

Invited Mini Review

Loss of gene function and evolution of human phenotypes

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Humans have acquired many distinct evolutionary traits after the human-chimpanzee divergence. These phenotypes have resulted from genetic changes that occurred in the human genome and were retained by natural selection. Comparative primate genome analyses reveal that loss-of-function mutations are common in the human genome. Some of these gene inactivation events were revealed to be associated with the emergence of advantageous phenotypes and were therefore positively selected and fixed in modern humans (the "less-is-more" hypothesis). Representative cases of human gene inactivation and their functional implications are presented in this review. Functional studies of additional inactive genes will provide insight into the molecular mechanisms underlying acquisition of various human-specific traits. [BMB Reports 2015; 48(7): 373-379]

INTRODUCTION

Humans diverged from the chimpanzee lineage approximately 5-7 million years ago (MYA) (1-3). Humans have many specific traits compared with closely related apes that must have resulted from genetic modifications acquired during evolution (2). For example, the human *FOXP2* gene shows accelerated amino acid sequence substitutions during human evolution and may have played an important role in language and speech evolution by altering transcription of genes responsible for the development of the central nervous system (4, 5). Human accelerated region 1 noncoding RNA shows accelerated nucleotide sequence substitutions in the human lineage and is highly expressed in human neocortex, possibly playing a role in the evolution of human brain development (6, 7).

Sequence comparisons of complete genomes of human and related primates has enabled the large-scale identification of genetic modifications in the human lineage, including accelerated sequence substitutions, novel transcript isoforms, and

acquisitions of posttranslational modification sites (6, 8-14). Molecular functions of most of these changes and their associated human-specific phenotypes are not yet established.

According to the "less-is-more" hypothesis, loss of gene function can be implicated in the evolution of human-specific traits (15). In general, loss of gene function by disrupting mutations would be deleterious to individual fitness, and purifying selection would remove the mutant allele from the population. However, environmental or behavioral changes of an organism may relax the selection constraints on a gene, which could then accumulate disrupted mutations without loss of fitness. Under certain circumstances, the absence of intact proteins can be more advantageous, and pseudogenizing mutations might then be favored and increase in frequency, eventually becoming fixed in a species.

In humans, one of the examples that potentially supports the adaptive pseudogenization hypothesis is the *MYH16* gene, for which inactivation has been suggested to be involved in human brain expansion (16). The loss of human *CMAH* gene function appears to be associated with the evolution of susceptibilities to malaria or typhoid toxins (17, 18). Loss of regulatory elements, as evidenced by hCONDEL.569 and HACNS1, can dramatically change expression patterns of nearby genes and confer human-specific anatomical traits (19, 20). In addition, a large number of human-specific gene losses have been identified thus far using comparative genomics studies (21-27). Here, we review representative cases of human gene inactivation and their functional implications.

LOSS OF THE *CMAH* GENE AND SUSCEPTIBILITIES TO PATHOGENS

The *CMAH* gene encodes for cytidine monophosphate-N-acetylneuraminic acid hydroxylase, which is an enzyme responsible for the biosynthesis of N-glycolylneuraminic acid (Neu5Gc), a hydroxylated form of the common sialic acid N-acetylneuraminic acid (Neu5Ac) (28). Sequence comparisons have shown that there is a 92-bp deletion in the coding region of the human gene, while the chimpanzee gene is intact, indicating the human gene was inactivated after the human-chimpanzee divergence (29). The absence of active *CMAH* enzyme in humans resulted in differences in the glycan composition between humans and other primates: human sialoglycans terminate in Neu5Ac, whereas those of other primates and most other mammals terminate in Neu5Gc (30). The human-specific

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<http://dx.doi.org/10.5483/BMBRep.2015.48.7.073>

Received 11 April 2015

Keywords: Human evolution, Gene inactivation, Pseudogene, Less-is-more hypothesis

pseudogenization of the *CMAH* gene occurred approximately 3 MYA through an Alu-mediated exon deletion (31-33).

The phenotypic consequences of the loss of *CMAH* are of great interest and have been studied extensively. Initially, it was proposed that the loss of Neu5Gc moiety might be associated with the brain expansion of humans (32). However, a study has suggested that the differences in sialoglycan might instead be associated with the differences in malaria susceptibilities between humans and chimpanzees (34). *Plasmodium falciparum* is the global cause of malaria mortality in humans; however, it does not cause severe infection in chimpanzees. *P. reichenowi*, which is the closest relative of *P. falciparum*, infects chimpanzees and gorillas but not humans.

To explain this interesting relationship, the inactivation of the *CMAH* gene in early humans and the subsequent evolution of the parasite has been proposed (17, 35). Inactivation of the *CMAH* gene rendered human ancestors incapable of synthesizing Neu5Gc from Neu5Ac. Abolishment of Neu5Gc from human erythrocytes might make humans resistant to *P. reichenowi*, which strongly prefers Neu5Gc. The resistance to *P. reichenowi* malaria could have been advantageous to human ancestors, and the inactive *CMAH* gene was fixed in the human lineage. More recently, however, a lineage of *P. falciparum* that is widely present in African great apes, especially in western gorillas, might have cross-infected humans (36). The strong preference for the overabundant Neu5Ac in human red blood cells might be responsible for the cross-species transmission of *P. falciparum* malaria and its emergence as the deadliest human pathogen (17, 34).

Glycans that terminate in Neu5Ac are associated with the host specificity of other exclusively human pathogens, such as *Salmonella* Typhi and human influenza A virus (IAV) (18, 37). The typhoid toxin selectively binds to Neu5Ac-terminated glycans and displays selective toxicity toward cells expressing them. Ferrets have also lost the *CMAH* gene through a nine-exon deletion, which is shared by the Pinnipedia and Musteloidia members of Carnivora, and they exhibit susceptibility to human-adapted IAV strains. Thus, the evolution of resistance to a certain pathogen through *CMAH* gene inactivation and subsequent development of susceptibility to other parasites is a good example of the evolutionary arms race between hosts and parasites (35, 38).

LOSS OF THE *MYH16* GENE AND REDUCTION OF MASTICATORY MUSCLES AND BRAIN EXPANSION

The *MYH16* gene encodes a sarcomeric myosin heavy chain, which is a major component of masticatory (jaw-closing) muscles (39). The human *MYH16* gene is a pseudogene and does not produce functional protein due to a two-nucleotide deletion in exon 18. This deletion occurred in the human lineage after the human-chimpanzee divergence and has been fixed in modern humans (16). The loss of the *MYH16* gene product in humans has been proposed to be associated with a marked re-

duction in masticatory muscle mass, which might have allowed humans to have bigger brains (16, 40, 41). Initially, this frameshift mutation in *MYH16* was estimated to have appeared approximately 2.4 MYA, predating the appearance of *Homo erectus/ergaster*, which had a relatively gracile masticatory apparatus. The age of the inactivating mutation appears to support the idea that the loss of the *MYH16* gene could be a crucial step for the enhanced encephalization of humans (16).

However, a more comprehensive analysis revealed that the human-specific deletion might have occurred approximately 5.3 MYA, which significantly precedes the first appearance of the genus *Homo* in the fossil record (42). The study also claimed that inactivation of the *MYH16* gene would have had little contribution to the expansion of the brain, as the majority of brain growth in humans occurs long before the development of the masticatory musculature (43). Another study has indicated that, although humans have relatively small jaws and jaw muscles compared with those of closely related great apes, the human masticatory apparatus is highly efficient and can produce relatively high bite forces using low muscle forces (44).

It is possible that a dietary shift, perhaps to consuming softer foods, might have permitted smaller jaws in early humans, which led to a lower dependency on the *MYH16* gene product (45). It is also likely that human ancestors evolved smaller jaws and chewing muscles without losing overall masticatory function. As a result, the *MYH16* gene might have simply become extraneous, and under relaxed selection pressure, the gene accumulated disruptive mutations. Although the pseudogenization event of the *MYH16* gene might not have directly driven encephalization, it is a compelling example of the association between gene inactivation and the acquisition of human-specific phenotypes.

LOSS OF BITTER TASTE RECEPTOR GENES AND DIETARY EVOLUTION

There are five major taste sensations in humans and most other vertebrates: salty, sour, sweet, umami (savory), and bitter. Each of these tastes is perceived by distinct sets of taste receptors (46). In various mammals, taste receptor genes are often pseudogenized as they adapt to different dietary habits and life styles. For example, cats are not able to detect the sweetness of sugars because of their loss of functional sweet receptors, likely a result of their carnivorous behavior (47). Giant pandas lack a functional umami taste receptor gene; the inactivation of which was reported to coincide with their dietary shift to bamboo (48). Bottlenose dolphins lost receptors for the three basic tastes, sweet, bitter, and umami, most likely as a result of swallowing food whole, without chewing (49).

Bitter taste sensation is mediated by type 2 taste receptors (TAS2Rs) and is usually associated with detection and avoidance of toxic chemical substances or putrid food (46). Sequence comparison of *TAS2R* genes among mammals re-

vealed that two of them, *TAS2R62* and *TAS2R64*, are pseudogenes in humans, which became inactivated due to nonsense and/or frameshift mutations after the human-chimpanzee divergence and fixed in the modern human population (50, 51). These two pseudogenes are shared with other archaic humans, Neanderthals and Denisovans, indicating their ancient origins (52). *TAS2R* genes with an intact coding region also show relaxation of selective constraints, implying that the bitter taste sensation is generally reduced in the human lineage in comparison with other mammals (53).

Loss and/or relaxed selection of *TAS2R* genes may be associated with the dietary shift of ancestral humans (52). Human ancestors consumed more starch-rich tuberous roots such as yams, which generally tasted bitter. Extra calories obtained from these bitter root vegetables may have allowed humans to develop bigger brains. Eventually, humans learned to cook in order to remove bitter substances, which might have further reduced selection pressure on bitter taste receptor genes. Therefore, loss and/or relaxed selection of *TAS2R* genes might be deeply interwoven with the evolution of human dietary habits.

LOSS OF OLFACTION-RELATED GENES AND EVOLUTION OF CHEMICAL SENSING AND SIGNALING

Olfaction, or the perception of smell, is a crucial sense for animals and plays an important role in avoiding predators, searching for foods, and recognizing the opposite sex. Olfactory receptors (ORs) in the olfactory epithelium are responsible for the detection and discrimination of various odorants (54). The olfactory perception capabilities of humans, other apes, and Old World monkeys (OWMs) are generally considered to be significantly diminished when compared with other mammals, based on observations that these species have relatively small olfactory apparatuses and high number of OR pseudogenes (55-57). It has been proposed that humans and other catarrhines (OWMs and apes) have become more dependent on vision rather than olfaction, which created a relaxed selection pressure on OR genes (57).

However, various studies have suggested that there is no direct link between the evolution of trichromatic color vision and the degeneration of OR genes in catarrhines (58, 59). Another study claimed that humans are capable of distinguishing between more than 1 trillion olfactory stimuli (60). Recently, this estimation has been questioned and should be scrutinized by further studies (61).

Interestingly, some olfaction-related genes other than OR genes are inactivated in humans. For example, transient receptor potential cation channel, subfamily C, member 2 (*TRPC2*), which is required for pheromone sensing, is a pseudogene in humans (62, 63). A comparative genomics study of primates revealed that the *TRPC2* gene is a pseudogene not only in humans but also in other catarrhines, indicating that the gene became inactivated in a common ancestor of OWMs and apes

(64). It has been speculated that the development of trichromatic color vision might have led to a reduced dependency on chemosensory communication for mediating a variety of social behaviors, although humans seem to still rely on chemosignals for certain interactions (65).

The *MOXD2* gene, which encodes a highly conserved monooxygenase DBH-like 2 protein in vertebrates, is another inactive gene that is proposed to be involved in olfaction in humans (21). The human *MOXD2* gene has an exon-deletion mutation, which occurred after the human-chimpanzee divergence. The mouse ortholog *Moxd2* gene has been reported to be highly expressed in olfactory epithelium, implying that vertebrate *MOXD2* could be involved in olfactory function (66). *MOXD2* and its paralogs, *MOXD1* and *DBH*, belong to the copper type II, ascorbate-dependent monooxygenase family. *DBH* is a dopamine β -hydroxylase, which converts dopamine to norepinephrine (noradrenaline) in the synaptic vesicles of postganglionic sympathetic neurons and for which deficiency or polymorphism is associated with various neuropsychiatric disorders (67-69). Vertebrate *MOXD2* might also be involved in metabolism of neurotransmitters, potentially during transduction of olfactory stimuli.

The *MOXD2* gene is also inactive in other apes: orangutans have multiple nonsense mutations and gibbons do not have the gene due to a genomic deletion that occurred in a common ancestor of all contemporary gibbons (21, 70). The gorilla gene shows an elevated non-synonymous substitution rate/synonymous substitution rate ratio, perhaps because its selection pressure has been recently relaxed, while the chimpanzee gene appears to be under purifying selection. Therefore, the loss of *MOXD2* enzyme function might be associated with the reduced olfactory capabilities of humans and other apes. However, it remains uncertain whether this gene inactivation caused the diminished olfaction or the reduced dependency on olfaction led to relaxed selection pressure on the gene (21).

Interestingly, *MOXD2* gene inactivation also occurred in another mammalian clade, the Cetacea (70). The dolphin and whale *MOXD2* genes underwent a broad range of disruptive mutations, including nonsense, frameshift, and complete deletion. In whales, the *TRPC2* gene is also a pseudogene, and many OR genes are not functional (71-73). The degeneration of these genes may coincide with the evolution of a fully aquatic lifestyle and highly sophisticated vocal communication and/or echolocation. Therefore, inactivation of olfaction-related genes of humans and other organisms may be a remarkable molecular signature of adaptive evolution to habitat shifts and/or sociobehavioral changes.

POLYMORPHIC GENE INACTIVATION AND ASSOCIATED PHENOTYPES IN THE HUMAN POPULATION

Sequence polymorphisms resulting in loss of function of a derived allele are frequently observed in the human population,

indicating that gene inactivation events are rather common (24, 26, 74-76). Population genetic studies have demonstrated that inactive allele frequencies range from rare to common, with some being nearly fixed (24, 26). Many of these inactive alleles have been reported to be associated with beneficial phenotypes in the individuals harboring them (75). Examples include: polymorphic pseudogenes in some *TAS2R* genes for bitter taste (51), a 32-bp deletion allele of the *CCR5* gene that is found in relatively high frequency in Europeans (77), a nonsense allele of the *CASP12* gene that is rare in Africans but very common in non-Africans (78, 79), and a nonsense allele of the *ACTN3* gene that is commonly detected in various human populations (80).

Humans generally have 26 intact *TAS2R* genes for bitter taste sensation (51). *TAS2R* genes exhibit a high level of polymorphisms among human individuals, including non-synonymous substitutions and copy-number variations. Interestingly, some polymorphisms involve loss-of-function mutations; there are polymorphic pseudogenes in *TAS2R2*, *TAS2R7*, *TAS2R45*, and *TAS2R46* as well as polymorphic whole-gene deletions in *TAS2R43* and *TAS2R45* (51, 53, 81-83). Loss of a specific *TAS2R* gene is involved in individual-specific phenotypes in bitter taste sensation. For example, the bitter taste receptor encoded by the *TAS2R43* gene responds to the artificial sweetener saccharin and contributes to the bitter aftertaste of saccharine and other related sweeteners (84). Lack of the *TAS2R43* gene renders affected individuals to be insensitive to the bitterness of saccharine and other natural plant compounds, including aloin and aristolochic acid (83).

The human *CCR5* gene encodes for C-C chemokine receptor type 5 (also known as CD195), which is a G-protein coupled receptor on the cell surface of white blood cells and acts as a receptor for chemokines (85). Human immunodeficiency viruses (HIVs) initially bind *CCR5* proteins to enter and infect mucosal CD4⁺ T cells, which eventually may cause acquired immune deficiency syndrome (AIDS) (86, 87). A 32-bp deletion mutation in the *CCR5* gene, referred to as the *CCR5-Δ32* allele, is almost exclusively found in approximately 5%-14% of the European population and their descendants (77). The mutant allele produces defective proteins that cannot be detected on the cell surface. This lack of *CCR5* protein on the cell surface protects individuals homozygous for the *CCR5-Δ32* allele from HIV infection (88). Because the frequency of this pseudogene allele is relatively high in Europeans, it was initially suggested that this allele appeared recently (approximately 1,000 years ago) and has undergone positive selection (77). However, a detailed study of the allele age and pattern of genetic variation revealed that the *CCR5-Δ32* allele may have arisen more than 5,000 years ago and was selected for another reason or neutrally evolved, indicating that its resistance to HIV was pre-adaptive (89). Nevertheless, the resistance to HIVs and protection against AIDS as a result of *CCR5* gene inactivation suggest that the *CCR5* protein is a promising therapeutic target for preventing the spread of HIV (90, 91).

The *CASP12* gene encodes caspase 12, which belongs to a family of cysteine proteases that cleaves their substrates at C-terminal aspartic acid residues (92). There is a nonsense polymorphism in the human *CASP12* gene, namely, a stop codon in exon 4 induces premature termination (79, 93). The inactive allele is very common in non-Africans, but rare in Africans. Intact full-length caspase 12 attenuates the inflammatory and innate immune responses to endotoxins, which can result in a severe septic response (79). Thus, the nonsense allele is advantageous as it confers resistance to severe sepsis, and it could have recently undergone positive selection in non-Africans (25, 78). The inactive allele appears to have originated in Africa and was initially neutral or approximately neutral. As human population size and density increased, individuals may have experienced more infectious diseases, for which the inactive allele was highly advantageous as a result of sepsis resistance (78). The human *CASP12* gene is another example of a pre-adaptive gene inactivation and subsequent positive selection.

The human *ACTN3* gene encodes α -actinin-3, an actin-binding protein found in skeletal muscle (94). *ACTN3* proteins are a major structural component of Z lines and regulate the function of fast twitch (type II) muscle fibers, which underlie forceful and rapid muscle contraction during athletic activities such as sprinting (95). There is a nonsense polymorphism R577X in exon 16 of the *ACTN3* gene (80, 95). This mutation results in undetectable *ACTN3* protein in skeletal muscle. Interestingly, the *ACTN3* genotype was suggested to be associated with human elite athletic performance; the 577X allele is associated with endurance, while the 577R allele is associated with sprinting and strength performance (96-98). The high frequency of the nonsense allele in human populations could have resulted from positive selection for improved endurance-running capabilities. This might have bestowed human ancestors increased opportunities for successful scavenging and/or persistence hunting (99-101).

Studies of human genomes have revealed a large number of loss-of-function mutations in humans that are polymorphic or fixed in populations. For example, a study on the genomes of Icelanders revealed a total of 6,795 autosomal loss-of-function alleles that are affected by nonsense or insertion/deletion mutations in 4,924 genes (24, 102). Some inactive alleles were found to be shared with archaic humans such as Neanderthals and are thought to be acquired by introgression (102). These loss-of-function mutations may contribute to the phenotypic variety of modern humans and could act as sources for adaptive evolution during various environmental changes (76).

CONCLUSIONS

The emergence of loss-of-function mutations and their subsequent expansion in modern humans indicate that gene inactivation is one of the mechanisms that confer novel advantageous phenotypes. A large number of gene inactivation events

have been identified thus far in the human genome. Several of these exhibited strong association with beneficial traits. However, many have been neglected since these genes are non-functional pseudogenes in humans. Molecular functional studies of these genes in model organisms will provide clues about the molecular mechanisms for the emergence of human-specific phenotypes.

ACKNOWLEDGEMENTS

This study was supported by the National Research Foundation of Korea (NRF) grant (NRF-2012R1A1B3001513) funded by the Ministry of Education, Science and Technology, Republic of Korea.

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