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0168-9525/$ – see front matter.
doi:10.1016/j.tig.2007.09.006 Available online 9 May 2008

Genome Analysis

Mutation of miRNA target sequences during human evolution

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It has long-been hypothesized that changes in non–protein-coding genes and the regulatory sequences controlling expression could undergo positive selection. Here we identify 402 putative microRNA (miRNA) target sequences that have been mutated specifically in the human lineage and show that genes containing such deletions are more highly expressed than their mouse orthologs. Our findings indicate that some miRNA target mutations are fixed by positive selection and might have been involved in the evolution of human-specific traits.

Selection on microRNA target sequences

The microRNA (miRNA) gene family is a highly conserved and important part of gene regulation in animals and plants [1]. The miRNAs are short (~22 bases long) RNA molecules, which are incorporated into a regulatory protein complex. Here, the miRNAs determine the binding specificity of the complex by hybridisation to partially complementary sequences, primarily in the 3′ untranslated region (UTR) of mRNAs. The majority of functional animal miRNA target sequence requires perfect base pairing between bases 2–7 of the miRNA and the target sequence, accompanied by an adenine in position 1 of the target or an additional base pair between the target and position 8 of the miRNA [2–4]. When recruited to a 3′ UTR, the miRNA complex destabilizes the mRNA and reduces translation [5,6] by mechanisms that are still not completely understood [7,8].

In humans (*Homo sapiens*), about one third of all protein-coding genes contain conserved target sequences for the 163 miRNA families that are conserved between humans and dogs [2,9,10]. These miRNA target sequences have been under strong purifying selection and are, on average, 3.5 times more conserved than control sequences [2] and depleted in single nucleotide polymorphisms (SNPs) [11]. Moreover, the 3′ UTRs of mRNAs co-expressed with tissue-specific miRNAs are depleted in target sequences of those miRNAs, indicating that a significant proportion of the miRNA target sequences resulting from mutations in the 3′ UTRs are selected against [12,13].

In contrast to transcription factors, which have degenerate binding specificity, miRNA target sequences have strict sequence requirements to be functional (i.e. presence of a specific 7mer sequence). It therefore requires only a single mutation to inactivate a miRNA target sequence. In

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most cases, such mutations will be selected against [11], but selection pressures are not static over time and some miRNA target sequence mutations can be neutral or even advantageous and be fixed by drift or positive selection acting on the mutation.

Deletion of miRNA target sequences during human evolution

We mapped 402 human-specific mutations in miRNA target sequences (miRNA 7mers) that are conserved in chimpanzee (*Pan troglodytes*), mouse (*Mus musculus*), rat (*Rattus norvegicus*) and dog (*Canis familiaris*) (see Table 1a in the online supplementary material). Of 402 mutations, 127 create a G-U wobble base pair instead of a Watson–Crick base pair (see Supplementary Table 1a); this ratio was not significantly different to that observed in the controls ($P = 0.82$). These mutations might not completely inactivate the miRNA target, but in general, there are relatively few conserved miRNA targets that lack perfect Watson–Crick seed pairing [2]. It is also possible that some of these miRNA target sequences are conserved for reasons other than miRNA regulation; however, miRNA target sequences predicted on the basis of conservation are thought to be 50–85% accurate [11,14,15]. Thus, most of the 402 miRNA target sequences will be real miRNA targets that mediate repression in rat, mouse, dog and chimpanzee, but are inactivated in humans. Because miRNA target predictions rely on conservation, the chimp, mouse, rat or dog miRNA target sites corresponding to the human-mutated targets are unlikely to

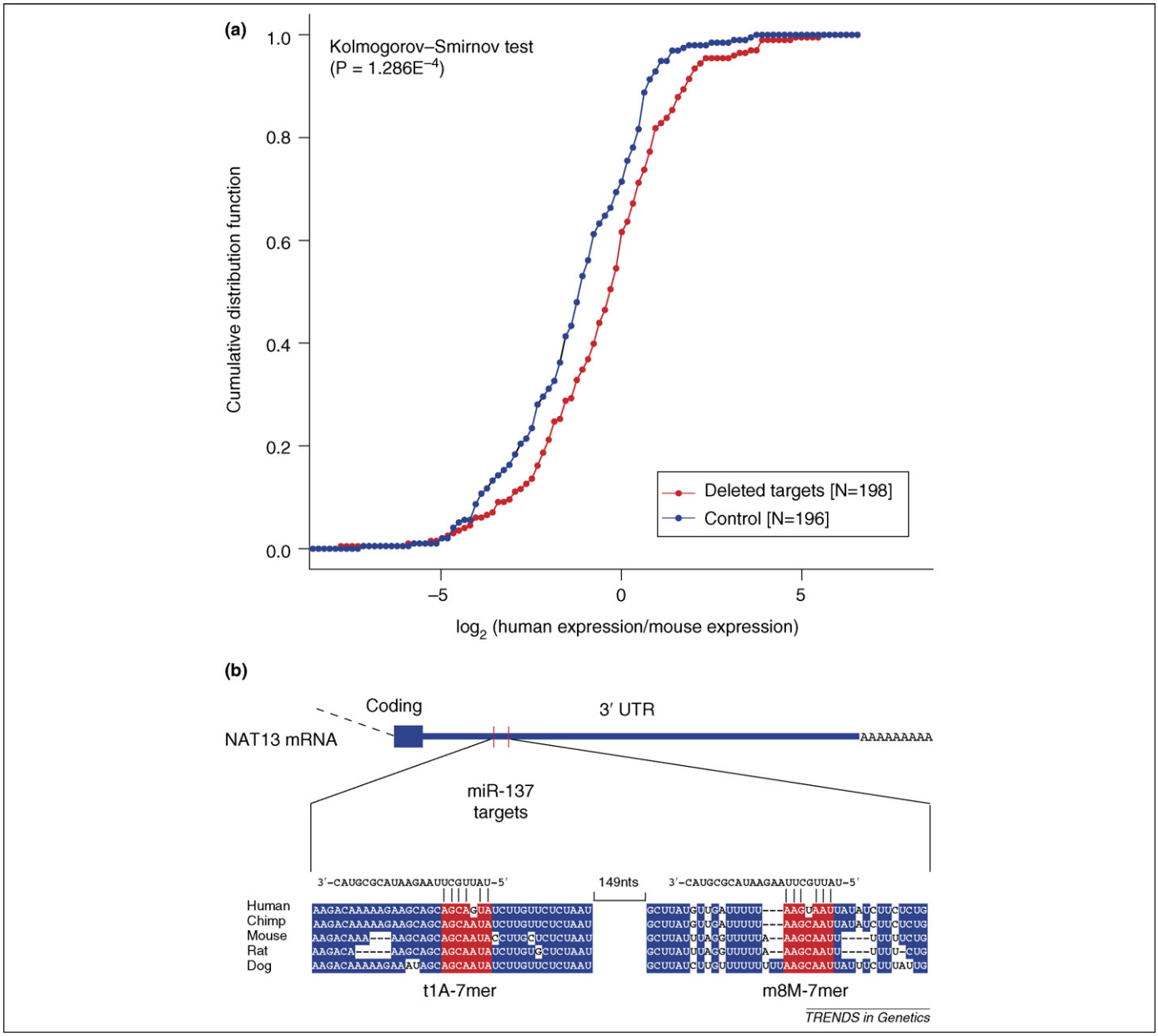

Figure 1. Human-specific mutations in microRNA (miRNA) target sequences. **(a)** Log ratios of human and mouse DNA microarray expression values [17] from genes that contain a human miRNA target sequence mutations are plotted as a cumulative distribution function and compared with the corresponding cumulative distribution function for genes containing deletions in control sequences. Only expression values from tissues known to express the miRNA in question were considered [18]. The two cumulative distribution functions are significantly different ($P = 1.286E^{-4}$) by the Kolmogorov-Smirnov test. **(b)** Two target sequences for miR-137 located in the human *NAT13* have been deleted since the human split from chimpanzee.

Table 1. miRNA[a] target mutations in the GABA pathway

miRNA target sequence mutated	Gene symbol	Gene name	Genome coordinates (hg18) of mutated miRNA target sequence	Entrez gene ID
miR-376c	*GABRA4*	GABA A receptor, α4	chr4:46624894–46624899	2557
miR-128	*GABRA6*	GABA A receptor, α6	chr5:161061663–161061669	2559
miR-27	*GABRA6*	GABA A receptor, α6	chr5:161061664–161061670	2559
miR-183	*GABRB3*	GABA A receptor, β3	chr15:24340171–24340176	2562
miR-326	*GABRB3*	GABA A receptor, β3	chr15:24340117–24340122	2562
miR-22	*GABRE*	GABA A receptor, ε	chrX:150872552–150872557	2564
miR-431	*GABRR1*	GABA receptor, ρ1	chr6:89945049–89945054	2569

[a]miRNA, microRNA.

have been investigated. However, an miR-7b target sequence located in the 3′ UTR of the *FOS* gene – a subunit of the transcription factor activator protein 1 (AP1) – has been characterized in mouse [16] and is mutated in humans. Interestingly, mouse miR-7 is upregulated in the hypothalamus after chronic hyperosmolar stimulation and inhibits FOS translation via the *FOS* mRNA 3′ UTR [16].

In addition to the 7mers corresponding to the miRNA target sequences, we also mapped mutations in conserved instances of ten carefully selected, control 7mer sequences for each miRNA family (for further details, see online supplementary material). Surprisingly, we found that the mutations in the miRNA 7mers and the controls are fixed at similar rates (1.07 versus 1.02 mutations per kilobase). Because miRNA targets are generally more conserved than control sequences [2] and are depleted in SNPs [11], we would expect mutations in miRNA target sequences to be being fixed at a lower rate. Some miRNA target sites might have experienced relaxed selection pressure because of redundant target sites for the miRNA in question. For the 402 3′ UTRs containing the miRNA target mutations, we found 128 that contain another site for the same miRNA. For the controls sites, we found a similar rate of sites being present in the same 3′ UTR ($P = 0.56$). It is also possible that some miRNA target mutations have been fixed by positive selection, acting to increase the expression of the targeted genes. Analysis of microarray expression data from human and mouse tissue [17] expressing the relevant miRNAs [18] revealed that genes containing a mutated miRNA target sequence are more highly expressed in the human tissue than in the equivalent mouse tissue ($P = 1.3E^{-4}$; Figure 1a). This expression difference is remarkable, because there might be differences in the miRNA expression levels between human and mice for the different tissues. In addition, the direct effect of the mutations of the miRNA target sequence will obviously only be observable in the tissues and cell types that express the miRNA in question. Thus, the difference that we observed probably reflects that genes with mutated miRNA sequences have been under selection for increased expression, which has been achieved by several means, one of them being mutation of the miRNA target sequence.

One gene – human *N*-acetyltransferase 13 (*NAT13*) – is noteworthy. Two of its miR-137 target sequences have mutated since humans diverged from chimpanzee ~5 million years ago (Figure 1b). Given the relatively few mutated miRNA sequences in the human genome, it is unlikely that both these mutations were fixed by random drift since humans split from chimpanzee. Human miR-137 is predominantly expressed in brain tissue [19] and is conserved back to Zebrafish (*Danio rerio*). It is expressed in a specific brain areas during the Zebrafish larval stage [20], suggesting that the human-specific selection pressure responsible for deleting the miR-137 target sequences in *NAT13* is related to neural development and/or function. NAT13 is an N-terminal acetyltransferase, which is required for centromeric cohesion in mitosis and correct timing of sister chromatid separation in HeLa cells [21,22].

Some human miRNA target sequences targeted by SNPs have been mapped [11,23]. Most SNPs identified in these studies are present at low frequencies in the human population, indicating that mutations in miRNA target sequences are predominantly selected against. We found that 47 of the 402 miRNA target sequence mutations present in the human reference genome overlap with known human SNPs. These SNPs were not included in previous studies, because the miRNA target sequences are not conserved in the human reference genome, and as expected, we found that many of these SNPs are present at high frequencies in the human population (see Figure S3 in the online supplementary material).

GABA(A) receptor genes have been targeted by mutations in miRNA target sequences

We used Gene Ontology (GO) analysis (http://david.abcc.ncifcrf.gov/home.jsp) to identify functional categories that were enriched within the genes that have had miRNA target sequence mutations [24] (see the full list in Supplementary Table 1b). One of the most significantly enriched GO categories was the GABA signalling pathway ($P = 8.98E^{-04}$), reflecting six miRNA target sequence mutations that have occurred in the genes encoding five different subunits of the GABA(A) receptor [α4, α6, β3 (two mutations), ε, ρ; see Table 1]. The GABA(A) receptor mediates inhibitory neurotransmissions in the central nervous system and are involved in sleep, anxiolysis, associative learning and memory, sensorimotor processing and consciousness [25]. Our results therefore indicate that there has been selection for increased expression of some of the GABA(A) subunits in specific regions of the brain.

Concluding remarks

It has long-been hypothesized that changes in non–protein-coding genes and the regulatory sequences that control expression could undergo positive selection [26], but such positive selection is difficult to demonstrate. Recently, is has been shown that positive selection has targeted the promoter regions of many genes involved in nutrition and neural development and function [27]. Here we show that 402 putative microRNA (miRNA) target

sequences have been specifically deleted in the human lineage and that the expression of the genes containing such deletions is increased compared with those in mouse.

Collectively, our findings indicate that mutations in human miRNA target sequences are functionally significant and that there might have been positive selection for deletion of miRNA target sequences in the human lineage. A single mutation is presumably enough to inactivate a miRNA target sequence and might be a rapid way for evolutionary fine tuning of gene expression. Our data should facilitate further studies into the functional relevance of miRNA target sequences that have been deleted during human evolution.

Acknowledgements

This work was funded by a Carlsberg Foundation Grant (21–00–0680) to the Molecular Evolution Group. The authors thank Rasmus Nielsen for useful discussions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tig.2008.03.009.

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0168-9525/$ – see front matter.
doi:10.1016/j.tig.2008.03.009 Available online 9 May 2008

Letters

Less is more: decreasing the number of scientific conferences to promote economic degrowth

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Today's scientists are confronted with a serious paradox: although the goal of their research is often to mitigate the negative impact of human activities (e.g. loss of biodiversity), the research community itself can be a significant contributor to the problem. Moreover, grant evaluation criteria (e.g. number of publications, number of presentations given at international conferences) strongly favour activities that have a significant impact on the environment. From an historical perspective, there is a strong positive correlation between scientific progress and environmental

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