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Commentary

Neuronal nicotinic acetylcholine receptors: From the genetic analysis to neurological diseases

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ABSTRACT

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated channels that mediate, in the peripheral nervous system, fast neurotransmission at the neuromuscular junction and in ganglia. Widely expressed in the central nervous system neuronal nAChRs are thought to contribute both to neurotransmission and modulation of neuronal activity. To date, eleven genes encoding for these receptors have been identified in the mammalian genome and their structure is well conserved throughout evolution. Progresses made in the field of genetics and the identification of a large number of small genetic variants such as single nucleotide polymorphisms raise new questions about the physiologic and pharmacologic consequences of such variations. The finding of associations between polymorphisms in the genes encoding for the neuronal nAChRs and neurological disorders such as schizophrenia and Alzheimer disease illustrate the importance of getting a better understanding of these receptors from the gene to function.

In this work we present an overview over the progress that has been made in understanding the role of nAChR genes in monogenic disorders such as familial epilepsy, and review the latest knowledge about genetic variants of the nAChR genes and their relationship with common disorders and behavioural traits of complex etiology.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are found in various neuronal (neuronal nAChRs) and non-neuronal tissues and organs (muscular and non-neuronal nAChRs). They have been implicated in various traits and behaviours as well as in rare monogenic and in common multifactorial disorders. Muscular nAChRs, are essential for neuromuscular synaptogenesis and neurotransmission between the nerve and the muscle at the muscular endplate. First identified in ganglia, were they mediate synaptic transmission, neuronal nAChRs have since then been recognized as important players in modulation and synaptic transmission in the central nervous system. The existence of functional non-neuronal nAChRs has long been known, and recent studies indicate that they might have important roles with regard to cell proliferation, angiogenesis, apoptosis or immunology [1,2]. Mutations in muscular nAChRs can cause either prenatal arthrogryposis (Escobar syndrome) or different types of congenital myastenic syndromes [3,4]. Neuronal nAChRs are involved in complex brain functions, such as memory, cognition and attention. Mutations in specific

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Abbreviations: nAChRs, nicotinic acetylcholine receptors; SNPs, single nucleotide polymorphisms; TM, transmembrane segments; ADNFLE, autosomal dominant nocturnal frontal lobe epilepsy.

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subunits of the neuronal nAChRs can cause a rare type of autosomal dominant seizure disorder, familial frontal lobe epilepsy [5]. Recent studies have found strong evidence for the involvement of neuronal nAChRs in the pathogenesis of common disorders. In most common disorders the influence of genetics is complex and no simple Mendelian patterns of inheritance lead to the hidden genes. Naturally occurring genetic variants in genes coding for different nAChR subunits are thought to act as susceptibility factors for disorders such as Alzheimers disease, Parkinson disease, schizophrenia, and depression. The number of possible neuronal nAChR subtypes is large and different receptor subtypes might be expressed in a given cell type, complicating the analysis of the diverse functional and pathofunctional roles the genetic variants present in most nAChR subunits are expected to have. Considerable progress can be expected from large-scale efforts such as HapMap, an international project that has already created a genome-wide database of human genetic variation (www.hapmap.org). It can be expected that the combined clinical, genetic and functional analyses of the different nAChRs (both neuronal and non-neuronal) will help us to understand molecular pathways for common diseases, refine estimation of individual genetic risks, and identify new therapeutic targets.

2. Genetic basic principles

In the human nuclear genome the genes are contained within microscopically visible structures, the chromosomes. It is estimated that the 23 pairs of chromosomes carry about 25 000–30 000 protein-coding genes, as well as an unknown number of so-called RNA-genes [6]. The latter ones are translated into functionally active RNAs that are not thought to serves as a blueprint for translation. Eukaryotic genes are usually comprised of different numbers of coding exons which are interrupted by non-coding introns. Most mutations change or interrupt one or more codons, but mutation types that affect regulating non-coding sequences or cause major structural changes of the chromosomal region harboring the respective gene are also a common pathogenetic mechanism. However, not all changes that occur in a given gene are mutations in the classical meaning of the term. Pathogenetically a spectrum exists with high-impact mutations that are sufficient to cause genetic disorders by themselves (such as the *CHRNA4* and *CHRNB2* mutations in familial epilepsy) at one end and low-impact mutations that contribute only weakly to disease susceptibility on the other end. The latter are often difficult to separate from the large number of non-functional polymorphisms that exist in our genome. Many of the low-impact mutations and non-functional polymorphisms are single base pair-exchanges, better known as single nucleotide polymorphisms (SNP) [7,8].

Most genetic studies published in the final decade of the last century concerned single-gene disorders that give rise to sharply defined clinical traits. However, these disorders are not responsible for the major part of the worldwide burden of common diseases. The heaviest load is contributed by disorders that are determined by a combination of multiple genetic susceptibility factors and interacting environmental factors [9,10]. Identifying the underlying genes is a far greater challenge in multifactorial disorders than in monogenic traits, and the respective methods to approach these disorders are different. In monogenic traits linkage studies and positional cloning are the most commonly used strategies, while the different types of association studies are favoured for genetically more complex disorders. Linkage studies make use of the recombinations between sister chromosomes and –chromatids that normally happen in each meiosis. The aim is to find a polymorphism that shows no recombination with the disorder in large family samples. If found a good chance exists that the unknown disease gene is located in the vicinity of this ''linked'' polymorphism.

Genetic studies of common disorders are a much greater challenge, and not everybody is convinced that it is a winnable game. High degrees of heterogeneity often exist within the same clinical phenotype and genetic susceptibility factors can vary considerably between different ethnic groups. Most genetic susceptibility factors contribute only small risks, and large cohorts of patients and matched healthy controls are needed to detect such weak genetic effects [11,12]. In recent years SNPs have become one of the major instruments that are used to determine whether a specific genotype is more common in patients than in controls. Several millions SNPs are known in our genome, but the challenge is still three-fold: find an association between an allele of a certain SNP and the disorder under consideration, replicate this finding in an independent cohort, and decide if the associated SNP allele is pathogenetically relevant or if it is functionally silent but physically close to an unknown functional variant. It is increasingly recognized that each of our chromosomes has an individual block-like structure with respect to meiotic recombination. Recombination is highly unlikely to occur within a given block, thus all alleles on such a block tend to remain together from generation to generation, creating a so-called haplotype block. International projects such as HapMap have put in great efforts to ascertain frequencies and patterns of association among roughly 3 million common SNPs [13]. For the analysis of disorders with a complex genetic background the haplotype block structure has both advantages and disadvantages. The advantage is that only one SNP for each block needs to be tested in association studies, which helps to reduce the size of the task considerably. However, once an association between a clinical phenotype and a SNP is found, other SNPs on the same haplotype block are likely to show the same tight association [14,15]. Only time-consuming functional studies might be able to separate the innocent bystanders from the true functional variants. This problem is especially obvious on chromosome 15q24, a genomic region containing three different nAChR subunit genes [16]. As discussed in the following paragraphs, several traits including nicotine addiction and smoking related disorders show promising associations with this region. However, three nAChR subunit genes ($\alpha3$, $\alpha5$ and $\beta4$) are located on the same haplotype block, thus the challenge will be to find out which of these subunits are the functionally relevant ones. Despite all these difficulties, the understanding of the genetic basis of common multifactorial disorders will remain one of the most important goals in genetics for the foreseeable future. Mastering this challenge will provide new

approaches for prevention and treatment, rendering genetics one of the most important tools in medicine.

3. Diversity of nAChRs subunits

Eleven neuronal (α2–α7, α9–α10, β2–β4) and five muscular (α1, β1, γ, ε, δ) nAChR subunits genes have been identified in the mammalian genome. They all go back to a common ancestor gene and belong to a large superfamily of homologous receptors that also include gabaergic, glycine and serotonin receptors. Two nAChR subtypes are expressed in muscle, the foetal and the adult form that differ only with respect to the γ- and ε-subunit, respectively. The diversity of nAChRs is much greater in brain, with its large number of possible different subtypes and the expression of multiple subtypes within the same tissue or cell type. Several of the neuronal nAChR subunits, such as α3, α5 and α7, are also expressed in various non-neuronal tissues, including glial cells, epithelial cells in airways, intestine, and epidermis, endothelial cells, placenta and circulating blood cells [1,17]. In brain, there are two main classes of nAChR subtypes, the homomeric pentamers build from α7–α10, and the heteromeric pentamers consisting either of various combinations of α2–α6 subunits with β2–β4 subunits, or are a mixture of either α7 with α9 or α10. In homomeric neuronal nAChRs five agonist-binding sites are present, one at each interface between two subunits. The heteromeric neuronal receptors are thought to have two binding sites that are located between a α- and β-subunit each. Exceptions are the α5 and the β3 subunits which are probably not able to form agonist binding sites. The stoichiometry of the individual receptor subtype determines its biophysical and functional properties as well as its pharmacological profile [18–20].

4. Expression pattern of neuronal and non-neuronal nAChR subtypes

The exact nAChR subtype composition in different brain parts is still not completely known. Studies are hampered by the fact that the various ligands are mostly not subtype-specific and that highly specific antibodies have not been produced for all subunits yet. Approaches using probes for *in situ* hybridisation on nAChR mRNAs also have problems with regard to their specificity, and false positive results are a common problem [21,22]. Furthermore, the expression patterns for specific nAChR subunits can differ between species, thus data gathered from one species might not be valid in another one. Examples are the nAChR subtypes that contain the α2-subunit. The α2β2 receptor is expressed at considerable levels in parts of the monkey and human cortex but is virtually absent from the respective areas of the rodent brain [23]. For many of the possible subtypes there is no evidence so far that they are indeed expressed in mammalian brain. Other subtypes are expressed, but only in selected parts of the brain. For example, expression of the α3β2 subtype is mainly restricted to the visual pathway, while α4α6β2β3 receptor subtypes are preferentially expressed in visual and mesostriatal pathways. Such subtypes are thought to have very specific roles in brain function, while subtypes such as the α4β2 nAChR are ubiquitously expressed and able to bind most nicotinic agonists with high affinity [24–29]. Different types of nAChR subunits are thought to be expressed in non-neuronal tissues, but little is known about the exact subunit composition of these nAChRs. Whole-cell patch-clamp studies of human bronchial epithelial cells demonstrated the presence of fast-desensitizing currents activated by choline and nicotine that could be blocked by the snake toxine α-bungarotoxin. This toxin specifically inhibits α7 or α9-α10 containing receptors, suggesting the expression of functional homomeric α7 nAChRs in non-neuronal tissues. Some bronchial epithelial cells also showed slowly decaying currents, consistent with the expression of functional α3β4 nAChRs [30–34]. Interestingly, α-bungarotoxin is also able to block the muscle nAChR, which shares many structural features with the homomeric α7-nAChR. It has been shown that a single amino acid exchange is sufficient to confer pharmacological properties of the muscle nAChR to α7-nAChRs [34].

5. Known mutations in nAChRs, their association with disease and altered function

5.1. *Muscular nAChR and congenital myasthenic syndromes*

While the majority of the muscle weakness associated with myasthenic symptoms occurs from the presence of auto-antibodies that are directed against the muscular nAChRs expressed at the neuromuscular junction (myasthenia gravis), a small fraction of patients have genetic disorders. The congenital myasthenic syndromes can be classified according to their target as presynaptic, synaptic basal lamina-associated or postsynaptic. The postsynaptic forms of congenital myasthenic syndromes constitute the largest group, and they are mainly due to mutations in the genes that encode for the muscular nAChR [3]. The ε-subunit, which is expressed in the adult form of the neuromuscular junction nAChR exhibits the highest frequency of alteration. This suggests a lower pressure of selection on this particular gene, either in evolutionary terms or with respect to the survival of the affected individual. The ε-subunit replaces the fetal γ-subunit around birth, thus mutations in the ε-subunit cannot interfere with the prenatal development of muscles. Accordingly, mutations in the γ-subunit of the muscular nAChR reduce fetal movement and cause severe arthrogryposis (Escobar syndrome). Patients with Escobar syndrome often survive because the ε-subunit is switched on around birth, restoring receptor function. Most gene alterations in congenital myasthenia result in a loss of function of the receptor. Interestingly, however, a small fraction of mutations cause a gain of function but which translate, nonetheless, in a reduction of the synaptic transmission efficiency [3]. This illustrates the need for a thorough understanding of the both the receptor function and the overall system to which it contributes.

5.2. *Neuronal nAChR and monogenic epilepsy*

CHRNA4 was the first gene identified to cause a form of idiopathic epilepsy in humans [35]. Mutations in this and other

nAChR subunit genes can cause autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), a seizure disorder characterized by clustered attacks of brief motor seizures. The seizures are of frontal lobe origin and occur mostly during light sleep [36]. They are often stereotyped and consist of brief stiffening of the limbs, accompanied by gradual turning of the head and dystonic movements of arms or legs. Grunting sounds, screaming and difficulty with breathing often occur with the seizures, and sleep-walking has also been reported for some ADNFLE patients. As in other frontal lobe epilepsies, ictal EEG recordings are usually normal and establishing the diagnosis often requires simultaneous nocturnal video and polygraphic recordings. The age of onset is mainly during childhood or early adolescence, but can vary considerably from infancy to old age, even within the same family [37]. The penetrance in most known ADNFLE families is about 70–80% but at least one mutation has been described that might be associated with a much lower penetrance [38]. Seizures tend to cluster, and the mean seizure frequency is about 20 per month. They are often, but not in all patients readily controlled by antiepileptic drugs, especially carbamazepine. Interestingly, seizure frequency generally decreases with age. It has been hypothesized that the observed reduction of seizure frequency in elderly patients correlates with the reduction of nAChR density in the aging brain.

By now *CHRNA4* and *CHRNB2* have been confirmed as ADNFLE genes in several independent families [35,38–43]. The surprising and so far not very well understood part is that both nAChR subunits are ubiquitously present in brain, and that the heteromeric nAChR composed from these subunits constitutes the major high affinity nicotine nAChR subtype in mammalian brain. Nevertheless, mutations in these two subunits cause a partial rather than a generalized type of epilepsy. A possible explanation would be that the frontal lobe shows a lower seizure threshold compared to other parts of the brain, but if this was correct, frontal lobe epilepsies should be a more common type of epilepsy in humans. Another possibility would be that the pathofunctional effect of ADNFLE mutations is better compensated outside the frontal lobe, because of the specific distribution of the nAChR subtypes as function of the brain areas.

All ADNFLE mutations identified so far are located within or in close proximity to the transmembrane regions (TM) of the respective nAChR subunit. A pattern seems to be emerging, with *CHRNA4* mutations firmly placed within TM2 while *CHRNB2* mutations are located 3′ of TM2 or in TM3 (Fig. 1a). Although the biological significance of this pattern remains to be confirmed it is possible to speculate that only mutations located in segments critical for the function of the receptor result in modifications of the receptor properties that are sufficient to trigger epileptic seizures. The TM2 builds the wall of the ion channel, while the other TMs contribute mostly indirectly to this central structure. Apparently only mutations that affect the ion pore are able to cause ADNFLE. Not surprisingly, these nAChR mutations not only cause epilepsy but can also be associated with additional neurological or psychiatric features [39,44,45]. Examples are the *CHRNA4* insertion mutation 776ins3 that is associated with nocturnal seizures and psychiatric disorders, especially schizophrenia-like symptoms [45], or the *CHRNB2* mutation I312M that seems to cause not only epilepsy but also very specific cognitive defects [42]. The observable spectrum of neurological symptoms in ADNFLE families reflects the importance of the cholinergic system in higher brain functions. To understand why other important functional domains such as the agonist-binding site are apparently not a mutational target in ADNFLE pathogenesis it is necessary to recall that mutations identified so far cause an increase in ACh sensitivity. If this is indeed the basic mechanism underlying the epileptic seizures it could be postulated that equivalent effects caused by a modification of the ligand-binding site are less probable and have not yet been identified.

As patients suffering from ADNFLE are heterozygous [35], meaning they carry on their chromosomes both a normal and a mutated allele, it is important to know how the mutations affect receptor function if coexpressed with the normal subunit. Unless the mutations affect gene expression (which is unlikely given their position within their respective genes) both the mutated and the normal allele can be expected to be expressed in comparable amounts in brain. In ADNFLE

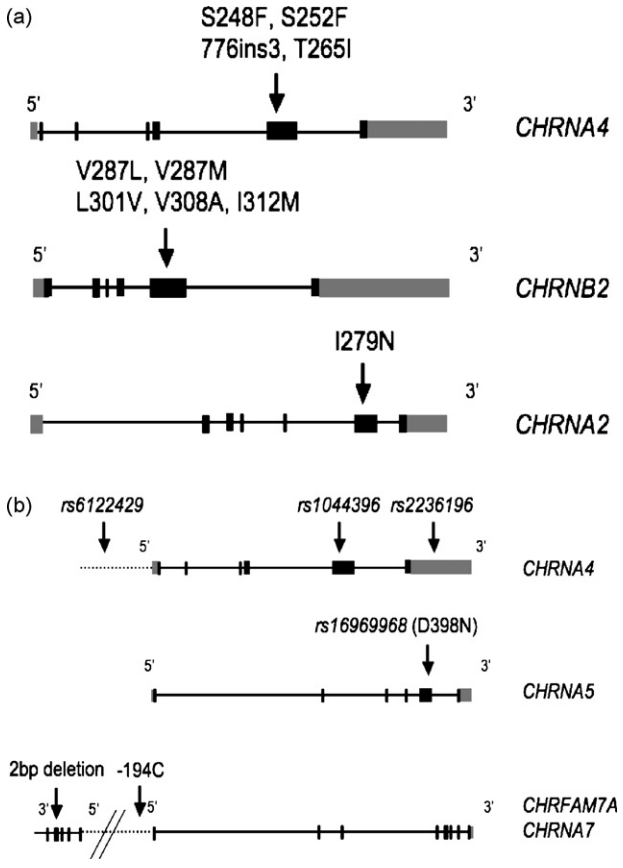

Fig. 1 – (a) Exon–intron structure of three nAChR subunit genes. Positions of ADNFLE mutations are indicated by arrows (for details see text). Black boxes, coding regions; grey boxes; untranslated regions. Introns and exons are not drawn to size. Orientations (5′–3′) of genes are indicated. (b) Polymorphisms in nAChR genes. The positions of SNPs and other polymorphisms mentioned in the text are indicated by arrows. For *CHRFAM7A* only the part homologous to *CHRNA7* is shown (Exon 5–10). Dotted lines indicate promoter regions.

patients the α4β2 nAChRs are therefore likely to be a mixture of receptor subtypes that carry either none, one or more (up to three for α-subunits) copies of the mutated subunit. To assess the overall functional effect of the mutations heterozygous receptor experiments were comparing expression of the single mutated allele or of the mutated allele together with the control allele [46]. Results obtained from this study have shown that the mutated allele affects receptor function both with or without the presence of the normal allele. Effects were most pronounced in the homozygous mutated state but remained significant in the heterozygous expression. The main difference was observed in the maximum amplitude of the ACh-evoked current that was significantly lower in the homozygous mutant receptor but remained at a level comparable to the wild type in the heterozygous receptor. Studies carried out by several laboratories and with different mutations have, since then, confirmed the effect of ADNFLE mutations in the heterozygous state [47].

Mutations identified to date in *CHRNA4* and *CHRNB2* together with their respective localisation in the receptor structure and their functional effects are summarized in Fig. 1a. Whereas mutations exhibit differences in their respective response profile and/or calcium permeability all mutations found so far cause an increase in receptor sensitivity to ACh [46]. This suggests that the cause triggering seizures in ADFNLE is attributable to the increase in ACh sensitivity. Because epileptic seizures reflect a higher degree of neuronal synchronisation it is interesting to note that α4β2 nAChR have been found to modulate interneurons GABAergic synapses. As interneurons are known to interact with a large number of pyramidal cells, alteration of synaptic transmission in such interneurons is expected to have a broad effect on the cortical function. The nAChRs carrying gain-of-function mutations would first increase the inhibitory effect of GABA, forcing a higher number of neurons to reduce activity. Release of the inhibition would cause sudden simultaneous firing of these neurons, and the resulting hypersynchronisation could trigger epileptic seizures [48].

ADNFLE displays a broad and so far mostly unexplained heterogeneity in clinical severity. Some patients only infrequently experience seizures, while others might be affected by frequent clusters of seizures, cognitive impairment or psychiatric symptoms. The clinical heterogeneity is best observed between different mutations but to a lesser degree, also found within the same family. Thus only part of the variability can be attributed to differences between the respective mutations. It is tempting to speculate that additional naturally occurring genetic variation in the *CHRNA4* and *CHRNB2* genes also contributes to the differences in clinical outcome. Analysis of single nucleotide polymorphisms (SNPs) and other gene modifications showed a high degree of variation in the *CHRNA4* gene but much less in *CHRNB2* (www.HapMap.org). An interpretation for such a difference is that nAChR β2, the protein encoded by *CHRNB2* associates with more subunits than α4 and is therefore under a greater pressure of selection. Although it is expected that additional variations either in *CHRNA4* or *CHRNB2* will be identified in a near future it is important to note that most variants identified so far by genome analysis are in the introns or untranslated domains. While speculation exists about the true role of intronic genetic variation and their relevance in protein synthesis, the preRNA splicing which takes place in the maturation of the mRNA is a key process that can be altered in many ways. As the mRNA encoding for the N-terminal domain of the nAChR α4- and β2-subunits encompasses four exons rearrangements and/or alternate splicing is possible. Such modification may, however, remain undetected for the following reasons. First, genetic screening studies, such as those conducted for ADFNLE, allow mainly detection of mutations that are able to cause a clinical phenotype rather than slight modification of the receptor function. Second, gene alteration that causes synthesis of non-functional proteins (or no proteins at all) may remain unnoticed due to compensatory mechanisms. Such mechanisms can be as simple as an increase of expression of the normal allele or more complex as suspected in the case of some genetically engineered mice. The complexity of compensatory genetic mechanisms is best illustrated in the case of $GABA_{A\text{-}1}$ receptor knock-out which was supposed to cause profound epileptic seizures in mice but turned out to be compensated by changes in expression of other genes [49]. The influence of modifier genes was also discussed as the most likely explanation for the phenotypic differences between two mice strains carrying the *CHRNA4*-S248F mutation [50,51]. Only one of these knock-in strains displayed frequent spontaneous seizures, although both showed nicotine-induced behavioural changes.

Two years ago, another nAChR subunit gene, *CHRNA2* has been reported as a third candidate for nocturnal frontal lobe seizures [52]. Like the above-described ADNFLE mutations the *CHRNA2*-I279N mutation causes a left shift in the receptor's concentration activation curve, indicating a significant gain-of-function effect. This effect had been first found in co-expression experiments with *CHRNB4*, which encodes for a structural subunit that is essentially expressed in ganglia but sparsely in human brain. This suggests that additional experiments are needed to determine the influence of the α2 mutation. More recent experiments demonstrated that the mutation I279N still increases the agonist sensitivity if partnered with more ubiquitously expressed β2 subunit (Bertrand and Steinlein, in preparation). Two replication studies did not reveal any additional *CHRNA2* mutations in samples of 47 and 46 ADNFLE families, respectively [53,54]. Thus *CHRNA2* is either an extremely rare cause of the disorder or mutations in this gene give rise to an epilepsy phenotype that differs from ''classical'' ADNFLE. The clinical phenotype described by Aridon et al. [52] might point towards the latter explanation since it showed some unusual aspects. Fear sensations, choreoathetoid tongue movements and nocturnal wandering were more frequent ictal features in the *CHRNA2*-I279N family than in previously described ADNFLE families. To access the true role of the nAChR α2 subunit in epileptogenesis additional families with *CHRNA2* mutations need to be identified.

6. nAChRs variants and association with common diseases

It is increasingly recognized that naturally occurring genetic variants in different nAChR subunits modulate cognition and behaviour, contribute to the risk for various neurological and

psychiatric disorders and increase the susceptibility for addictions such as nicotine dependence. The *CHRNA4* and *CHRNA7* genes have been the focus of research because they encode the most abundant nAChR α subunits in mammalian brain. Both genes contain several sequence polymorphisms each, mostly non-coding SNPs. One of these SNPs, *CHRNA4* rs1044396 in exon 5 is associated with individual differences in attention tasks, and variability in the α4 subunit is discussed as a potential risk factor for attention deficit hyperactivity disorder [55]. The role of the *CHRNA4* gene in Alzheimer disorder is controversially discussed. Some authors found an association between different *CHRNA4* polymorphisms and Alzheimer disorder while other studies did not [56,57]. These divergent findings might at least in part be due to differences in the study designs. Some studies did not exclude patients with an age of onset before age 65, although early onset Alzheimer disease is believed to have a stronger and maybe different genetic background compared to the late onset type of the disorder.

Addictive behaviours such as smoking and alcoholism seem to overlap genetically and nAChR polymorphisms are strong candidates for addiction susceptibility factors. Recently two functional *CHRNA4* SNPs, one within the promoter sequence (*rs6122429*) and one (*rs2236196*) within the 3′ UTR were found to be associated with smoking endophenotypes including nicotine-dependence, smoking abstinence as well as acute nicotine effects [58,59]. Interestingly, these two SNPs do not share the same haplotype block, suggesting that both polymorphisms can act independently from each other (Steinlein and Bertrand, unpublished results). The non-synonymous SNP *rs16969968* in *CHRNA5* causes the alteration of a single amino acid, D398N, which is localized in the intracellular domain connecting TM3 and TM4 of the α5 subunit. Individuals homozygous for this allele show a two-fold increase in the risk of developing nicotine dependence [60,61]. Principal SNPs and their respective localisation on the genes are summarized in Fig. 1b.

Recent publications have revealed important associations of nAChR polymorphisms with lung cancer (both smoking triggered and in never-smokers) and peripheral arterial disease. Three genome-wide association studies reported in last April issues of Nature and Nature Genetics found an association between these disorders and the region on chromosome 15q24 containing the cluster of genes *CHRNA5*, *CHRNA3*, and *CHRNB4* [60,62,63]. Several SNPs, including the above mentioned non-synonymous *CHRNA5* SNP *rs16969968* (see Fig. 1b) showed strong associations with the phenotypes under consideration. In view of the large size of the tested cohorts, independence of the studies, and significance of these associations it is reasonable to predict that *CHRNA5* and/or one of the neighbouring nAChR genes play a determinant role both in nicotine dependence and in disorders caused by smoking.

Two possibilities must at least be considered in the interpretation of these results. First, these associations might indicate that the pathologies under consideration closely depend on the gene polymorphism and therefore protein alteration. Second, and alternatively, the associated polymorphisms might not be causative themselves but share the same haplotype block with the causative gene. Albeit it may be precocious to speculate on these issues it is worth to shortly review the current knowledge of *CHRNA5* and its expression in the central nervous system or the lung. As antibodies against nAChRs do not display enough specificity, information about the distribution of protein expression relies mainly on *in situ* hybridisation and immunoprecipitation [24]. These studies reveal that expression of α5 is rather limited and that, in the brain, it co-immunoprecipitates with the α4 and β2 subunits. Functional studies attempting to co-express the α5 subunit with α4 and β2 lead to the proposal that α5 is a modifier of the basic properties displayed by the α4β2 nAChR and may allow a fine tuning of the receptor properties [20,64,65]. Importantly, the α5 subunit was found to be expressed in small cells lung cancer (SCLC) and nicotine was found to promote proliferation of these particularly aggressive cells [66–68]. These preliminary data would be in accordance with a direct involvement of *CHRNA5* in smoking-related disorders, but further functional studies are needed to understand the interplay between genetic variation on chromosome 15q24 and clinical phenotypes.

Several studies have reported an association between *CHRNA7* and endophenotypes of psychiatric disorders and nicotine dependence, rendering α7 one of the clinically most interesting nAChR subunits. *CHRNA7* was one of the first genes for which a possible involvement in schizophrenia had been discussed. An early study found evidence for linkage between *CHRNA7* and auditory gating deficits [69,70]. The so-called P50 wave can be used to measure the failure to inhibit the response to irrelevant sensory input that is characteristic for schizophrenia and other psychiatric disorders. Later studies identified several *CHRNA7* promotor polymorphisms that reduce the genes transcriptional activity to be associated with schizophrenia [71,72] (see Fig. 1b). This would be consistent with the finding of reduced levels of α7 nAChRs in the brain of schizophrenic patients [73]. Again, the association data are not easy to interpret, since another study produced contradictory results showing that one of the functional promotor polymorphisms, −194C, was associated with protection against loss of auditory gating capacity [74]. The role of *CHRNA7* in smoking is also still a matter of debate. Some authors reported a positive association of this gene with smoking in schizophrenic patients, but others were not able to find the same association in smokers without psychiatric disorders [75,76]. Progress is hampered by the rather complicated genomic structure of the *CHRNA7* gene. The human locus not only contains the active gene but, in most individuals, also a hybrid gene that is a partial pseudogene of *CHRNA7*, *CHRFAM7A* [77]. The functional relevance of *CHRFAM7A* and its frequent 2 base pair deletion polymorphisms is still unknown. Nevertheless, clinical studies are already testing the safety and efficacy of chronic *CHRNA7* agonist application in schizophrenic patients. First results indicate that such a treatment might improve the positive and negative symptoms of schizophrenia [78].

7. A vision of the future

Nature and nurture are both involved in the pathogenesis of complex traits such as psychiatric disorders, behaviour and addiction. Lifestyle, social and cultural norms interact with naturally occurring genetic variation, which itself can show

differences between ethnic groups. Furthermore, our genomes are not static but are subject to recombination when passed to the next generation. Compared to their parents, children will carry new combinations of genetic variants. On a much larger time scale, positive and negative selective evolutionary forces are still changing our genome. Evolution already created a considerable number of homologous nAChR genes (α1–α10, β1–β4, γ, ε, δ) from one ancient gene. Some of these subunit genes are ubiquitously expressed, but many of them have found specialized roles for themselves. The functional diversification of nAChR genes can be attributed to gene duplication mostly by segmental duplication, followed by the accumulation of small genetic changes in coding and non-coding parts of the homologous genes. Most of these genetic variants are functionally neutral. Some interfered with gene function and were quickly removed from the gene pool. But the rare genetic variant provided advantage, and subtly changed either the function or the expression pattern of the duplicated gene. The step-by-step accumulation of genetic variants allowed the duplicated genes to grow into their own functional role, a prerequisite to survive in evolution. It also helped to make nAChRs one of the most diverse and functionally important receptor superfamilies.

The complexity presented by the network of nAChR genes and environmental factors creates a big challenge for genetic research. Finding association between a certain gene and a (endo) phenotype is no longer the final goal. More important are the next two steps, successful replication of the association in an independent sample and identification of the functionally relevant genetic variant. Genome-wide association studies have by now mostly replaced smaller candidate gene association studies, and large samples of patients are increasingly used to detect even modest genetic effects. Variants within the nAChR genes were among the first susceptibility factors that could be unequivocally linked to specific health risks by the new generation of association studies [60,62–63]. Still, the question remains how and when to use this genetic information in the clinic. For each variant that increases a specific risk there are probably several others hidden in the genome that decrease the same risk. Our knowledge of the functional relevance of naturally occurring genetic variation is still too sketchy to make the personalized genetic profile a useful clinical tool today. We do not know enough yet about the interactions between predisposing and protecting genetic factors to assess an individuals risk for common disorders and life style risks. Nevertheless, unexpected rapid progress has been made in the analyses of genetic variation in complex disorders within the last couple of years. It is therefore likely that the identification of individuals with a high risk for certain common disorders will become routine in the not so far future. The next challenge will then be to understand how gene variations are affecting receptor distribution and their function and to develop preventive therapies or effective treatments for those who are going to be identified as at risk subjects.

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