

## REVIEW

## Personalized genetics of the cholinergic blockade of neuroinflammation

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**Abstract**

Acetylcholine signaling is essential for cognitive functioning and blocks inflammation. To maintain homeostasis, cholinergic signaling is subjected to multi-leveled and bidirectional regulation by both proteins and non-coding microRNAs ('CholinomiRs'). CholinomiRs coordinate the cognitive and inflammatory aspects of cholinergic signaling by targeting major cholinergic transcripts including the acetylcholine hydrolyzing enzyme acetylcholinesterase (AChE). Notably, AChE inhibitors are the only currently approved line of treatment for Alzheimer's disease patients. Since cholinergic signaling blocks neuroinflammation which is inherent to Alzheimer's disease, genomic changes modifying AChE's properties and its susceptibility to inhibitors and/or to CholinomiRs regulation may affect the levels and properties of inflammasome components such as NLRP3. This calls for genomic-based medicine approaches based on genotyping of both coding and non-coding single nucleotide polymorphisms (SNPs) in the genes involved in cholinergic signaling. An

example is a SNP in a recognition element for the primate-specific microRNA-608 within the 3' untranslated region of the AChE transcript. Carriers of the minor allele of that SNP present massively elevated brain AChE levels, increased trait anxiety and inflammation, accompanied by perturbed CholinomiR-608 regulatory networks and elevated prefrontal activity under exposure to stressful insults. Several additional SNPs in the AChE and other cholinergic genes await further studies, and might likewise involve different CholinomiRs and pathways including those modulating the initiation and progression of neurodegenerative diseases. CholinomiRs regulation of the cholinergic system thus merits in-depth interrogation and is likely to lead to personalized medicine approaches for achieving better homeostasis in health and disease.

**Keywords:** acetylcholinesterase (AChE), Alzheimer's disease, genetics, neuroinflammation, single nucleotide polymorphisms (SNPs).

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**Acetylcholine signaling supports cognition and blocks inflammation**

Acetylcholine (ACh) signaling is best characterized as a neurotransmitter-derived signaling pathway. Within the nervous system, ACh operates both as a neurotransmitter and as a neuromodulator, and in the periphery, it conveys messages of neurons or other ACh-producing cells to their parasympathetic effectors (Picciotto *et al.* 2012; Soreq 2015). Cholinergic signaling at large, and the ACh hydrolyzing enzyme AChE specifically, is simultaneously involved in central cognitive processes such as learning, memory, and stress responses and in activating the parasympathetic system and mediating both neuromuscular and anti-inflammatory responses (Soreq 2015). In brain neurons, ACh signaling

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*Abbreviations used:* ACh, acetylcholine; AChE, acetylcholinesterase; AD, Alzheimer's disease; ADHD, attention Deficit–Hyperactivity Disorder; BChE, butyrylcholinesterase; ChE-Is, Cholinesterase inhibitors; CholinomiRs, non-coding microRNA regulators of ACh signaling; CHRN, nicotinic Cholinergic Receptor; IBS-D, diarrheal Predominant Irritable Bowel Syndrome; IL, interleukin; LPS, lipopolysaccharide; miR, microRNA; PD, Parkinson's Disease; PPAR $\gamma$ , peroxisomal proliferation receptor gamma; PTSD/S, post-traumatic stress disorder/symptoms; SLITRK1, slit and Trk-like 1; SNPs, single nucleotide polymorphisms; TZD, thiazolidinedione; UTR, untranslated region.

elevates under anxiety (Graef *et al.* 2011) and suppresses inflammation (Rosas-Ballina *et al.* 2011). At neuromuscular junctions of skeletal muscles, ACh determines channel opening (Fambrough 1979); in peripheral cells including pancreatic alpha cells (Rodriguez-Diaz *et al.* 2011), placenta cells (Wessler *et al.* 2001), thrombocytes (Schedel *et al.* 2011) and lymphocytes (Kawashima and Fujii 2004; Olofsson *et al.* 2016), non-neuronal ACh controls numerous signaling processes. The diverse roles of ACh, and the need to achieve homeostasis predict complex regulatory processes over its levels.

Recently, microRNA(miR) regulation emerges as a new level of control for ACh signaling (Simon *et al.* 2008; Hanin *et al.* 2014; Nadorp and Soreq 2014). MiRs are short (~22 nucleotides), non-coding RNAs that regulate various molecular pathways by post-transcriptional gene silencing (Bartel 2009; Krol *et al.* 2010). Each miR may target several mRNAs via interacting with short 'seed' motifs, often in specific locations on their 3'-untranslated region (3'-UTR) and can rapidly and effectively modulate entire pathways in a rheostat-like manner (Chen *et al.* 2004). MiRs are hence particularly suitable for controlling the rapidly adjustable physiology of the parasympathetic system, and could modulate both the neuronal and immune functions of ACh by controlling its production and destruction (Shaked *et al.* 2009). However, the great majority of current miR studies focus on the interaction and silencing activities of one miR and one selected target, whereas individual mRNA transcripts may be silenced by many different miRs (Boudreau *et al.* 2014; Hsu *et al.* 2014). This complexity provides checks and balances ensuring that silencing would work efficiently, while implying that many miRs may share part of their targets. Therefore, when a particular miR interacts with one specific target, its availability for interaction with other targets may be reduced; and inversely, when one mRNA interacts with a specific miR, the probability of that mRNA to be silenced by other miRs would likely be reduced, albeit in a cell- and tissue-specific manner. Very little is still known about the effects of such competition on basic physiological processes, although some studies from recent years have started unveiling the context dependence of this complexity (Hanin and Soreq 2011; Khella *et al.* 2013; Bracken *et al.* 2014; Wojtowicz *et al.* 2016); and we believe that the parasympathetic system is especially suitable to test this concept of complexity and appreciate its impact.

### Bidirectional cholinergic signaling regulators include 'CholinomiRs'

We have previously designated those miRs which regulate key cholinergic proteins 'CholinomiRs' (Nadorp and Soreq 2014). CholinomiRs extend regulatory functions over various cholinergic transcripts including nicotinic (Simon *et al.* 2008) and muscarinic (Scarr *et al.* 2013) receptors, as well as

ACh packaging and degrading enzymes (Hanin and Soreq 2011). Many of these sequences are shared predicted targets of several miRs (Nadorp and Soreq 2014). This implies the existence of a common regulatory mechanism over cholinergic signaling at both pre- and post-synaptic sites, keeping it in balance. Notably, many of the CholinomiRs are primate-specific and their levels are altered in various conditions, including inflammation, anxiety, and neurodegeneration.

CholinomiR regulation over the various transcript variants of AChE, a central regulator of cholinergic signaling, is of clinical, pharmacological, and physiological significance. The 'synaptic' AChE-S (or AChE-T) is active in the synapse, whereas both the soluble variant AChE-R and the erythrocyte-expressed AChE-E (AChE-H) are active in the periphery (Soreq and Seidman 2001). For example, miR-186 is an evolutionarily conserved predicted regulator of the both, the anxiety-induced AChE-R and the peripheral butyrylcholinesterase (BChE), which shares much of its protein sequence with AChE and hydrolyzes ACh as well. BChE's expression is elevated in the intestine of mice exposed to stressful conditions, predicting limited cholinergic blockade of intestinal inflammation (Nadorp and Soreq 2014). This may possibly be relevant for the prodromal stages of Parkinson's Disease (PD), which likely involve gastrointestinal symptoms that are part of the disease pathogenesis (Fasano *et al.* 2015). The unique entity of central nervous system inflammation is often referred to as neuroinflammation, and the cholinergic involvement in its physiological aspects (Filiou *et al.* 2014), is also relevant for other mental and neurodegenerative diseases. Thus, miR-132 targets AChE in both the brain and the periphery, its levels decrease sharply in Alzheimer's disease (AD) and the disruption of its regulation causes behavioral and learning-related phenomena (Shaltiel *et al.* 2013), as well as exacerbated inflammation (Shaked *et al.* 2009). Hence, CholinomiRs are key controllers of the interface between cholinergic signaling, neuroinflammation, and neurodegeneration.

### AChE inhibitors potentially serve as genomics-based modulators of AD therapeutics

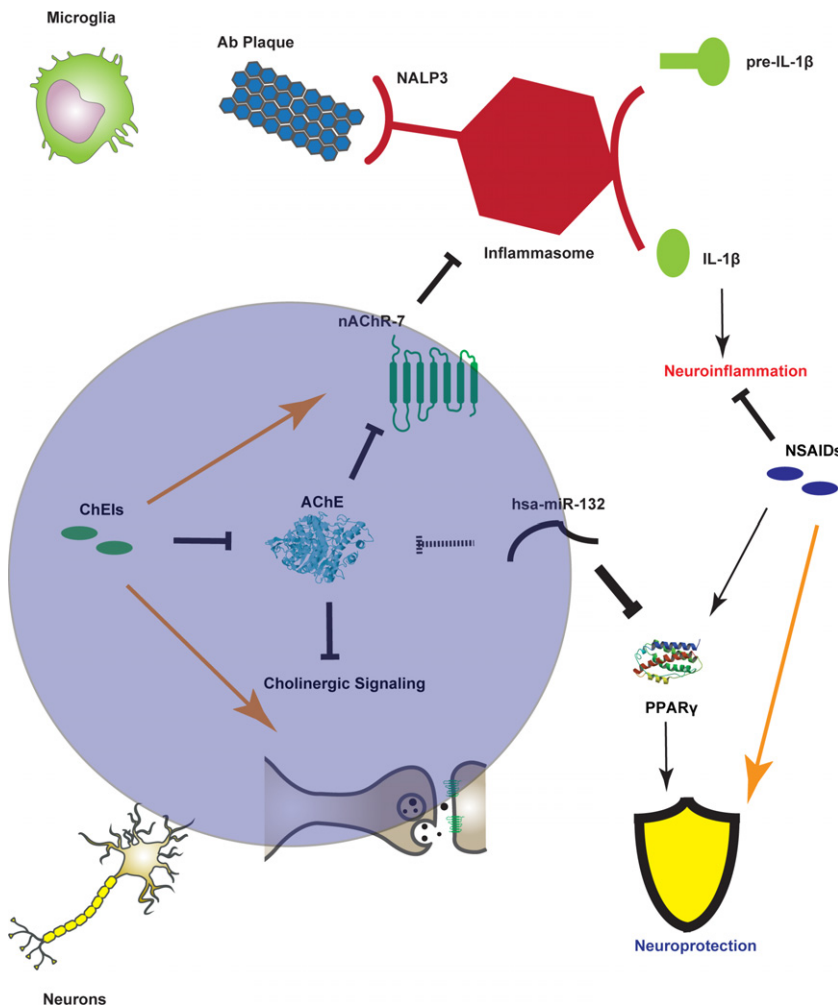
Neuroinflammation is involved in AD and many other neurodegenerative conditions such as PD (Simchovitz *et al.* 2016), frontotemporal dementia, amyotrophic lateral sclerosis, Huntington's Disease and more (Heneka *et al.* 2014). The diminished risk of AD among users of non-steroidal anti-inflammatory drugs (in t' veld *et al.* 2001; Wang *et al.* 2015) attests to its importance, but steroids do not exert a similar effect; which calls for a closer inspection of the neuroinflammatory pathway in AD (Heneka *et al.* 2015a,c; Wang *et al.* 2015). In this context, AChE inhibitors (ChE-Is) are currently the only effective line of treatment for AD patients (Birks 2006). ChE-Is may delay the consistent ACh depletion because of cholinergic cell death (McGeer *et al.* 1984),

decreasing the excessive activation of microglia that promotes neuroinflammation-mediated damage in response to the formation of A $\beta$  plaques (Heppner *et al.* 2015). Some of the non-steroidal anti-inflammatory drugs found to decrease the risk for AD were also identified as agonists of peroxisomal proliferation receptor gamma (PPAR $\gamma$ ), a target of the thiazolidinedione class of antidiabetics (Lehmann *et al.* 1997). Strikingly, activation of PPAR $\gamma$  was protective in AD model mice (Heneka *et al.* 2005), activation of all PPARs was even more protective (Kummer *et al.* 2015), and thiazolidinediones were protective against dementia in human patients (Heneka *et al.* 2015b). Of note, PPAR $\gamma$  is repressed in the liver indirectly by down-regulation of miR-132 (Mann *et al.* 2010), which is down-regulated in AD (Lau *et al.* 2013), and importantly is also a regulator of AChE (Shaked *et al.* 2009). It is hence tempting to speculate that the failure of endogenous PPAR $\gamma$  to protect against AD without pharmacological aid involves CholinomiRs contribution (Fig. 1).

Inflammatory reaction to A $\beta$  entails the release of Interleukin-1 $\beta$  (IL-1 $\beta$ ), a primary pro-inflammatory cytokine activated via cleavage by caspase-1, a part of the

inflammasome (Halle *et al.* 2008). The inflammasome is a soluble cytoplasmic complex of several proteins mediating the production and effect of pro-inflammatory cytokines (Martinon *et al.* 2002), activated by the NALP3 protein as a reaction to toxins, ATP (Mariathasan *et al.* 2006) and also fibrillar A $\beta$  (Halle *et al.* 2008). The inflammasome activation in AD is reflected as higher caspase activity in the post-mortem brains of AD patients and mild cognitively impaired volunteers, compared to cognitively spared age-matched controls (Saresella *et al.* 2016). Furthermore, mice lacking the NLRP3 gene coding to the NALP3 protein, as well as mice lacking caspase-1, show less cognitive deterioration when carrying familial AD mutations, indicating that the inflammasome is causally involved in the pathogenicity itself (Heneka *et al.* 2013). Neuroinflammation and its regulation are hence key elements in the initiation and progression of AD.

The anti-inflammatory cholinergic pathway is an active controller of neuroinflammation. Vagus nerve signaling through the alpha-7 nicotinic receptor inhibits the activation of the inflammasome (Lu *et al.* 2014); and nitric

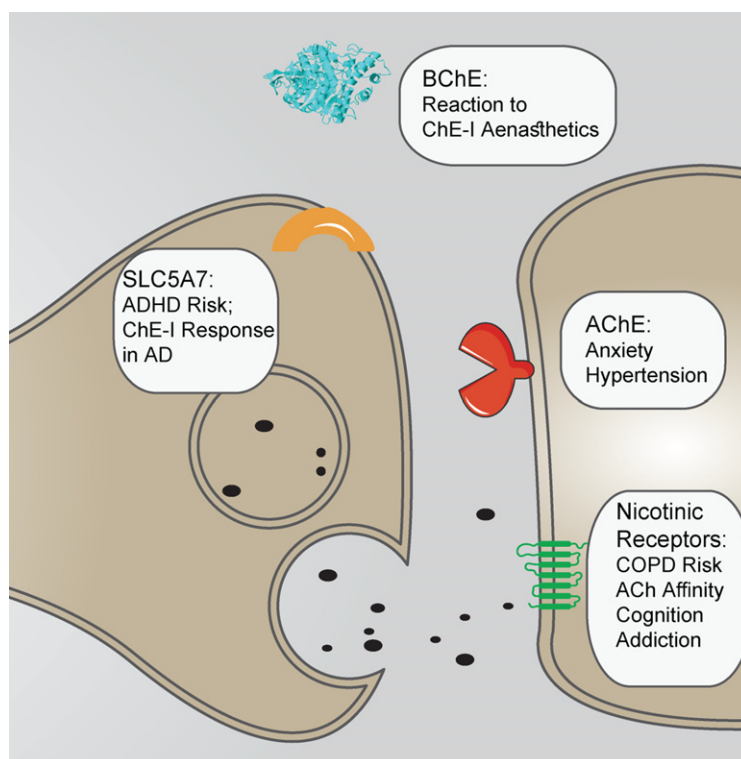


**Fig. 1** The network of Alzheimer's disease (AD), neuroinflammation, and the cholinergic system. Alzheimer's Disease-related processes occur in both microglia (upper panel) and neurons (lower panel); The cholinergic system and ChE-I treatment might modulate both these aspects, as does the treatment with certain non-steroidal anti-inflammatory drugs (NSAIDs). Microglia are activated by A $\beta$  plaques through the NALP3 receptor of the inflammasome. The activated inflammasome then promotes the cleavage of pre-IL1 $\beta$  to mature IL1 $\beta$ , which induces neuroinflammation. The nicotinic receptor inhibits inflammasome formation; Therefore, ChE-Is, which increase cholinergic signaling, might inhibit the inflammasome as well. NSAIDs, on the other hand, directly inhibit neuroinflammation by their anti-inflammatory action. In cholinergic neurons, synaptic transmission initiates signaling processes, which are altered in AD and might be potentiated by ChE-Is. Finally, NSAIDs involvement in neuronal activity might be mediated by neuroprotective peroxisomal proliferation receptor gamma (PPAR $\gamma$ ), which is activated by NSAIDs and inhibited by miR-132, which also targets acetylcholinesterase (AChE) and is down-regulated in AD.

oxide-mediated central muscarinic activity is anti-inflammatory as well (Pavlov *et al.* 2009), suggesting co-amplified anti-inflammatory role for ChE-Is in AD treatment. Correspondingly, ChE-I treatment reduces cytokine levels in AD patients (Reale *et al.* 2004). Nevertheless, the vast variability in response to ChE-Is among AD patients (Birks 2006) contrasts this potential robustness of ChE-Is against various aspects of AD, even when considering general pharmacokinetic-related inter-individual differences, such as polymorphisms in the CYP2D6 gene (Xiao *et al.* 2016). This raises the question of whether other inter-individual genomic differences, for example, changes in the 3'-UTR regions interacting with CholinomiRs can be responsible for the variable efficacy of ChE-Is (Martinelli-Boneschi *et al.* 2013). AChE, therefore, presents an attractive target for studying the personalized genetic aspects in the junction between the cognitive and neuroinflammatory pathways of AD pathology.

### Both coding and non-coding mutations in cholinergic genes modify signaling

The personalized medicine implications of inter-individual genomic differences represent a prominent trend in modern medicine (Hamburg and Collins 2010). Specifically, relatively common genomic variations that are not necessarily disease-causing but rather disease and treatment modifying, rapidly become a focus of intensive research. Single nucleotide polymorphisms (SNPs) constitute a large portion of these changes, with some of those existing in cholinergic genes and their regulators (Fig. 2). For example, a coding SNP in the neuronal nicotinic cholinergic receptor  $\alpha$ -5 subunit (CHRNA5) changes aspartate to asparagine and associates with increased risk of chronic obstructive pulmonary disorder (Cui *et al.* 2014), and coding SNPs in the CHRN4 gene modify receptor affinity to ACh, with consequent implications on cognition and addiction (Liang



**Fig. 2** Single nucleotide polymorphisms (SNPs) in Coding and non-coding regions in cholinergic genes and their reported impact. Cholinergic transcripts include, but are not limited to SLC5A7, responsible for choline transport into the cell [SNPs described in – (English *et al.* 2009; Harold *et al.* 2006; Lee *et al.* 2015)]; Acetylcholinesterase, responsible for central degradation of ACh (Hasin *et al.* 2004; Lin *et al.* 2016) (Hanin *et al.* 2014); Butyrylcholinesterase, responsible for peripheral degradation of ACh (Loewenstein-Lichtenstein *et al.* 1995); and several nicotinic and

muscarinic receptors, responsible for signal transmission across the synapse (Cui *et al.* 2014; Liang *et al.* 2005; Luo *et al.* 2005; Schlaepfer *et al.* 2008; Wang *et al.* 2004; Mobascher *et al.* 2016; Russo *et al.* 2015). These are all major players in the cholinergic signaling pathway, and may well be affected by miR-interfering SNPs in both their coding and non-coding regions. These effects modulate protein activity and regulation and may induce significant systemic impact, as reported in the cited references.

*et al.* 2005). Also, an aspartate to glycine substitution in the BChE gene reduces the capacity for ACh hydrolysis by the BChE protein, such that carriers of this substitution are more susceptible to side-effects of ChE-Is and cholinergic-related general anesthetics (Loewenstein-Lichtenstein *et al.* 1995). Similarly, a common variant in the SLC5A7 gene, coding for the Sodium/Choline cotransporter protein, associates with an increased rate of attention deficit–hyperactivity disorder (English *et al.* 2009), whereas an alanine to threonine substitution in SLC5A7 associates with better response to ChE-Is treatment in AD (Harold *et al.* 2006; Lee *et al.* 2015). Individual variability in the cholinergic network genes may hence be pivotal for the individual variability in the efficacy of AD therapeutics.

Several synonymous SNPs in ACh receptor genes have been associated with other brain-mediated phenotypes. SNPs in CHRNA4 associate with smoking, PD symptoms (Zhang *et al.* 2015), and electroencephalography (EEG)-measured reaction to stimuli (Mobascher *et al.* 2016). SNPs in non-coding intron domains of the CHRNA4/A5/B3 cluster associate with early tobacco and alcohol initiation (Schlaepfer *et al.* 2008); intron SNPs in CHRN4 associate with alcohol and drug dependence, as well as with affective disorders (Wang *et al.* 2004; Luo *et al.* 2005); and intron SNPs in the CHRNA7 gene regulate patients' response to ChE-Is treatment in AD (Russo *et al.* 2015). SNPs in cholinergic genes can therefore have a large impact on systemic and cognitive function, potentially also effecting the variability in individual responses to AD treatment, whether they reside in protein-coding or non-coding regions of these transcripts.

### CholinomiR–target interactions as potential modulators of neuroinflammation in AD

How SNPs in the non-coding regions of cholinergic genes exert global systemic effects and increase the risk for certain pathologies is largely unclear, but a recent study demonstrated one possible mechanism. This study predicted that SNPs which modify miR binding to the 3'-UTR of their target genes may initiate a domino-like cascade involving several layers of regulation. Publicly available SNP databases revealed 250 SNPs in the 3'-UTRs of miR target genes (Saunders *et al.* 2007); however, those SNPs do not necessarily lie in the region interacting with the 'seed' region (Hariharan *et al.* 2009). Nevertheless, some of these SNPs may interfere with miR/target interactions and thus affect the expression of their targets. This might be because of a modified secondary structure near the miR-binding site, which changes the accessibility of that miR to its binding site (Kertesz *et al.* 2007). Examples include a SNP in the 3'-UTR of the human ATF1 gene that associates with essential hypertension because of weakened binding by miR-1283, leading to the target ATF1 transcript up-regulation in

peripheral blood (Yang *et al.* 2015). A variant of the aldosterone synthase gene Cyp112, in which an A>G SNP interrupts miR-590-3p regulation, is also associated with essential hypertension in Chinese and German cohorts (Xiao *et al.* 2015).

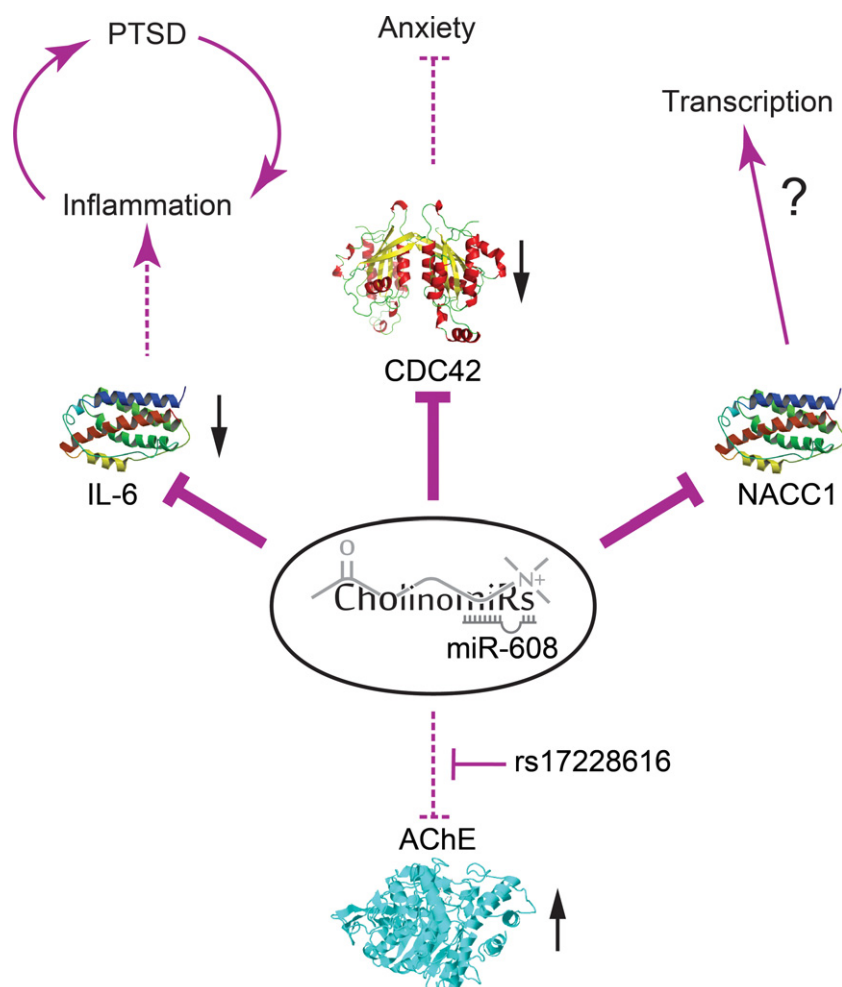
MiR-interacting SNPs are also of relevance to higher cognitive functioning and CNS-related disorders, as is seen for a variant in the 3'-UTR of the Slit and Trk-like 1 (SLITRK1) gene which strengthens an existing miR-189 target site. The SLITRK1 gene is expressed at high levels in the brain and supports neurite growth, and this SNP is involved in Tourette's syndrome (TS) and attention deficit–hyperactivity disorder (Abelson *et al.* 2005). In PD, a 3'-UTR SNP in the fibroblast growth factor 20 (FGF20) gene was identified as a risk factor for the disease by disrupting a binding site for miR-433, increasing translation of FGF20 which is associated with PD (Wang *et al.* 2008). Both strengthening and weakening of miR/target interactions may therefore be physiologically relevant.

At the inflammatory level, diarrheal predominant irritable bowel syndrome (IBS-D) is generally associated with dysfunctions in the serotonergic system. A 76G>A variant in the 3'-UTR of the serotonin receptor type 3 subunit gene (HTR3A) showed a strong association with female IBS-D by affecting binding of miR-510 to the HTR3E 3'-UTR, resulting in high expression of the receptor subunit (Kapeller *et al.* 2008). Strikingly, genes involved in neurodegeneration were shown to have higher prospects of carrying SNPs with a potential to modulate miR binding (Saba *et al.* 2014). These novel findings make SNPs which potentially disrupt miR-binding sites in 3'-UTRs of cholinergic genes promising candidates for research. Supporting this notion, recent work described below has shown their relevance and the major complexity that arises from the disruption of large miR networks, compatible with the dual paradigm of single miR/many targets; single target/many miRs. The genomics-affected interactions between miRs and their targets are therefore of special importance to therapeutic efforts aimed at addressing the various aspects of neurodegenerative diseases, including cognitive decline and inflammation.

### A SNP interrupting CholinomiR regulation has wide systemic effects

The complex nature of miR regulation over a network of transcripts is particularly evident in the case of miR-608 and its disrupted regulation of AChE by the rs17228616 SNP (Fig. 3). The minor allele of this SNP associates with highly elevated AChE activity in the brains of homozygous carriers. Also, this is accompanied by reduced levels of other hsa-miR-608 targets including CDC42, CD44, and IL-6 (Hanin *et al.* 2014). Systemic effects of this SNP include elevated anxiety and blood pressure, as well as relative resilience to post-traumatic stress symptoms (Lin *et al.*

**Fig. 3** AChE single nucleotide polymorphisms (SNP)-induced propagation of imbalanced miR-608 network. As previously reported (Hanin *et al.* 2014; Lin *et al.* 2016), a SNP in the 3'-UTR of AChE diminishes miR-608 binding and regulatory effect, causing both significant elevation of brain acetylcholinesterase (AChE) activity and down-regulation of other miR-608 targets attributed to 'unemployed' miR-608 molecules. These include the anxiety-inhibiting CDC42, the inflammatory-promoting IL6, and the neuronal transcription factor NACC1, with possibly complex effects over brain transcriptome and activity. Thick arrows represent an increase in positive or negative regulation, whereas stroked arrows represent diminished regulation.

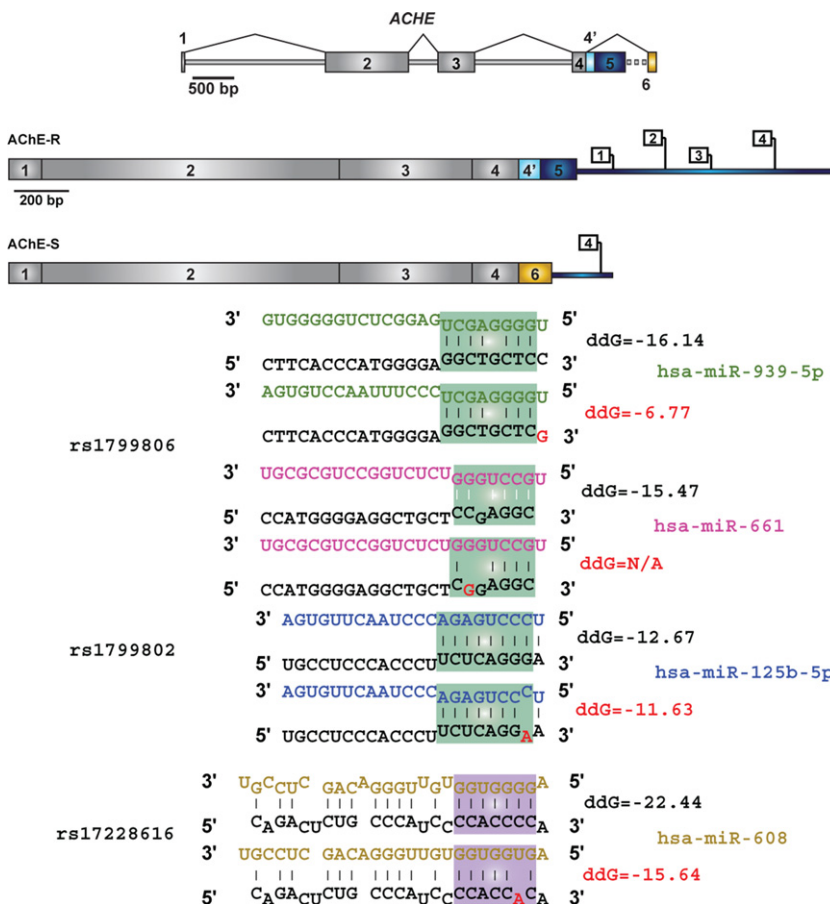


2016). Reduced CDC42 levels were anxiogenic in mice, and reports by others suggest that reduced levels of the pro-inflammatory IL-6 might attenuate post-traumatic stress disorder-related symptoms (Gill *et al.* 2009; Wilson *et al.* 2013) and modulate inflammation (Zimmerman *et al.* 2012; Gill *et al.* 2013). Down-regulation of IL-6 might also explain why despite excessive AChE levels, which might limit the cholinergic anti-inflammatory pathway, SNP carriers present only slightly increased inflammatory markers (Hanin *et al.* 2014). Skewing homeostasis by tilting a single miR/target interaction is therefore shown as a possible mechanism for the initiation of a complex phenotype because of dual changes in the level of the single affected target as well as in the multiple other targets of that miR and the interactions between these targets.

### The future prospects of genomics-based cholinergic-targeted medicine

The physical proximity of individual SNPs on a single transcript may affect the potency of the relevant miRs to

suppress their targets, because of competition on the binding interactions. In this context, several of the identified SNPs in the AChE gene (Hasin *et al.* 2004) reside close to each other in the 3'-UTR region, such that interaction with one miR might possibly interfere with other miR/AChE interactions. Of note are two specific SNPs – rs17228602 and rs1799806 (Fig. 4). Rs17228602 resides in the recognition element for miR-125b, which predictably targets both the soluble AChE-R variant (suppression of which would reduce ACh destruction) and the vesicular acetylcholine transporter (suppression of which would inversely reduce ACh production) (Nadrop and Soreq 2014). This indicates homeostatic bidirectional impact of miR-125b on cholinergic signaling. Intriguingly, miR-125b is down-regulated in response to bacterial lipopolysaccharide exposure, possibly through the activation of NFκB (Tili *et al.* 2007). This creates a seemingly self-controlled mechanism, by which NFκB suppresses hsa-miR-125b, which in turn re-balances cholinergic signaling, keeping the inflammatory response to anxiety under continuous control. However, deficient miR-125b/AChE interaction in carriers of the rs17228602 SNP might impair the



**Fig. 4** Four single nucleotide polymorphisms (SNPs) in predicted miR-binding sites of the AChE 3'-UTR. Shown are common SNPs in the 3'-UTR of acetylcholinesterase (AChE) splice variants according to the 1000 genome project, and the bioinformatics prediction of the effect of three of them on binding of miRs (Kertesz *et al.* 2007). The presented ddG values predict the difference in free energy between the bound and unbound conditions of the miR and mRNA. For example, rs1799806 would slightly enhance miR-939-5p binding, but reduce miR-661 binding; similar effects are observed for rs1799802 and miR-125b and for rs17228616 and miR-608 (the latter prediction was experimentally validated) (Hanin *et al.* 2014).

capacity for keeping homeostatic cholinergic signaling under both stress and inflammation, leading to a vicious anxiety-inflammatory cycle (Zimmerman *et al.* 2012).

In addition to its direct impact on AChE transcripts, the rs17228602 SNP might leave 'unemployed' miR-125b chains that fail to bind AChE mRNA, similarly to the rs17228616 miR-608/AChE-interfering SNP (Hanin *et al.* 2014). These chains will be free to target other transcripts carrying the same 'seed' domain, decreasing the levels of other miR-125b targets which would likely impair synaptic plasticity (Edbauer *et al.* 2010). The anxiety phenotype of mice lacking the AChE-3'-UTR (Shaltiel *et al.* 2013), where miR-125b would fail to exert its effect, is compatible with this prediction. Interestingly, miR-608 and miR-125b interact with closely positioned sites on the AChE 3'-UTR and share some targets and pathways, indicating a synergistic function of the SNPs interrupting their AChE binding; for example, miR-608 regulates IL-6 (Hanin *et al.* 2014), whereas miR-125b regulates its receptor, IL-6R (Gong *et al.* 2013), implying a synergistic anti-inflammatory effect of the two miRs and an amplified inflammatory phenotype in co-carriers of the two SNPs. However, this is not always the case. For example, miR-608 down-regulates the anxiolytic Rho

GTPase CDC42 (Zhang *et al.* 2016), which would predictably elevate anxiety; whereas, miR-125b predictably targets the learning-associated NR2A, the levels of which decrease upon miR-125b over-expression (Edbauer *et al.* 2010), with a seemingly inverse predicted outcome. In this context, the two SNPs may counterbalance each other's impact. Thus, interrogating the outcome of SNP interruptions of brain targets is rather complicated and probably depends on many different parameters.

The complex bidirectional effects of AChE 3'-UTR SNPs comprise only part of the genomic variability in this one gene. Along with other SNPs in the AChE gene, these SNPs may provide a useful example for studying the personalized outcome of different SNPs in the non-coding domains of brain-expressed genes. Additional examples include the common rs1799806 SNP [minor allele frequency 26.9% (Auton *et al.* 2015)], which localizes at both the coding region of the erythrocyte AChE-E variant, and at the non-coding 3'-UTR region of the AChE-R protein. This SNP abolishes a predicted recognition element for miR-661, which targets the pregnancy-related histocompatibility complex variant HLA-G (Castelli *et al.* 2009). Additionally, this SNP might enhance the potency of a putative binding site for

miR-939-5p, which regulates nitric oxide production by suppressing the translation of the inducible nitric oxide synthase gene (Guo *et al.* 2012). Different SNPs in the AChE 3'-UTR might therefore dysregulate diverse miRs and implement multi-leveled imbalances in those processes that are regulated by these miRs. This, in turn raises the question of potential inter- and intra-haplotype interactions between these SNPs, both at the mechanistic and the population level, which awaits further research.

Of note, AChE SNPs might skew the balance between different cholinergic signaling pathways, including but not limited to AD-related events. This provides a possible explanation for the vast variability in AD patients' reactions to ChE-Is. However, AChE is only one member of the cholinergic signaling pathway, and many other modulators of this system should be added to this equation, such as the nicotinic and muscarinic ACh receptors, the vesicular ACh transporter and choline acetyltransferase (Soreq 2015). Therefore, treatment with ChE-Is might only be effective in patients with particular genotypes, in whom it may limit some of the pathology-related and delay the cognitive decline, as was originally intended; however, in others, existing SNPs may interfere with this treatment's impact. Interrogating the genomic profiles of patients who are responsive and unresponsive to ChE-Is, with special attention to the 3'-UTR of the AChE gene may provide answers to this and related questions. Also, the involvement of AChE in other phenotypes suggests similar considerations for other medical conditions, providing an opportunity to develop individual, genome-based therapy strategies.

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