and M indicate human and mouse, respectively, and the subscripted "e" and "n" indicate essential and nonessential genes, respectively.

Gene Duplication Is Not the Cause of Gene-Essentiality Changes. What caused the dramatic change in essentiality between human and mouse in $>$20% of the examined genes? Previous studies in yeast (21) and nematode (22) suggested that when a gene is deleted, its paralogous gene(s) can often provide functional compensation such that an otherwise essential gene would appear to be nonessential. The H_eM_n and H_eM_e orthologs studied here are one-to-one orthologs and hence do not have paralogs that were generated since the human–mouse separation. Nevertheless, it is possible that a paralog that was generated before the human–mouse separation is retained in mouse but lost in human, rendering the effect of functional compensation present in mouse but absent in human. We thus examined whether H_eM_n-type mouse genes tend to have (*i*) more paralogs and (*ii*) closer paralogs in the mouse genome than H_eM_e-type mouse genes, which could explain why some orthologs of human essential genes are nonessential in mouse. We found that the proportion of mouse genes that have paralogs is not significantly different between the H_eM_n group (18 of 27 = 66.7%) and the H_eM_e group (55 of 93 = 59.1%) (P = 0.512, Fisher's exact test). Among the mouse genes that have paralogs, H_eM_n-type mouse genes do not have significantly more paralogs (average number of paralogs = 4.33) than H_eM_e-type mouse genes have (average = 3.78) (P = 0.415, Mann–Whitney U test). Moreover, H_eM_n-type mouse genes are not more similar to their closest paralogs (average protein sequence identity = 56.2%) than H_eM_e-type mouse genes are to their closest paralogs (average = 58.3%; P = 0.568, U test). Because divergent paralogs are unlikely to compensate one another, we repeated our analysis by considering only paralogs with relatively high protein sequence identities, but our results remain unchanged (Table S1). These observations, consistent with recent reports of a general lack of functional compensation between paralogs in mammals (23, 24), indicate that the dramatic changes of gene essentiality between human and mouse orthologs are not due to differential functional compensation from paralogs. Rather, it is more likely that the evolutionary changes of gene essentiality have resulted from alterations of the genes themselves.

Gene Essentiality Changes Are Associated with Accelerated Protein Sequence Evolution. Were changes of gene essentiality more frequently caused by alterations of protein function or gene expres-

Table 1. (continued)

Human gene name	Human disease name	Mouse gene knockout phenotypes
von Willebrand factor-cleaving protease (*ADAMTS13*)	Atypical hemolytic uremic syndrome; congenital thrombotic thrombocytopenic purpura†	Normal development, viability, and fertility; prolonged vWF-mediated platelet–endothelial interactions
Acid sphingomyelinase (*SMPD1*)*	Niemann–Pick disease, type A and B†	Males could breed until 20 weeks of age and females until 10 weeks of age with normal litter size; life span of 4–8 months; impaired coordination; mild tremor, and ataxia after 8 weeks of age; abnormal lipid homeostasis; decreased body weight
β-Glucosidase-1 (*GLB1*)*	GM1-gangliosidosis; mucopolysaccharidosis IVB†	Normal fertility and litter size; life span of 7–10 months; progressive spastic diplegia; emaciation; accumulation of ganglioside GM1 and asialo GM1 in brain tissue
α-1,4-glucosidase (*GAA*)*	Glycogen storage disease II†	Normal growth and fertility; reduced mobility and strength; impaired coordination, hindlimb paralysis and muscle weakness for the mutants >8 months of age
Cytochrome p450, family 7, subfamily b, polypeptide 1 (*CYP7B1*)*	Giant cell hepatitis, neonatal†	Normal survival, physical appearances, and behaviors; normal bile acid metabolism, plasma cholesterol and triglyceride levels; sterol biosynthetic rates were unaffected in multiple tissues with the exception of the male kidney, which showed an ≈40% decrease
Coagulation factor VIII (*F8*)	Hemophilia A†	Females exhibit normal fertility and pregnancy; males show reduced ability to clot blood; no spontaneous bleeding into joints or soft tissues is observed up to 12 weeks of age
Hexosaminidase B (*HEXB*)	Sandhoff disease†	Normal growth and fertility; mutants exhibit spasticity, muscle weakness, rigidity, tremors, and ataxia beginning ≈4 months of age and resulting in death ≈6 weeks later
GM2 activator protein (*GM2A*)*	GM2-gangliosidosis, AB variant†	Normal growth, survival and fertility; abnormal accumulation of glycolipid and ganglioside in various brain regions with impaired balance, coordination, and learning
Very long-chain acyl-CoA dehydrogenase (*ACADVL*)	Deficiency of Acyl-CoA dehydrogenase, VL†	Normal gross appearance, survival, behavior and fertility; normal body and heart weight at 2 months of age.
Alanine:glyoxylate aminotransferase (*AGXT*)	Hyperoxaluria, primary, type 1†	Normal growth and development; no histological differences between mutants and wild types in multiple tissues; increased oxalate urine levels and higher chance to develop bladder stones for males.
Neuraminidase 1 (*NEU1*)*	Sialidosis, type I and type II†	27% of the pups in the NMRI background and 10–15% in the C57BL/6 background died suddenly around weaning age; mice that survived past the 21 days were fertile, but stopped producing offspring by the age of 10 weeks; death occurred between the ages of 8 and 12 months.
Galactose-1-phosphate uridylyltransferase (*GALT*)	Galactosemia†	Normal embryonic survival; normal fertility in both sexes; abnormal galactose metabolism, but lack symptoms of acute toxicity.

*Protein product localized to vacuole.
†Death before puberty.
‡Infertility.

sion? To address this question, we first estimated the nonsynonymous distance (d_N) between each pair of human and mouse orthologs. We found that d_N of the H_eM_n group is significantly greater than that of the H_eM_e group ($P = 5.04 \times 10^{-5}$, *U* test) (Fig. 1*a*), whereas the synonymous distances (d_S) are not significantly different between the two groups ($P = 0.697$, *U* test) (Fig. 1*b*). For comparison, let us also define an H_aM_n group, which includes any human–mouse orthologous pair in which the mouse gene is known to be nonessential. Our H_aM_n group comprises 864 nonessential non-immune-system mouse genes and their human orthologs. The relatively large d_N of the H_eM_n group compared with that of the H_eM_e group must be due to accelerated nonsynonymous substitutions caused by (*i*) weaker purifying selection in the mouse lineage on H_eM_n genes than on H_eM_e genes, because mammalian nonessential genes are subject to weaker purifying selection and consequently have higher d_N than essential genes (25) and/or (*ii*) positive selection associated with the change of function and essentiality of H_eM_n genes. If (*i*) is the primary reason, the d_N of the H_eM_n group should be lower than that of the H_aM_n group, because the latter is composed of H_nM_n and H_eM_n genes. However, we found that the d_N of H_eM_n genes is significantly greater than that of H_aM_n genes ($P = 1.62 \times 10^{-4}$, *U* test) (Fig. 1*a*), suggesting that (*i*) cannot be the primary reason of greater d_N for H_eM_n genes than for H_eM_e genes. Consequently, (*ii*) must have contributed to a large degree. As expected, there is no significant difference in d_S between H_eM_n and H_aM_n genes ($P = 0.770$, *U* test) (Fig. 1*b*).

To reconfirm (*ii*), we used a maximum-likelihood method (26) to estimate branch-specific d_N/d_S values in a phylogeny of seven placental mammals for each of the 27 H_eM_n genes (Fig. 2). Besides human and mouse, five additional mammals (chimpanzee, macaque, rat, dog, and cow) were chosen because they can divide the evolutionary path linking human and mouse and because they have publicly available high-quality (i.e., at least 6× coverage) genome sequences so that the orthologous sequences of the H_eM_n genes can be retrieved. We then compared the d_N/d_S values for the five branches connecting human and mouse (Fig. 2). An earlier genomic study showed that orthologous genes have, on average, lower d_N/d_S in rodents than in primates, likely because of a larger population size and consequently increased efficacy of purifying selection in rodents than in primates (27). To make a fair comparison here, we multiplied the estimated d_N/d_S values for the two rodent branches (d and e in Fig. 2) by 1.23, which is the mean d_N/d_S value for 5,286 primate genes relative to that for their one-to-one rodent orthologs analyzed in an earlier study (27). Under no positive selection, H_eM_n

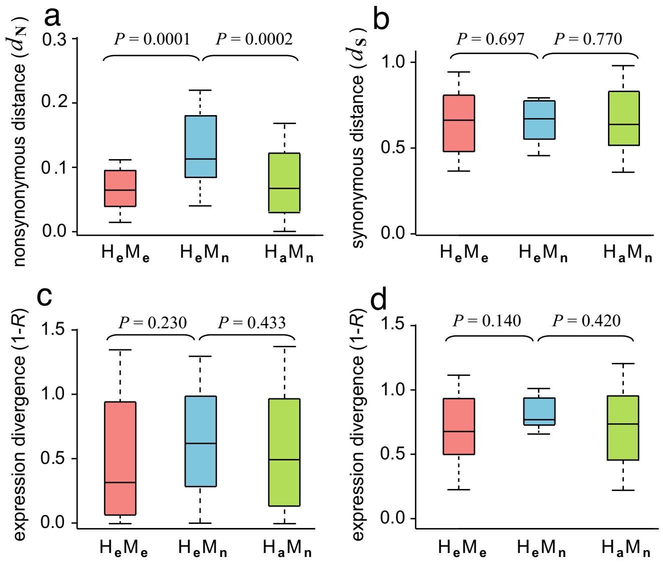
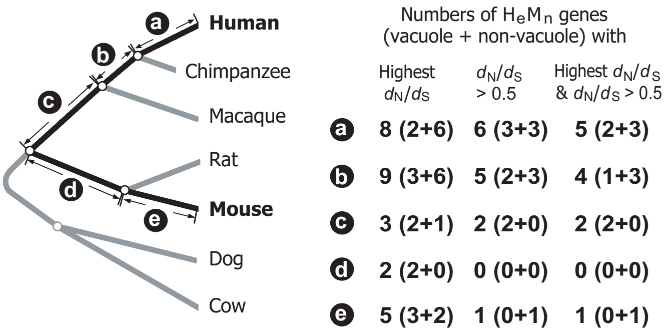


Fig. 1. The quartile plots of sequence divergence [d_N (*a*) and d_S (*b*)] and expression-profile divergence [ExonArray (*c*) and GeneAtlas v2 (*d*)] between human and mouse one-to-one orthologous genes. Values of upper quartile, median, and lower quartile are indicated in each box. The bars indicate semiquartile ranges. H and M indicate human and mouse, respectively, and the subscripts e, n, and a indicate essential, nonessential, and any genes, respectively. The *P* vales are determined by two-tailed Mann–Whitney *U* tests.



Numbers of H_eM_n genes (vacuole + non-vacuole) with	Highest d_N/d_S	d_N/d_S > 0.5	Highest d_N/d_S & d_N/d_S > 0.5
a	8 (2+6)	6 (3+3)	5 (2+3)
b	9 (3+6)	5 (2+3)	4 (1+3)
c	3 (2+1)	2 (2+0)	2 (2+0)
d	2 (2+0)	0 (0+0)	0 (0+0)
e	5 (3+2)	1 (0+1)	1 (0+1)

Fig. 2. Variation of branch-specific d_N/d_S values among the five branches (marked a to e) that connect human and mouse in the mammalian phylogeny. The branch lengths are not drawn to scale. The d_N/d_S values for branches d and e have been adjusted to correct for the intrinsically low d_N/d_S in rodents (see *Methods*).

genes are expected to exhibit relatively low d_N/d_S values in branches closer to human and relatively high d_N/d_S values in branches closer to mouse, along the evolutionary path connecting human and mouse, because essential genes tend to have lower d_N/d_S than nonessential genes (25). However, we observed the opposite pattern. That is, the fraction of H_eM_n genes that have their highest d_N/d_S values in the two branches closest to human (a and b in Fig. 2) is significantly greater than the chance expectation of 2 in 5 ($P = 0.014$, binomial test). A recent analysis of a high-exchangeability group of amino acid changes suggests that $d_N/d_S > 0.5$ likely indicates positive selection (28). Again, we found that the fraction of incidences where d_N/d_S of an H_eM_n gene is >0.5 in branch a or b is greater than the chance expectation ($P = 0.004$, binomial test; Fig. 2). The same is true when both highest d_N/d_S in a branch and $d_N/d_S > 0.5$ are considered ($P = 0.015$, binomial test; Fig. 2). Taken together, these results suggest that accelerated protein sequence evolution driven by positive selection was associated with changes of gene essentiality in at least an appreciable fraction of H_eM_n genes and that most H_eM_n genes had their gene essentiality changed during primate evolution.

Gene Expression Evolution Is Not the Cause of Gene Essentiality Changes. Next, we measured the expression-profile divergence between human and mouse orthologous genes by $1 - R$, where R is Pearson's correlation coefficient between their expression levels across homologous tissues of the two species (see *Methods*). Two independent microarray gene-expression datasets were used. The ExonArray dataset has a higher accuracy in interspecific comparisons (29), whereas the GeneAtlas v2 dataset contains more homologous tissues between the two species (30). Neither dataset shows a significant difference in $1 - R$ between H_eM_n genes and H_eM_e genes ($P = 0.230$ and 0.140 in Fig. 1 *c* and *d*, respectively, *U* test). Furthermore, $1 - R$ is not significantly different between H_eM_n and H_aM_n genes in these datasets ($P = 0.433$ and 0.420 in Fig. 1 *c* and *d*, respectively, *U* test). In short, we did not find accelerated gene-expression evolution to be associated with the essentiality changes of H_eM_n genes. Use of other measures of gene expression divergence gave similar results (Fig. S1).

Gene Essentiality Changes and the Vacuole. To better understand the biological reasons behind the changes of gene essentiality, we compared the Gene Ontology of the human genes in the H_eM_n group and the H_eM_e group using FatiGO (31). There is only one category that is significantly different between the two groups after the control for multiple testing. A much greater fraction of H_eM_n genes (12 of 27 = 44.4%) than H_eM_e genes (4 of 93 = 4.3%) have their protein products localized to the vacuole (false discovery rate $q = 5.52 \times 10^{-5}$), a cellular compartment primarily responsible for containing and degrading wastes and toxins. The absence of vacuole proteins in humans tends to cause the accumulation of cellular wastes and toxins that often leads to fatal neurological diseases (Table 1). The mass-corrected basal metabolic rate in human is ≈12% of that in mouse (32), but human reproductive age is ≈150 times that of mouse (Table S2). Consequently, the total amount of waste produced until reproduction for every gram of body mass is ≈18 times higher for human than for mouse. Hence, waste management is much more important in human than in mouse for maintaining proper cellular functions until the time of reproduction. This may have rendered the orthologs of many nonessential mouse vacuole proteins essential in humans. Consistent with this idea, deficiencies of vacuole proteins tend to cause defects at a later life stage in mouse than in human (Table 1). Furthermore, the evolution of the prolonged life history of humans probably generated selective pressures for better vacuole proteins, which may be part of the reason behind the accelerated protein sequence evolution observed in H_eM_n genes. Comparison of the product of the metabolic rate and the starting reproductive age among primates suggests that the importance of vacuole functions gradually increased in the primate lineage leading to humans, beginning from the common ancestor of all extant primates (Table S2). Consistent with this pattern, H_eM_n vacuole proteins show accelerated sequence evolution in the three primate branches (a, b, and c) in Fig. 2. However, because of the small sample size, only one comparison yielded statistically significant enrichment in the three branches. That is, for H_eM_n vacuole proteins, incidences of branch-specific $d_N/d_S > 0.5$ occurs more frequently in these three branches than expected by chance ($P = 0.028$, binomial test).

Approximately 55% of H_eM_n genes are not vacuole proteins. We confirmed that the results in Fig. 1 remain qualitatively unchanged after the removal of vacuole proteins (Fig. S2). Although the biological reason behind the change of gene essentiality of these nonvacuole proteins is unclear, the association between the essentiality change and accelerated protein-sequence evolution may be similarly caused by an increase in the importance of a particular biological process during human evolution since the human-mouse split, which rendered a nonessential gene essential and at the same time generated selective pressures for the improvement of the gene function. Consistent with this idea, analysis of branch-specific d_N/d_S indicates that nonvacuole H_eM_n proteins are significantly more likely to have rapid evolution and highest d_N/d_S in branch a or b of Fig. 2 than expected by chance (P = 0.002, 0.019, and 0.019, respectively, for the three properties shown in Fig. 2, binomial test).

Final Remarks. It is possible that the frequency and direction of gene essentiality changes are not the same among evolutionary lineages. For example, the proportion of essential genes in a genome is much greater in mouse than in yeast, which is, in turn, much greater than that in *E. coli* (24). In the present work, although only H_eM_n genes are systematically examined, anecdotes of H_nM_e genes are known. For example, humans with homozygous *RECQL* null alleles display viable and fertile Bloom's syndrome, whereas targeted deletion of the ortholog in mouse causes embryonic lethality (33). Unfortunately, it is not possible to identify H_nM_e genes systematically, owing to the difficulty in proving the nonessentiality of human genes. This obstacle notwithstanding, it is almost certain that the prevalence of distinct null phenotypes of human and mouse orthologs is underestimated here. The first reason is that genes with unaltered essentiality could still have altered phenotypic effects. For instance, *Adamts2*, *Acox1*, and *Fancg* are considered essential for human because of the mutant phenotype of premature death (20, 34), but they are essential for mouse because of the knockout phenotype of infertility of adult mice (35–37) (Dataset S1). Second, the phenotypes associated with nonessential genes are probably more labile in evolution than those associated with essential genes, because changes of nonessential genes are expected to be more tolerable than changes of essential genes. Therefore, it is likely that significantly >20% of one-to-one orthologs between human and mouse have different phenotypic effects when deleted. However, we caution that the gene sample analyzed here is relatively small, and thus our results should be reconfirmed when more data become available. In the future, it may also be possible to verify our results by comparing the essentiality of one-to-one orthologous genes from several bacterial species that have been subject to genome-wide gene deletion experiments (38, 17, 39). However, because of high incidences of horizontal gene transfer (40) and nonorthologous gene replacement (41) in prokaryotes, caution should be taken in such comparisons. When studying functional changes in orthologous gene evolution, it is important to distinguish among changes of molecular function, changes of involved biological processes, and changes of physiological importance. By comparing gene essentiality, we are addressing the physiological importance of a gene. A careful examination of Table 1 suggests that the molecular functions and the involved biological processes are likely to be unaltered for the majority of the 27 H_eM_n genes, whereas their physiological importance has changed dramatically.

Potential implications of our findings are manifold. First, gene annotation based on mutant phenotypes in other species may often be wrong, especially about gene essentiality. Second, comparative and evolutionary analysis depending on the assumption of conservation of gene function or importance between orthologs should be interpreted carefully. Third, alteration of gene essentiality between species could be a cause of the observation that some mutations pathogenic to one species are nevertheless fixed in other species (42, 43). Fourth, it is possible that mouse models of a large number of human diseases will not yield sufficiently accurate information, although they might provide some basic knowledge. The scientific community may need to strategically and systematically consider establishing a primate model organism for studying many human diseases. In this regard, it is particularly important to choose appropriate animal models for the study of human neurological disorders that involve malfunctioning vacuole proteins, because of the opposite essentiality of many vacuole proteins between human and mouse. Finally, the association between changes of gene essentiality and the prolonged life history of humans sheds light on the mechanisms of some human-specific disorders that accompany apparently beneficial human traits.

Although a recent literature survey found otherwise (44), many believe that changes of gene expression are more important than changes of protein function in generating phenotypic differences between species (45, 46). We found that changes of gene essentiality were accompanied by accelerated evolution that was likely driven by positive selection at the protein sequence level but did not find such a signal at the gene-expression level. Although we cannot exclude the possibility that our result regarding expression evolution is caused by the relatively large noise of microarray expression data or the lack of relevant tissues in the datasets analyzed, we can conclude that protein sequence and function changes are important in the change of gene essentiality in evolution. It remains possible, however, that gene expression changes are more important for phenotypic evolution that does not involve a change in gene essentiality.

Methods

Genomic Data and Annotations. Human genome version NCBI36 and mouse genome version NCBIM36 were used. Annotations of 31,545 human and 28,390 mouse known or predicted genes by Ensembl (release 44) (www.ensembl.org/) were retrieved through BioMart (www.biomart.org/). We considered 14,423 pairs of human–mouse orthologous genes that were annotated as ''ortholog_one2one.'' This annotation was not based on reciprocal best BLAST hits but was based on phylogenetic analysis (www.ensembl.org/info/about/docs/compara/homology_method.html). The number of synonymous nucleotide substitutions per synonymous site (d_S) and the number of nonsynonymous substitutions per nonsynonymous site (d_N) between human and mouse orthologs, estimated by the likelihood method, were retrieved from BioMart. The paralog information, including the percentage of sequence identity, was also obtained from BioMart. Because retroduplicates are expected to have unrelated expression patterns from their mother genes and thus are not expected to compensate the loss of the mother genes, we did not consider retroduplicate copies as paralogs of a gene. Retroduplicates were recognized by the absence of introns that are present in their mother genes. Our results remained unchanged when retroduplicates were not excluded.

Human Essential Genes. There are 1,716 human genes in Ensembl that are associated with heritable human diseases in OMIM (www.ncbi.nlm.nih.gov/omim/). Among them, 1,450 have unambiguous mouse orthologs, and 756 have phenotypic descriptions from gene knockout mice. Following Jimenez-Sanchez and colleagues (20), we categorized essentiality of a gene by the most life-threatening disease that the gene is associated with. Of the 162 human essential genes that have corresponding mouse knockout phenotypes, 24 are immunity related (MP:0005387 in MGI) and were excluded in further analysis, because the sterilized laboratory environment may underestimate the fitness reduction associated with the deletion of immunity genes in mice. To compare with mouse knockout phenotypes, we require that human diseases considered here are due to null (or at least not gain-of-function) mutations. Null mutations are defined as nonsense or frameshift mutations or the absence of gene products in patients as determined by biochemical assays. Eighteen genes were removed because of the lack of evidence for the association between human diseases and null mutations. We manually verified the human and mouse phenotypes for the remaining 120 orthologous genes by reading relevant literature, especially ensuring that the mouse abnormal reproductive system phenotypes annotated in MGI are infertility. The complete list of these 120 genes is provided in Dataset S1.

Mouse Essential and Nonessential Genes. Mouse phenotypic data were downloaded from MGI version 3.53 (www.informatics.jax.org/). We limited our analysis to null mutants generated by random gene disruption, gene trap mutagenesis, and targeted deletion, together referred to as gene knockout here. Only those genes with one-to-one matches between MGI symbol names and Ensembl gene IDs were kept for subsequent analysis. Genes with phenotypes of embryonic lethality (MP: 0002080), prenatal lethality (MP: 0002081), survival postnatal lethality (MP: 0002082), abnormal reproductive system morphology (MP: 0002160), or abnormal reproductive system physiology (MP: 0001919) were grouped as essential genes. Genes with phenotypes of premature death or induced morbidity (MP: 0002083) were manually inspected and classified according to the literature. If the mutant has a life span of <50 days, the gene is considered to be essential. Genes associated with all other phenotypes (at least MP: 0000001), including the normal phenotype, were grouped as nonessential genes. The dataset included 2,022 essential and 1,655 nonessential mouse genes.

Estimating Branch-Specific d_N/d_S Values. Coding sequences (CDS) of H_eM_n genes from human and mouse and their ''one2one'' orthologs from chimpanzee (*Pan troglodytes*), macaque (*Macaca mulatta*), rat (*Rattus norvegicus*), cow (*Bos taurus*), and dog (*Canis familiaris*) were obtained from BioMart and the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/). If multiple transcripts were annotated for one gene, the longest CDS was chosen. The sequences were aligned by MEGA4 (47) with manual adjustment. Alignment gap sites were subsequently removed. With the known phylogeny of the seven mammals (Fig. 2) (48), the program ''codeml'' in PAML (49) was used to estimate d_N/d_S for the H_eM_n orthologs in each branch of the tree, with the option of ''model = 1'' chosen in the control file. We then compared the d_N/d_S values of the five branches (a to e in Fig. 2) that connect human and mouse in the tree. Rodents are known to have intrinsically low d_N/d_S compared with primates (27). To make a fair comparison of d_N/d_S among branches, we multiplied the d_N/d_S estimates for branches d and e by 1.23, which is the mean d_N/d_S value for 5,286 primate genes relative to that for their one-to-one rodent orthologs (1.28/1.04; see figure S6 of ref. 27).

Microarray Data Analysis. The GeneAtlas v2 dataset (http://symatlas.gnf.org/) contains the expression data obtained by hybridization of RNAs from 73 human nonpathogenic tissues and 61 mouse tissues onto the Affymetrix microarray chips (human: U133A/GNF1H; mouse: GNF1M) (30). We assigned the probe sets to the human and mouse genes following a previous study (50). The expression level detected by each probe set was obtained as the signal intensity (*S*) computed from the MAS 5.0 algorithm. The dataset contains 26 common tissues between the two species. They are adipocyte, adrenal gland, amygdala, bone marrow, cerebellum, dorsal root ganglion, heart, hypothalamus, kidney, liver, lung, lymph node, ovary, pancreas, pituitary, placenta, prostate, skeletal muscle, spinal cord, testis, thymus, thyroid, tongue, trachea, trigeminal ganglion, and uterus. Mouse lower spinal cord was used as the homologous tissue of human spinal cord. We measured the expression-profile divergence between a pair of orthologs by $1 - R$, where *R* is Pearson's correlation coefficient between human *S* and mouse *S* across the 26 common tissues. We also used another parameter, $1 - ICE$, to measure the expression-profile divergence between orthologous genes. *ICE* (index of coexpression) between two genes is defined as the number of tissues in which both genes are expressed divided by the geometric mean of the number of tissues where each gene is expressed (51). Following convention (30), we used a cutoff of $S = 200$ to determine whether a gene is expressed in a tissue or not when estimating $1 - ICE$.

The ExonArray data were generated by using a microarray platform with more than six million probes targeting all annotated and predicted exons in a genome and were obtained from Xing *et al.* (29). The data include six common tissues between human and mouse (heart, kidney, liver, muscle, spleen, and testis). We used the expression signals *S* computed from GeneBASE (http://biogibbs.stanford.edu/~kkapur/genebase/). The *S* values were averaged among the three replicated experiments performed for each tissue. Because the quality of the ExonArray data are higher than that of GeneAtlas (29), a cutoff of $S = 150$ was used to determine whether a gene is expressed in a tissue or not in the estimation of 1-*ICE*. Under this cutoff, the numbers of genes expressed in a given tissue are similar between the GeneAtlas and ExonArray datasets.

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