Cell. Mol. Life Sci. (2014) 71:2815–2835
DOI 10.1007/s00018-013-1553-4

Cellular and Molecular Life Sciences

REVIEW

Transcription factor 4 (TCF4) and schizophrenia: integrating the animal and the human perspective

Boris B. Quednow · Magdalena M. Brzózka · Moritz J. Rossner

Received: 12 September 2013 / Revised: 4 December 2013 / Accepted: 30 December 2013 / Published online: 12 January 2014

Abstract Schizophrenia is a genetically complex disease considered to have a neurodevelopmental pathogenesis and defined by a broad spectrum of positive and negative symptoms as well as cognitive deficits. Recently, large genome-wide association studies have identified common alleles slightly increasing the risk for schizophrenia. Among the few schizophrenia-risk genes that have been consistently replicated is the basic Helix-Loop-Helix (bHLH) transcription factor 4 (*TCF4*). Haploinsufficiency of the *TCF4* (formatting follows IUPAC nomenclature: TCF4 protein/protein function, *Tcf4 rodent gene cDNA mRNA, TCF4 human gene cDNA mRNA*) gene causes the Pitt-Hopkins syndrome—a neurodevelopmental disease characterized by severe mental retardation. Accordingly, *Tcf4* null-mutant mice display developmental brain defects. *TCF4*-associated risk alleles are located in putative coding and non-coding regions of the gene. Hence, subtle changes at the level of gene expression might be relevant for the etiopathology of schizophrenia. Behavioural phenotypes obtained with a mouse model of slightly increased gene dosage and electrophysiological investigations with human risk-allele carriers revealed an overlapping spectrum of schizophrenia-relevant endophenotypes. Most prominently, early information processing and higher cognitive functions appear to be associated with *TCF4* risk genotypes. Moreover, a recent human study unravelled gene × environment interactions between *TCF4* risk alleles and smoking behaviour that were specifically associated with disrupted early information processing. Taken together, TCF4 is considered as an integrator ('hub') of several bHLH networks controlling critical steps of various developmental, and, possibly, plasticity-related transcriptional programs in the CNS and changes of *TCF4* expression also appear to affect brain networks important for information processing. Consequently, these findings support the neurodevelopmental hypothesis of schizophrenia and provide a basis for identifying the underlying molecular mechanisms.

Keywords Schizophrenia · Neurodevelopment · Transcription factor 4 · Cognition · Mouse models · Endophenotype · Polymorphism · Basic Helix-Loop-Helix protein

B. B. Quednow (✉)
Department of Psychiatry, Psychotherapy and Psychosomatics, Experimental and Clinical Pharmacopsychology, Psychiatric Hospital, University of Zurich, Lenggstrasse 31, 8032 Zurich, Switzerland
e-mail: quednow@bli.uzh.ch

B. B. Quednow
Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland

M. M. Brzózka · M. J. Rossner (✉)
Department of Psychiatry, Molecular and Behavioral Neurobiology, Ludwig-Maximillians-University, Nussbaumstr. 7, 80336 Munich, Germany
e-mail: moritz.rossner@med.uni-muenchen.de

M. J. Rossner
Research Group Gene Expression, Max-Planck-Institute of Experimental Medicine, Hermann-Rein-Str. 3, Goettingen 37075, Germany

Introduction

Schizophrenia is still an unsolved genetic enigma. Although the disease is clearly heritable and great effort has been undertaken in the past decades to elucidate the genetic basis of this disorder, no major risk genes that would be suitable for prediction of the illness have been identified.

The reason for this failure might arise from complex multigenetic interactions of risk alleles with minor individual contributions. It is also likely that a disease entity 'schizophrenia' does not exist behind the phenomenology-based disease classifications, which have been useful for the clinical requirements but not for biological research. Thus, in the following text and for improved readability, the word 'schizophrenia' actually means 'schizophrenia-spectrum disorder'. Nevertheless, in recent years, some interesting and replicable findings with population-wide significance have suggested that variations in a few genes might serve as risk markers in a subgroup of schizophrenia patients. Among the most validated genes is the basic Helix-Loop-Helix (bHLH) transcription factor 4 (*TCF4*). The preclinical and clinical findings regarding the connection between the *TCF4* gene and schizophrenia will be reviewed and discussed.

The basic-Helix-Loop-Helix protein TCF4

TCF4 belongs to the superfamily of bHLH transcription factors that can act as a transcriptional repressor or activator in a context-specific fashion [1]. The bHLH domain comprises the basic region mediating DNA binding and a dimerization interface provided by the HLH domain with two amphipathic helices separated by an unstructured loop region forming left-turned four-helix bundles in dimers [2, 3]. bHLH proteins are involved in various developmental processes, including control of proliferation, determination of cell fate and specifications, but have also been shown to be transcriptional integrators of adaptive cellular processes in terminally differentiated cells [1, 4–6]. TCF4 (also known as E2-2/SEF2, ITF2, ME2) is an ubiquitously expressed protein and subgrouped with two additional so-called E-proteins, TCF3/E2A and TCF12/HEB, as class I bHLH factors [2] (for complete lists of gene name assignments, see, e.g. http://www.ihop-net.org or http://www.ncbi.nlm.nih.gov/gene). Ubiquitously expressed class I bHLH factors (TCF3, TCF4 and TCF12) are capable of forming homo-dimers and hetero-dimers with numerous cell-type-specific (or class II) bHLH and dominant-negative (or class V) HLH factors of the ID family (ID1-4) that lack a basic region and are therefore inhibiting DNA binding by sequestering bHLH factors [1].

Of particular practical importance is that the acronym or gene name alias *TCF4* is unfortunately also widely used for T Cell Factor 4 (official gene symbol *TCF7L2*). TCF7L2 belongs to the high mobility group (HMG) family of transcription factors and interacts with β-catenin of the WNT signalling pathway [7]. Therefore, great care should be taken when using software tools that automatically annotate key words from literature entries with '*TCF4*' and when manually scanning the '*TCF4*' literature. In consequence, the 'bHLH-*TCF4*-schizophrenia- and Pitt-Hopkins Syndrome (PTHS)'-related literature is likely to be contaminated by some false associations and authors, and reviewers and readers should be sensible to this fact. We could not find evidence for a function of the bHLH factor TCF4 in glia/oligodendrocyte development, which has unfortunately been mentioned in several previous reviews.

Dimeric bHLH complexes bind to partially palindromic short DNA elements called Ephrussi-boxes (E-boxes) with the core sequence 5′-CANNTG-3′ located in regulatory regions [8]. For structural reasons, individual bHLH proteins display a preference towards particular E-box half-sites, which, however, does not necessarily predict the exact binding site of a given hetero-dimer which can even vary at different sites of the genome [9]. Class II bHLH factors cannot form homo-dimers and exert their transcriptional function only in concert with a class I or E-protein such as TCF4 [1]. TCF4 may thus exert pleiotropic functions depending on its dimerisation partner(s) at a given developmental stage and in a particular cell type. In consequence, TCF4 functions have been shown to be modulated by spatio-temporal expression patterns of its various interaction partners, differences in DNA-binding specificities, post-translational modifications and associated co-factors [10–13].

Mammalian E-proteins have been shown to at least partially complement for each other, and gene dosage effects have been described in lymphocyte development that further enhances the pleiotropic functions of these genes and complicates the assignment of dedicated roles for individual E-proteins [14, 15]. In contrast to mammals, only one E-protein is found in *Drosophila melanogaster* and *Caenorhabditis elegans*, i.e. daughterless (da) and helix-loop-helix protein 2 (hlh-2), respectively. Still, the corresponding mutants revealed multiple phenotypes including deficits in nervous system development indicating phylogenetic conservation of E-protein functions [16–18]. The human and mouse genes coding for TCF4 are located on chromosome 18 in both species. The mouse gene *Tcf4* encompasses >360 kB (chr 18E2, forward strand) and the human *TCF4* >440 kB (chr 18q21.2, reverse strand) (www.ensembl.org, rel. 72). More than 18 coding splice variants with alternative N-termini have been described in humans [10], and the Ensembl genome browser (www.ensembl.org, rel. 72) lists 43 potentially protein encoding variants with the majority including the bHLH domain. Moreover, Sepp and colleagues [10] subgrouped 22 exons that are alternatively spliced, particularly in the 5′ region with multiple alternative transcription initiation sites. Up to date, two putative antisense and one microRNA transcripts (miR4529) have been annotated on the opposite strand within the human *TCF4* locus (none so far in the

mouse) potentially indicating regulation at the RNA level of increased complexity in the human genome. *TCF4* mRNA abundance levels and/or control of translation may be regulated by complementary microRNAs (miRs) shown to bind to the 3′ region of *TCF4* transcripts including the schizophrenia-associated risk factor miR-137 as well as miR-155 and miR-204 [19–22], while the number of predicted and, however, so far experimentally not validated binding sites for additional miRs is much longer [23]. Besides the C-terminal located bHLH domain, TCF4 shares with the other class I/E-proteins additional regions of homology including two more N-terminally located transcriptional activation domains (AD1 and AD2) [24, 25]. These domains have been shown to provide protein–protein-interaction surfaces to recruit chromatin remodelling complexes and transcriptional co-factors such as CBP/p300 [26, 27]. Moreover, TCF4 may be part of a SWI/SNF chromatin-remodelling complex which might be of relevance for the etiopathology of schizophrenia [28]. A knockdown of endogenous *TCF4* in the human neuroblastoma cell line SH-SY5Y by siRNA altered the expression of multiple genes corresponding to various signalling pathways and affected cell survival, epithelial to mesenchymal transition and neuronal differentiation [29]. The siRNA approach yielded a highly efficient reduction of endogenous *TCF4* protein to levels below 20 %. Therefore, these findings that were obtained in a proliferating neuroblastoma cell line could be of relevance for PTHS, where a loss-of-function of *TCF4* is most probable. Nonetheless, it is unclear which bHLH interaction partners/dimeric complexes were affected in SH-SY5Y cells, e.g. affecting neuronal differentiation properties of this cell line and whether these processes mimic developmental defects causing PTHS in vivo. Because of the presumably very subtle effects by the extragenic common alleles in *TCF4* that have been associated with an increased risk of schizophrenia, mechanistic studies with relevance for schizophrenia are most likely technically more challenging.

In summary, the mammalian class I bHLH protein TCF4 can be considered as an integrator ('hub') of several bHLH networks controlling critical steps of various developmental and possibly also plasticity-related transcriptional programs in the CNS (see Fig. 1). The deregulated splicing events and/or mRNA misexpression or altered stability of one or more distinct TCF4 protein isoform(s), which could be of particular relevance for schizophrenia, are still unknown.

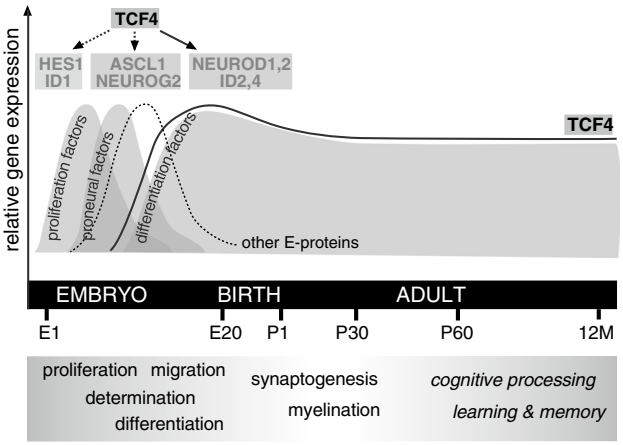

Fig. 1 Different bHLH transcription factors direct central nervous system (CNS) development at embryonic stages and may be involved in adult brain plasticity. Inhibitory bHLH factors (HES1, ID1) and proneural factors ATOH1, ASCL1 and NEUROG1,2 as well as E-proteins TCF3 and TCF12 are involved in early developmental stages. The temporal expression patterns and mutational analyses of the neurogenic differentiation factors (NEUROD1,2 and 6) and inhibitors of differentiation ID2 and ID4 suggest instead a function in later stages of neuronal differentiation and in the adult CNS. The spatiotemporal expression pattern of *Tcf4* overlaps substantially with all other bHLH factors involved in brain development. Moreover, TCF4 is capable of forming hetero-dimers with most involved neuron expressed bHLH factors although direct evidence is thus far only available for NEUROD1 and -2 (as indicated by a *solid line* in contrast to *dashed lines*). It should be noted that this schematic drawing is thought to be an overview representation not claiming detailed spatial and temporal expression domains of single genes (for citations, see main text)

Schizophrenia

The main symptoms of schizophrenia can be distinguished into three major domains: (1) positive symptoms such as hallucinations, perceptual disturbances, delusional phenomena and formal thought disorder; (2) negative symptoms mostly presented as flat affect, poverty of speech, avolition, anhedonia, lack of motivation and inappropriate emotional responses; and (3) cognitive dysfunction including impairment of attention, memory, social cognition and executive functions [30]. The highest risk period for developing schizophrenia is during young adulthood, while both sexes are equally affected by the disorder, although the age of onset is typically younger for men than women [31–33]. Although incidence rates vary depending on classification criteria, schizophrenia affects approximately 1 % of the population across cultures [34, 35]. Individuals with parents or siblings suffering from schizophrenia have an increased risk for developing the disorder (8–12 %). For monozygotic twins, the concordance rate is approximately 50 % [36, 37]. The elevated familial incidence of schizophrenia strongly indicates that there must be a genetic contribution to the disorder, although the fact that concordance rates for monozygotic twins are lower than 100 % suggests that environmental factors are also considerably involved. Thus, it is likely that a combination of genetic risk and environmental factors are required for the disorder to develop [37]. Initially, family-based linkage

studies have identified several chromosomal regions and candidate genes that are associated with the risk for schizophrenia [38, 39]. However, none of the results of the linkage studies has passed a genome-wide significance level so far [40]. Subsequently, a multitude of association studies that were recently extended by genome-wide association studies (GWAS) identified only a few common variants that contribute a very small increase in the susceptibility for schizophrenia [41–43]. Among the most replicable genes are the zinc finger binding protein 804A (*ZNF804A*), several genes from the major histocompatibility (MHC) region on chromosome 6, neurogranin (*NRGN*), and *TCF4* [44]. Most recently, several rare submicroscopic chromosomal alterations—called copy number variants (CNV)—have also been detected to cause schizophrenia or schizophrenia-like symptoms (e.g. as the case in 22q11-syndrome) [43, 44]. However, these rare chromosomal abnormalities cannot explain the pathogenesis of the majority of schizophrenia patients and are often also associated with physical abnormalities and mental retardation.

Although there is evidence for enlarged ventricles and decreased cerebral (cortical and hippocampal) volume associated with schizophrenia, there is not a distinct "diagnostic" neuropathology associated with the disease [38, 45, 46]. However, misplaced and clustered neurons, particularly in the entorhinal cortex, indicate problems of neuronal migration and suggest an early developmental anomaly [47–49]. Moreover, pyramidal neurons in the hippocampus and neocortex have been shown to have smaller cell bodies and fewer dendritic spines and dendritic arborisations, and there are also reports of decreases in cell numbers in the thalamus and a decreased number of oligodendrocytes (reviewed in [39]. Additionally, decreased presynaptic proteins such as synaptophysin, SNAP-25, and complexin II have been observed in schizophrenia brains [50, 51], as well as decreased density of interneurons (e.g. parvalbumin-immunoreactive cells; [52, 53]. Neuroimaging data and post-mortem studies have shown that N-acetylaspartate (NAA), a marker of neuronal integrity, is decreased in first episode and never-medicated patients [54, 55]. Based on these neuropathological changes, investigators have conceptualised schizophrenia as a disease of functional "dysconnectivity" [56–58], or a "disorder of the synapse" [59, 60], affecting the machinery of the synapse and subsequent neurotransmission [50, 51].

Finally, accumulating evidence suggests that schizophrenia might be a neurodevelopmental disorder that is—at least in part—caused by aberrant early brain development that could be partially genetically determined: (1) many schizophrenia patients exhibit delayed developmental milestones in childhood, including cognitive, motor, and behavioural abnormalities, which indicates abnormal brain function prior to diagnosis of schizophrenia, (2) obstetric complications and prenatal infections increase the risk for schizophrenia, (3) post-mortem studies did not find indicators for neurodegenerative processes such as gliosis or loss of neurons in the brain of schizophrenia patients, and (4) several anatomical and functional disruptions are associated with exacerbation of schizophrenia in adulthood and these disruptions can be simulated in developmental animal models [61, 62]. As suggested by Murray et al. [63], aberrant developmental processes may play a major role, especially in the congenital subform of schizophrenia that shows a gradual increase in behavioural disturbances until the disorder is diagnosed in adolescence or early adulthood. Maynard and colleagues [64] have proposed a two-hit hypothesis of schizophrenia. According to their suggestion, a lesion occurring in early neurodevelopment (first hit), caused by genetic risk factors or adverse embryonic and perinatal events, in combination with a second hit, arising from hormonal events, excitotoxicity, psychosocial stress or oxygen radical formation, may cause schizophrenia. Immunocytochemical and ultrastructural post-mortem studies have demonstrated neuronal alterations in schizophrenia, such as decreased size of the neuronal cell body, increased cellular packing density, fewer dendritic spines and synapses, and distortions in neuronal orientation [65]. The abnormalities in the cytoarchitecture, such as neuronal disarray, heterotopias and malpositioning, indicate disruption of proliferation or migration at the gestational period [62]. In agreement, it has consistently been shown that the expression of reelin, a glycoprotein that regulates neuronal migration, is strongly decreased in schizophrenia patients [66, 67]. Thus, these morphological and cytoarchitectural changes are likely to arise during brain maturation. In sum, several lines of evidence suggest that abnormalities in brain development may contribute to the pathogenesis of schizophrenia at least in a subset of patients.

Genetic association of TCF4 with schizophrenia

For the last 14 years, chromosome 18 has been repeatedly proposed as a possible location for schizophrenia and bipolar disorder risk genes [68–72]. As bipolar disorder and schizophrenia show a high genetic correlation [73], it is not surprising that *TCF4*, which is located on this chromosome, was initially associated with bipolar disorder: The first study found that bipolar disorder was associated with a CTG triplet repeat expansion in an intronic region of the *TCF4* gene [74]. The second study demonstrated that moderate expression of such repeats in this region was linked to severity of bipolar I disorder [75]. Subsequently, Pickard and colleagues [72] identified a pericentric inversion of chromosome 18 in a small Scottish family whose members are suffering from mental retardation and schizophrenia,

and the breakpoint of this inversion was located close to the *TCF4* gene. More recently, several large but also partially overlapping meta-analyses of GWAS consistently identified that common variants of the *TCF4* gene contribute to the risk of schizophrenia (see also Table 1) [19, 76, 77]. In these analyses, two single nucleotide polymorphisms (SNPs) located in the intron between the internal exon 4 and internal exon 5 of the human *TCF4* gene, according to the gene structure of Sepp et al. [10] (see below), on chromosome 18q21.2 (rs9960767, rs17512836) and an intragenic SNP near the *TCF4* gene (rs4309482) have shown the strongest association with the disease [19, 76, 77]. All three GWAS meta-analyses included data from the SGENE-plus study of schizophrenia, from the International Schizophrenia Consortium (ISC) and from the Molecular Genetics of Schizophrenia (MGS) group, but the later reports of Steinberg et al. [77] and Ripke et al. [19] also included additional patient and control samples that are not overlapping. Additionally, three more studies have replicated schizophrenia-*TCF4* gene associations in independent samples: (1) a study in Han Chinese (in which the rs9960767 SNP is not polymorphic) identified a further intronic *TCF4* SNP (rs2958182) that showed a significant association with schizophrenia [78]; (2) in a discovery sample from Ireland and a replication sample including non-overlapping samples from the Psychiatric GWAS Consortium (PGC), the SGENE-plus consortium and the Wellcome Trust Case Control Consortium 2 (WTCCC2), two intronic *TCF4* SNPs (again rs9960767 and rs17594526, which wer among the top ten significant *TCF4* SNPs in the so-far largest megalo-analysis of Ripke et al. [19]) passed the significance threshold of $p < 5 \times 10^{-8}$ [79]; and (3) in a recent family-based linkage meta-analysis, a further *TCF4* SNP was identified (rs1261117) as being significantly associated with schizophrenia [80]. The use of the family-based approach is a critical advantage here, given that all other GWAS employed only case–control designs that are susceptible for artefacts produced by population stratification [81], while using nuclear families in a replication study is robust against population stratification-induced false-positive findings [80].

Moreover, in a phenotype-based association study applied to the German GRAS (Göttingen Research Association for Schizophrenia) sample, *TCF4* rs9960767 (but not rs4309482) displayed some signals regarding a multivariate schizophrenia phenotype including PANSS positive and negative scores, a cognitive score, neurological soft signs, and age of prodromal onset [82]. Although the direction of the effect was similar to previous GWAS (risk allele C was associated with a more pronounced phenotype), the association was not strong enough to pass multiple testing adjustments. In addition, a small post-mortem study suggested that at least the rs9960767 SNP is neither functional nor affects mRNA expression in the adult human brain, indicating that such polymorphisms may yield their effects on gene expression through post-transcriptional pathways or in a developmental context by gene × environment interactions [41, 42]. In contrast, a more recent study reported that *TCF4* expression level in peripheral blood was significantly increased in patients with schizophrenia and bipolar disorder compared to controls. Additionally, peripheral *TCF4* mRNA concentration was positively correlated with severity of positive and negative symptoms. However, *TCF4* expression levels were only nominal and non-significantly correlated with some *TCF4* SNPs that have not so far been named as schizophrenia risk variants [83]. In the same study, after correction for multiple testing, more than ten *TCF4* SNPs, which have not been identified in previous GWAS, were significantly associated with the expression of negative symptoms [83].

It was also investigated whether the *TCF4* polymorphism rs9960767 modulates the response to antipsychotic drug treatment in schizophrenia, but in two independent samples, comprising more than 200 patients in total, the clinical improvement across 4 weeks was not influenced by *TCF4* genotype [84], suggesting that this TCF4 SNP is probably not a suitable predictor for antipsychotic drug effects.

Taken these findings together, SNPs from the *TCF4* gene together with common variants in the major histocompatibility complex (MHC) region are currently the best replicated schizophrenia susceptibility genes. However, the odds ratios for single variants are still small (OR around 1.2; see Table 1) and not useful for prediction of the disorder. Moreover, TCF4 SNPs cannot so far predict antipsychotic drug response. Thus, either *TCF4* as well as the other schizophrenia risk genes only contribute a very small fraction to the total risk, together with many other genetic and environmental risks, or there is a distinct subpopulation of patients for which the *TCF4* abnormalities might be major contributors to the etiopathogenesis of schizophrenia.

TCF4, information processing, and cognition: human studies

Kraepelin [85] and Bleuler [86] proposed that attentional and information processing deficits constitute core symptoms of schizophrenia. Following the early idea of Arvid Carlsson that schizophrenia might be a "thalamic filter deficit disorder" [87], impairments in early information processing have been repeatedly suggested to play a critical role in the pathogenesis of schizophrenia [88–91] Consequently, electrophysiological measures of early information processing—such as sensory gating or sensorimotor gating—have been proposed as promising

Table 1 Single nucleotide polymorphisms of the transcription factor 4 (TCF4) associated with schizophrenia and schizophrenia endophenotypes

Phenotype/endophenotype	TCF4 SNPs	Significant association with minor allele	Study type and samples	Ethnicity	References
Schizophrenia	rs9960767 rs17512836 rs4309482	OR = 1.20–1.23 OR = 1.23 OR = 1.09	GWAS with partially overlapping samples: 12,945–18,206 SZ patients 34,591–42,536 controls	European ancestry (also including European-Americans and European -Australians)	Stefansson et al. [76] Ripke et al. [19] Steinberg et al. [77]
Schizophrenia	rs2958182	OR = 0.78	Single association study: 2,496 SZ patients 5,184 controls	Han Chinese	Li et al. [78]
Schizophrenia	rs9960767 rs17594526[a]	OR = 1.18 OR = 1.77	GWAS: 1,606 SZ patients 1,794 controls	Irish	Strange et al. [79]
Schizophrenia	rs1261117	OR = 1.6	Family-based linkage meta-analysis: 6,298 individuals (including 3,286 SZ patients) from 1,811 nuclear families	European ancestry	Aberg et al. [80]
Multivariate schizophrenia phenotype including positive, negative, and cognitive symptoms, neurological soft signs, and age of prodromal onset	rs9960767	Risk allele C was associated with more pronounced schizophrenia phenotype (only trend, not surviving correction for multiple testing)	Phenotype-based association study: 1,041 SZ patients 1,144 controls	German	Papiol et al. [82]
Antipsychotic drug response in SZ patients	rs9960767	No effect	Pharmacogenetic association study: 214 SZ patients in total (two independent samples wit $n = 70$ and $n = 144$ SZ patients)	German	Lennertz et al. [84]
Prepulse inhibition of the acoustic startle response (sensorimotor gating)	rs9960767	Risk allele was associated with schizophrenia-like endophenotype OR = 6.82 (SZ)[b] OR = 4.93 (controls)[b] OR = 4.81 (total sample)[b]	Endophenotype-based association study in two independent samples: 105 SZ patients and high risk subjects 98 controls	SZ patients: German Controls: British	Quednow et al. [113]
P50 suppression of the auditory evoked potential (sensory gating)	rs9960767 rs17512836 rs17597926[a] rs10401120	Risk alleles were associated with schizophrenia-like endophenotype OR = 1.23–1.46 (never-smokers)[b] OR = 2.10–2.44 (light smokers)[b] OR = 3.21–5.50 (heavy smokers)[b] OR = 1.81–1.94 (total sample)[b]	Endophenotype-based association study: 1,821 controls	German	Quednow et al. [114]
Word recognition	rs9960767	Risk allele C was associated with enhanced performance	Endophenotype-based association study: 401 SZ patients	German	Lennertz et al. [127]
Attention and vigilance Working memory Processing speed Visuo-motor speed/set-shifting Verbal fluency	rs9960767	No effect	Endophenotype-based association study: 198 SZ patients 205 controls	German	Lennertz et al. [84]

Table 1 continued

Phenotype/endophenotype	TCF4 SNPs	Significant association with minor allele	Study type and samples	Ethnicity	References
Verbal fluency	rs12966547 rs4309482	Risk alleles were associated with poor performance	Endophenotype-based association study: 596 psychotic patients (including patients with SZ, bipolar disorder, or other psychoses) 385 controls	Norwegian	Wirgenes et al. [83]
Intelligence test (WAIS-RC)[c] Several attention-related tasks	rs2958182	Risk allele was associated with better performance in patients but worse in controls	Endophenotype-based association study: 580 SZ patients 498 controls	Han Chinese	Zhu et al. [128]
Reasoning and problem solving Processing speed	rs9960767	Risk allele was associated with lower performance	Endophenotype-based association study: 173 first-episode psychosis patients	Canadian	Albanna et al. [129]

GWAS genome-wide association study, *SZ* schizophrenia, *OR* odds ratio

[a] This SNP was also among the top ten significant *TCF4* SNPs in the so-far largest megalo-GWAS-analysis of Ripke et al. [19]

[b] Criterion one standard deviation from the control population

[c] Wechsler Adult Intelligence Scale-Revised

behavioural endophenotypes of schizophrenia [92]. Such gating mechanisms have been conceptualised as important pre-attentive filter functions protecting cognitive processes from interfering with irrelevant information [93]. Schizophrenia patients, and to a lesser extent also their unaffected first-degree relatives, consistently display disrupted sensory and sensorimotor gating, commonly demonstrated by either lower P50 suppression of the auditory evoked potential (AEP) or reduced prepulse inhibition (PPI) of the acoustic startle response [88, 89, 94–100]. Both measures have been shown to be heritable and to be disturbed before onset of the illness [101–107]. Although sensory (P50 suppression) and sensorimotor (PPI) gating are conceptually related, and both were in parallel suggested as useful endophenotypes of schizophrenia, they are not equivalent and usually also not correlated [94, 108–110]. However, a recent meta-analysis confirmed that electrophysiological gating measures differentiate best between healthy individuals, relatives of schizophrenia patients and the patients themselves when compared to other proposed endophenotypes such as ventricle size, neurological soft signs or neuropsychological dysfunction [111].

As described above, transgenic mice moderately overexpressing *Tcf4* in the postnatal brain display profound reductions in sensorimotor gating as measured by PPI [112]. Accordingly, the impact of the schizophrenia risk SNP *TCF4* rs9960767 on PPI was investigated in human samples (Table 1). In fact, the risk allele C of this SNP was strongly associated with reduced sensorimotor gating in two independent samples of healthy volunteers and schizophrenia patients [113]. Interestingly, low PPI levels (>1.5 SD below normal) have shown a much stronger associations (OR = 4.81) with the *TCF4* risk allele C than schizophrenia per se (OR = 1.23) [76]. When considering effect size measures, a similar pattern arises: whereas the association of a diagnosis of schizophrenia with *TCF4* genotype displayed only a very small effect size of $w = 0.09$ [76], the association of the schizophrenia endophenotype PPI with *TCF4* showed a strong effect size of $d = 0.90$ averaged across both samples. Impressively, of the 23 subjects carrying the C-allele across both investigated samples, 14 (61 %) displayed low PPI levels (>1.5 SD below normal), when compared to the merged total sample, which is again an expression of the strong genotype effect of *TCF4* on PPI. The authors hypothesised that the impact on PPI might arise from developmental changes of brain stem nuclei induced by the *TCF4* polymorphism (see Fig. 3, below, for an illustration of involved brain structures) [113].

Subsequently, we also investigated the influence of 21 *TCF4* polymorphisms—which were most strongly associated with schizophrenia in a recent meta-analysis [19]—on sensory gating as assessed by P50 suppression of the AEP

[114]. We used a multi-centre study including six academic institutions throughout Germany with 1,821 subjects (1,023 never-smokers, 798 smokers) randomly selected from the general population (Table 1). Given that smoking is highly prevalent in schizophrenia [115] and has been shown to affect sensory and sensorimotor gating [116], several parameters for smoking behaviour were additionally assessed. Like PPI P50 suppression was also significantly decreased in carriers of schizophrenia risk alleles of the *TCF4* polymorphisms rs9960767, rs10401120, rs17597926, and 17512836—the latter two were the most significant SNPs in the mega-analysis of Ripke et al. [19]. Importantly, these gene effects were strongly modulated by smoking behaviour as indicated by significant interactions of *TCF4* genotype and smoking status: heavy smokers (Fagerström score ≥4) showed stronger gene effects on P50 suppression than light smokers and never-smokers. Moreover, the genotype × smoking interaction seems to be dose-related as the *TCF4* genotype effect grows with increasing smoking severity. Interestingly, *TCF4* genotype effects on sensory gating were more evident at frontal (*Fz*) than vertex (*Cz*) electrodes. Previous studies have reported that the prefrontal cortex substantially contributes either to the sensory gating process per se [117] or at least to the generation of the P50 amplitude [118]. Additionally, data from a recent EEG source localization study suggest that the sensory gating deficit of schizophrenia patients could be explained by dysfunction of the dorsolateral prefrontal cortex [41]. Thus, *TCF4* mutations (in combination with smoking) might affect PFC function in schizophrenia. Accordingly, deficits of PFC functions have recently also been described in *Tcf4tg* mice [119].

In conclusion, these results imply that the schizophrenia risk alleles of *TCF4* variants interact with smoking behaviour with regard to auditory sensory gating. However, if smoking behaviour strongly modulates the *TCF4* genotype effects on a proposed endophenotype of schizophrenia, it might also modulate the risk for schizophrenia itself. We therefore suggested the investigation of potential moderating effects of dimensional and binary measures of smoking behaviour on genetic risk factors of schizophrenia. In fact, preliminary data from 882 schizophrenia patients and 2,163 controls now suggest that the risk allele C of the *TCF4* rs9960767 is indeed more frequent in smoking schizophrenia patients (8.3 %) than in non-smoking patients (5.3 %) or smoking (5.5 %) and non-smoking controls (5.8 %), transferring to an OR of 1.55 for smoking patients in contrast to OR of 0.90 for non-smoking patients (Dan Rujescu, University of Munich, Germany, personal communication of unpublished data). These results have certainly to be replicated in further and larger samples, but nevertheless these data indicated that stratification for smoking behaviour in case–control association studies potentially adds power, resulting in stronger gene effects. Moreover, it should be further explored whether nicotine use itself might enhance the risk for schizophrenia as indicated by longitudinal studies showing that, beyond cannabis and alcohol use, early consumption of tobacco also increases the risk for psychosis [120, 121]. Finally, an extended endophenotype, including electrophysiological gating measures such as PPI or P50 suppression, smoking behaviour, and risk genes such as *TCF4*, may be suitable as an early indicator for a developing psychosis [114]. Moreover, dedicated gene × environment studies could be performed in mouse models, providing additional evidence for *TCF4* × smoking interactions and allowing the investigation of underlying molecular mechanisms.

Neurocognitive dysfunctions have also been proposed as promising endophenotypes of schizophrenia [122]. In particular, impaired verbal memory, which is among the most prominent and consistently reported cognitive deficits of schizophrenia [123], has been emphasised as a potential intermediate schizophrenia phenotype, as studies with unaffected relatives from multiple affected families ("multiplex families") and twin studies demonstrated an increasing memory deficit along with an increasing genetic load [124–126]. Lennertz et al. [127] therefore investigated the impact of the *TCF4* rs9960767 variant on verbal memory performance in a sample of 401 schizophrenia patients [127]. While no effect of the schizophrenia risk allele C on immediate recall and total learning was found, a weak trend regarding delayed verbal memory appeared, surprisingly indicating superior performance in carriers of the risk allele compared to non-carriers. Moreover, in the cued recall condition (word recognition), schizophrenia patients carrying at least one C-allele also significantly recognized more words compared to patients without the risk allele. These results were unexpected considering the supposed impact of *TCF4* on brain development and assuming that an endophenotype should display a similar association with the risk gene (e.g. impaired memory in carriers with the schizophrenia risk gene) as an endophenotype with the complex disease phenotype (e.g. memory deficit in schizophrenia patients). Given that the effects sizes of the genotype effects were rather small (Cohen's $d = 0.34$, and after correction for several covariates, $d = 0.27$), and that the results would not have become significant if the statistical threshold had been corrected for multiple test parameters, these results should not be over-interpreted. These authors also explored functional effects of the same *TCF4* variant on a comprehensive neuropsychological test battery in a sample of about 200 schizophrenia patients and a control sample of 205 healthy volunteers [127]. The assessed cognitive functions attention and vigilance, working memory, processing speed, visuo-motor speed and set-shifting, as well as verbal fluency, were all unaffected by *TCF4* rs9960767 in

both groups (unpublished data). Thus, although haploinsufficiency of the *TCF4* gene is associated with severely disrupted intellectual functions as presented in the PTHS, no considerable effect of the *TCF4* rs9960767 polymorphism on neuropsychological function was found in this sample with the exception of a weak and unexpected association with word recognition (Table 1).

In contrast, Wirgenes et al. [83] recently reported from a large sample of patients with schizophrenia spectrum disorders (total $n = 596$ including patients with schizophrenia, bipolar disorder, other psychoses) and healthy controls ($n = 385$) that the risk alleles of the *TCF4* risk variants rs12966547 and rs4309482 were associated with worse verbal fluency in the total sample. They also found some trends that the schizophrenia risk alleles from rs43094882 and rs9960767 were associated with ventricular and/or hippocampal volume, but these results did not survive correction for multiple testing. In the exploratory analyses, there were also some significant associations of other *TCF4* SNPs with verbal learning, executive functioning, and several brain abnormalities [83].

A study in Han Chinese investigated the impact *TCF4* rs2958182 SNP, previously associated with schizophrenia in the same ethnicity [78], on cognitive functions in 580 schizophrenia patients and 498 controls [128]. The authors reported that the schizophrenia risk allele was associated with better performance in patients but worse performance in controls regarding an IQ test as well as in attention-related tasks. Because of this unexpected result pattern, the authors speculated that *TCF4* and cognition might follow an inverted U-shaped function. However, it is not fully clear at the moment whether previous European studies and this Chinese study can be adequately compared.

Most recently, a Canadian study explored the association of the *TCF4* rs9960767 SNP with neurocognitive function in 173 first-episode psychosis patients (affective and non-affective psychosis). The authors reported that carriers of the rs9960767 C allele performed worse in the cognitive domains of "reasoning and problem solving" and "speed of processing", adumbrating that TCF4 polymorphisms might also contribute to deficits in higher cognitive function in schizophrenia patients [129].

In a lymphocyte-based gene expression study in healthy Mexican Americans, it has recently been shown that peripheral expression of *TCF4*—among seven further genes (*IGFBP3*, *LRRN3*, *CRIP2*, *SCD*, *IDS*, *GATA3*, and *HN1*)—predicted cortical grey matter thickness measured by magnet resonance tomography [130]. Notably, *TCF4* expression was particularly correlated with grey matter thickness in the prefrontal cortex. The authors concluded that a progressive decline in the regenerative capacity of the brain contributes to normal cerebral aging including thinning of the grey matter [130]. A critical role of TCF4 specifically for development of the prefrontal cortex was also supported by recent post-mortem data showing a significant association between *TCF4* expression and *cis* eSNPs (previously identified in an expression quantitative trait loci analysis) in tissue of the prefrontal cortex (rs1261085, rs1261134, rs1261073) and the thalamus (rs1261134), while in the hippocampus, temporal cortex and cerebellum, no such associations were found [131].

Taking the human gating and cognition data together, it appears that *TCF4* SNPs likely affect early information processing in such a way that schizophrenia risk alleles are consistently associated with a schizophrenia-like phenotype, i.e. reduced gating functions (for an overview, see Table 1). At the least, the effect for auditory sensory gating was strongly modulated by smoking, suggesting a possible gene × environment interaction that might be also relevant for the development of schizophrenia. There are also initial data arguing that common *TCF4* variants might have an impact on brain morphology specifically regarding the prefrontal cortex, which is also in line with the impact on gating functions that might involve the prefrontal cortex, and also in accordance with neurodevelopmental phenotypes obtained with *Tcf4* and neuronal bHLH mouse models as discussed above. Whether common *TCF4* variants also influence higher cognitive functions in healthy volunteers or schizophrenia patients is not clear at the moment as existing studies are controversial, present rather weak associations, and are currently not replicated. In contrast, more severe *TCF4* mutations, as occurring in the PTHS, are definitely accompanied by strong cognitive dysfunction, suggesting that a considerably disturbed *TCF4* function is associated with strong changes in brain development (see following section).

TCF4 and neurodevelopmental disorders

Heterozygous hypomorphic, null mutations or deletion (haploinsufficiency) of the *TCF4* gene in humans causes the rare PTHS (an autosomal-dominant neurodevelopmental disorder characterized by severe mental, motor and language retardation, epilepsy, facial dysmorphisms, intermittent hyperventilation, and rarely also postnatal microcephaly), pointing to the fact that TCF4 is also critical for normal development of the mammalian nervous system [132–135]. Currently, only 200–300 diagnosed cases with PTHS exist worldwide [134]. A small proportion of patients suspected to have the Angelman syndrome, which displays a similar phenotype as PTHS, also have mutations in the *TCF4* gene [136]. A recent study with ten young PTHS patients revealed strong intellectual and motor disabilities together with a behavioral phenotype that overlaps with autism spectrum disorders [137]. The autism-like

behaviour was characterised by difficulties in engaging and communicating with others, frequent occurrence of repetitive motor stereotypies, repetitive play and fascination with specific objects, and difficulties with changes in daily life routines. The real age of the PTHS patients ranged from 32 to 289 months, whereas the estimated developmental age lay between 3.5 and 15 months for the mental abilities and between 4 and 19 months for the motor abilities [137]. Notably, in a recent study investigating balanced chromosomal abnormalities in patients with autism convergent genomic information, suggested that the *TCF4* gene might also be involved in the pathogenesis of autism-spectrum neurodevelopmental disorders [138].

Surprisingly, prominent macroscopic brain abnormalities are not common in PTHS: only subtle hypoplasia of the corpus callosum has been consistently reported [139–141], while enlarged ventricles (similar to schizophrenia, [142]) and thin hindbrain [140, 141], as well as enlarged caudate nuclei and a lower hippocampus volume, have also been reported [143]. Whalen et al. estimated that only about 50 % of the PTHS patients display abnormalities in structural brain imaging, while only about 7 % reveal a microcephaly [208].

Tcf4/TCF4 expression in brain development

The function of TCF4 in nervous system development and adult brain must be seen in the context of its numerous proposed and the few experimentally validated interaction partners of the bHLH family (see Fig. 1) and its complex spatio-temporally regulated expression pattern. All E-proteins are expressed during embryonic stages including neural structures (http://www.brain-map.org/), with *Tcf4/TCF4* showing the highest expression levels in mouse and human brain tissue (http://www.brainspan.org/) [13, 144]. In contrast to *Tcf3* and *Tcf12*, *Tcf4* expression remains at substantially high levels in the adult and aged rodent brain [112, 145, 146]. *Tcf4* expression is sustained particularly in areas of high neuronal plasticity, such as the cerebral cortex, hippocampus and cerebellum [13, 112]. Human *TCF4* expression has been detected in the prosencephalon and the ventricular zone of the embryonic CNS [133], in the telencephalon at all stages of fetal development as well and in the adult forebrain (http://www.brainspan.org/). In summary, *TCF4* is the only E-protein being expressed at all stages in the developing and adult mouse and human brain.

In contrast to the constitutive and broad expression of *Tcf4*, all putative interaction partners in the nervous system show a much more spatio-temporally restricted expression profile (Fig. 1). The expression of the bHLH inhibitors *Hes1* and *Id1* are transiently expressed in embryonal stages and their function indeed seems to be confined to inhibit premature differentiation initiation [147, 148]. Neurogenesis-associated pro-neuronal class II bHLH proteins of the achaete scute (e.g. ASCL1/MASH1), atonal (e.g. ATOH1/MATH1) and neurogenin families (NEUROG1,2) are transiently expressed at early stages of development, whereas the gradual onset of expression of group II bHLH proteins involved in terminal neuronal differentiation (e.g. NEUROD family members NEUROD1, -2, and -6) is confined to later stages and remains sustained in the adult brain [5, 149]. All type II neuronal bHLH proteins are thought to depend on the hetero-dimerisation with an E-protein [1]. Thus, at least in later stages of neuronal differentiation and selected brain regions, TCF4 appears to be the obligate interaction partner of neuronal class II bHLH proteins. This selective availability as unique interaction partner may explain dosage susceptibility of TCF4 observed in genetic model systems such as zebrafish [150] and mouse [151], and in human patients suffering from PTHS [134] and potentially also schizophrenia (see below).

Tcf4 functions in neurodevelopment and cognitive processing: lessons from mouse models

Tcf4 heterozygous null mutant mice ($Tcf4^{+/-}$) are viable, fertile and display no obvious phenotype [151]. Although subtle defects cannot so far be excluded, $Tcf4^{+/-}$ mice do not replicate the profound effects observed in humans where haploinsufficiency causes severe developmental disturbances including PTHS phenotype (Fig. 2 and see below). As reported by Zhuang et al. [15, 152], *Tcf4* homozygous null mutant mice ($Tcf4^{-/-}$) were born with extremely low frequency and did not survive longer than 1 week after birth. In contrast, Flora et al. [151] did not observe embryonic lethality of null mutants and obtained expected Mendelian ratios of $Tcf4^{-/-}$ mice at birth; however, animals died within the first 24 h. Nevertheless, both studies showed that the complete inactivation of both *Tcf4* alleles has strong developmental consequences in mice with evident morphological defects detected so far only in pontine nuclei development, which has been specifically attributed to the interaction of TCF4 with the proneural transcription factor ATOH1/MATH1 [151]. Therefore, in mice, TCF4 function during early brain development may be partially compensated by the other class I bHLH factors, TCF3 and TCF12. However, in mosaic $Tcf4^{+/-}$ $Tcf4^{-/-}$ mice, only pups displaying maximal proportion of 30 % of *Tcf4* null cells are viable [153]. Nonetheless, conditional knockouts enabling targeted deletions at various stages of CNS development will be essential to better understand more subtle phenotypes possibly caused by the loss of function of *Tcf4*. Such mouse models may allow the study of embryonal TCF4 dysfunction even in the adult

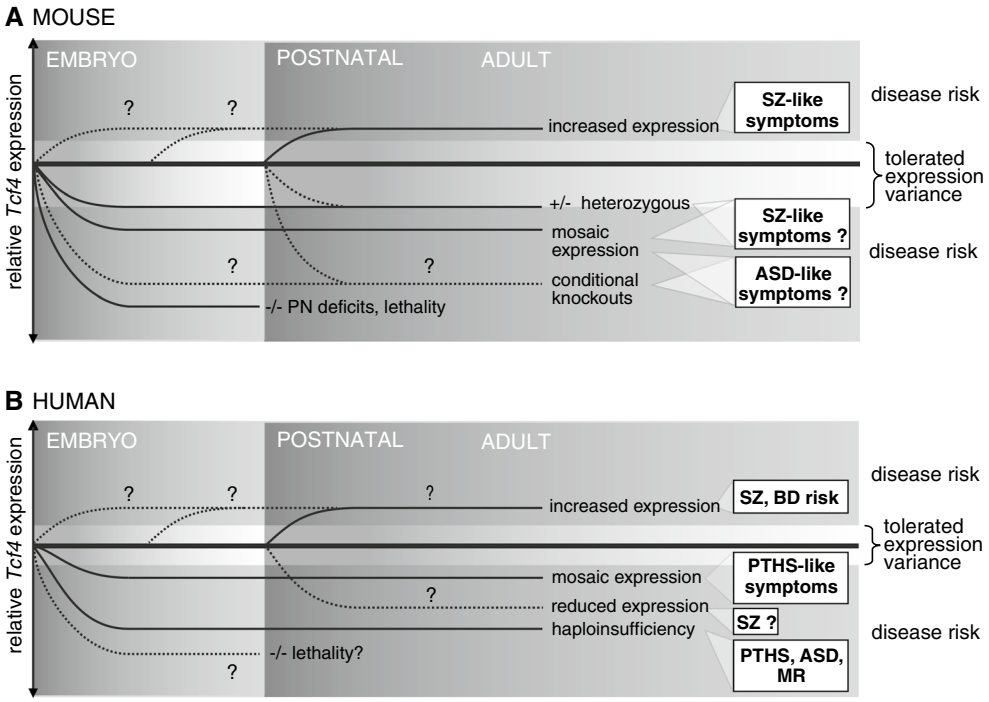

Fig. 2 Phenotypical comparisons reveal different *TCF4* gene dosage dependences in mice (**a**) and humans (**b**) in neurodevelopment related diseases including schizophrenia. Gain-of-function and loss-of-function analyses in mice and corresponding risk alleles and mutations in humans suggest that *TCF4* expression differences are tolerated in a narrow range (range depicted in *light blue*). Exceeding critical thresholds increases disease risks (depicted in *grey*). Slightly increased postnatal expression of *Tcf4* has been found to cause schizophrenia (SZ)-associated symptoms in mice. Phenotypic consequences of increased *Tcf4* expression during embryonal stages are not yet known (**a**). Indirect evidence from human post-mortem brain and blood sampling suggests that elevated expression may be associated with SZ and bipolar disease (BD). The critical period of enhanced *TCF4* expression in humans is unknown (**b**). The tolerance range for reduced gene dosage effects might potentially be higher in mice compared to humans, since heterozygous animals appear to be largely unaffected, although a thorough behavioural phenotyping has not so far been performed. Thus, it is unknown if reduced gene dosage in mice may cause SZ-like symptoms. The analysis of null mutants is hampered by perinatal lethality, but structural deficits in brain development have already been described, although not thus far representing Pitt-Hopkins-like symptoms (**a**). Loss-of-function of *TCF4* (haploinsufficiency and mosaic deficiency) causes severe neurodevelopmental diseases including PTHS and possibly other autism-like syndromes. Given many examples of inverted-U-shape relationships of gene dosage with disease severity in autism-related neurodevelopmental diseases, it appears possible that slightly reduced expression levels of *TCF4* may be implicated in SZ (**b**) (for citations, see main text). *SZ* schizophrenia, *BD* bipolar disorder, *PTHS* Pitt-Hopkins Syndrome, *NDD* neurodevelopmental disorder, *MR* mental retardation

brain without being hampered by embryonic or perinatal lethality.

So far, insight into the role of TCF4 on adult brain function in the mouse is restricted to a model with slightly increased expression levels in the forebrain [112]. In addition to loss-of-function models (see above), gain-of-function studies may be of particular relevance for schizophrenia, as *TCF4* mRNA expression is significantly increased in post mortem cortical samples and peripheral blood cells of psychosis patients [83, 154]. Furthermore, *TCF4* mRNA expression level is elevated in neurons derived from human-induced pluripotential stem cells of schizophrenia patients versus unaffected subjects [155]. Therefore, *Thy-1* promoter driven overexpression of *Tcf4* mRNA in brain structures involved in cognition, such as the cortex and hippocampus of the mouse [112], may partially replicate molecular alterations of increased schizophrenia risk in humans. Subsequently, we will refer to these mice as *Tcf4tg*. The onset of transgenic *Tcf4* expression is confined to early postnatal stages, and neither breeding problems nor any overt abnormalities have been observed. Nonetheless, adult *Tcf4tg* mice displayed profound deficits in contextual and cued delay fear conditioning indicating hippocampal deficits. Alterations in activity, anxiety or exploratory drive were not observed, thus postnatal *Tcf4* overexpression affected only early information processing and cognitive functions [112]. Fear-associated learning deficits were erased upon applying stronger aversive stimuli arguing for a subtle defect [112]. In addition, *Tcf4tg* mice display deficits in trace fear memory most likely paralleled by reduced levels of attention and behavioural anticipation [119]. It has been shown that these higher order cognitive processes depend on both the hippocampus and the anterior cingulate cortex (ACC) [156]. Thus, impaired interactions between

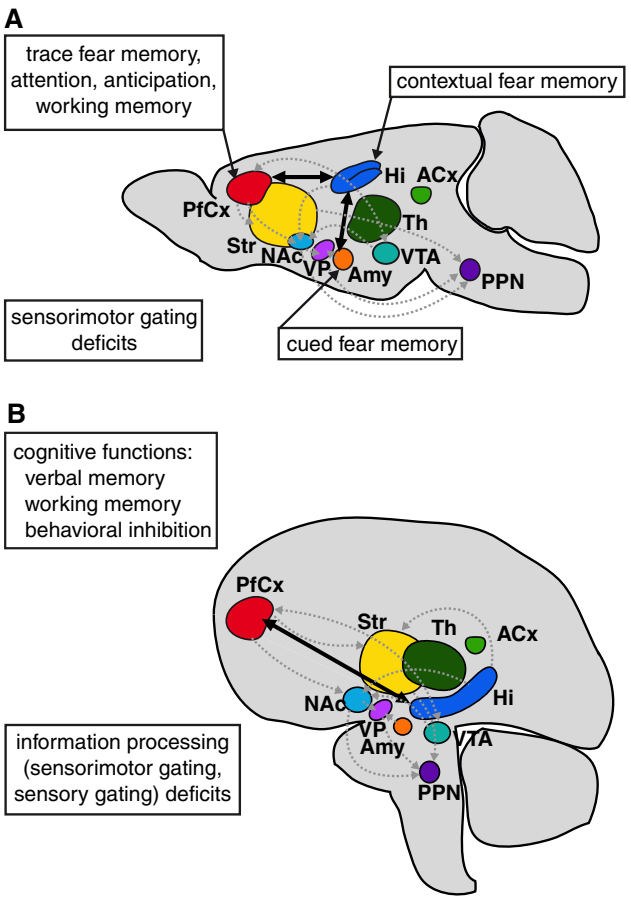

Fig. 3 Brain structures involved in postulated deficits of information processing in mice and men. Behavioural and neuropsychological phenotypes obtained in mice (**a**) and human subjects (**b**) suggest a function of TCF4 in brain networks that are important for cognition (*bold lines*) and sensory processing (*dotted grey lines*). Deregulation of *TCF4* expression levels during development interferes with proper functional connectivity within corresponding brain networks (for citations see main text). *ACx* auditory cortex, *Amy* amygdala, *Hi* hippocampus, *NAc* nucleus accumbens, *PCx* prefrontal cortex, *PPN* pedunculopontine nucleus, *VTA* ventral tegmental area, *VP* ventral pallidum

the prefrontal cortex and the hippocampus likely contribute to the reduced cognitive performance in *Tcf4tg* mice. Similar disturbances between remote brain regions have been described in the Df(16)A+/− strain that harbour a microdeletion in mice corresponding to a human chromosome 22 (22q11.2) deletion described in schizophenia [157]. Moreover, altered functional cortical–hippocampal connectivity has been frequently reported in schizophrenia patients [158, 159]. In addition, *Tcf4tg* mice display sensorimotor gating deficits correlating with a frequent endophenotype of SZ patients [88, 107, 160–162]. In summary, the analysis of *Tcf4tg* mice has provided accumulating evidence to support the role of TCF4 in brain circuits involved in cognition and higher order information processing, which is independently strengthened by human studies (Fig. 3 and see below).

Discussion

The schizophrenia-associated gene *TCF4* belongs to a subfamily of bHLH transcriptional factors that recognize E-box binding sites on regulatory DNA elements in the genome [1, 8]. At early developmental stages, class I/E-protein transcription factors such as *Tcf3, Tcf4*, and *Tcf12* show wide expression throughout the brain, but only *Tcf4* displays sustained expression in the adult brain of mice, which is most prominent in the cerebellum, hippocampus and cortex [112, 145]. In conclusion, TCF4 is, at least during later stages of neurodevelopment and in the adult brain, the obligate interaction partner of multiple class II neuronal bHLH factors of, e.g., the NEUROD family [112]. Therefore, TCF4 must be considered as an interaction 'hub' in neuronal bHLH protein networks important for different aspects of neurodevelopment and adult plasticity [149, 163]. Due to potentially competing functions, it is retrospectively not surprising that control of *TCF4* gene dosage and protein function, in contrast, e.g., to other neuronal bHLH factors, is particularly susceptible to interference. Thus, TCF4 availability for unknown homo- and/or heterodimeric bHLH complexes represents a critical bottleneck in neurodevelopmental processes that might be associated with an increased risk of schizophrenia. Reduced *TCF4* activity (haploinsuffiency) has been shown to cause severe mental retardation, as observable in the PTHS, and may also be associated with other autism-spectrum disorders in humans [134, 138]. More subtle gene dosage alterations are likely to be associated with schizophrenia and possibly also bipolar disease. Gene dosage sensitivity may not be as pronounced in rodents compared to humans, as heterozygous null mutant mice display only subtle neurodevelopmental disturbances, although, for example, a thorough behavioural analysis of these mice is still missing [151].

Given the enormous complexity of *TCF4* splice variants and biochemical properties of different PTHS-associated mutations [10, 11], it is still possible that dominant-negative effects beyond dosage effects contribute to the severity of the neurodevelopmental disturbances in humans. In a transgenic mouse model (*Tcf4tg*) with slightly elevated expression of *Tcf4* in the forebrain and displaying cognitive and sensorimotor deficits, such effects were observed supporting the critical gene dosage sensitivity [112]. However, potentially dominant negative effects by the corresponding C-terminally tagged protein expressed in the transgenic animals cannot be formally excluded, since C-terminal frame shift mutations have been shown to alter TCF4 functions [11]. Nonetheless, *Tcf4tg* mice display a disbalance

of *Neurod1* versus *Id2* expression ratios, and it is thus plausible that even a slight disturbance of a delicate balance of bHLH transcription factor gene expression in the adult brain impairs cognition and information processing. In line with that, heterozygous *Neurod2* null mutants also display cognitive deficits [164]. Notably, the dominant HLH factors *Id2* and *Id4* display similar to *Tcf4*, *Neurod2* and *Neurod6* sustained expression in the adult brain indicating a dynamic control of adult *TCF4* function at the level of dimerisation, possibly coupled to neuronal activity possibly via nuclear Ca^{2+} signalling. It has been shown that TCF4 interacts with the Ca^{2+} binding protein calmodulin at physiological concentrations inhibiting DNA binding of E-protein homodimers in non-neuronal cells [165–169]. The mode of the Ca^{2+}-mediated regulation of TCF4 function in neurons is not known but should, for several reasons, be of high interest for future attempts to understand the mechanisms of how TCF4 contributes to endophenotypes of schizophrenia. Firstly, localised Ca^{2+} signalling has been identified as a key player in communicating synaptic activity to the nucleus and to be critically involved in mediating transcription-dependent adaptive responses in neurodevelopment, plasticity and cognitive processes [170, 171]. Secondly, recent cross-disorder analyses of GWAS data combined with pathway analysis provided strong evidence for the importance of L-type voltage-gated Ca^{2+} channels (VGCC) and Ca^{2+} signalling in schizophrenia and bipolar disorders [172]. Most prominently, the intronic polymorphism rs4765914 in *CACNA1C* has been previously associated independently with bipolar disorder [173, 174], schizophrenia [19] and major depressive disorder [175]. Genetic imaging approaches linked *CACNA1C* variants along an endophenotypic spectrum similar to that observed for *TCF4* including attention deficits [176] and memory formation [177]. Of note, the C-terminus of CACNA1C encodes a transcription factor that is implicated in activity-transcription coupling by regulated proteolysis at the membrane [178]. Moreover, several other Ca^{2+}-regulated transcription factors (CREB1, MECP2, MEF2, FOSB, NPAS4, CREST among others) have been associated with psychiatric diseases such as Rett-Syndrome, autism and bipolar disorder [170, 171, 179]. In addition, the validated TCF4 interaction partners NEUROD1 and NEUROD2 are themselves regulated by Ca^{2+} [179–182]. Although there is no direct experimental evidence for a particular mechanism by which synaptic activity/Ca^{2+} could modulate TCF4 activity in neurons, several non-exclusive modes of action are possible which are based on studies with different class I and II bHLH factors in non-neuronal cells (see above): (1) regulation of transcription or splicing of *TCF4* or its interaction partners by Ca^{2+}-regulated transcription or splicing factors, (2) Ca^{2+} controlled cytoplasm to nucleus shuttling of TCF4, (3) modulation of dimerization selectivity and DNA binding efficiency or specificity by either interaction with Ca^{2+} binding proteins such as calmodulin or posttranslational modifications via Ca^{2+} regulated kinases, which (4) could also alter the recruitment of transcriptional co-factors such as p300/CBP, and (5) transcriptional regulation of gene products involved in Ca^{2+} signalling.

The importance of a tightly controlled gene expression program in the context of schizophrenia is further supported by the findings that *mirR-137* is also a genetic risk factor and has been shown to target 3′ regions in the human mRNA of *TCF4* [22]. Due to the promiscuity of miRs, it is not surprising that *miR-137* most likely regulates abundance levels of several mRNAs among which, however, may be a substantial fraction of schizophrenia risk-associated gene products [183]. Similar to *TCF4*, *miR-137* has been shown to be involved in the regulation of neuron maturation [184] and adult neurogenesis [185]. Most recently, a post mortem study demonstrated that a decreased *miR-137* expression—caused by the TT genotype of the SNP rs1625579—was associated with increased *TCF4* expression [154]. Although the identification of putative *miR-137* targets is mainly based on in silico predictions and has only partially been validated by reporter gene assays, it is possible that *mir-137* also represents another 'hub' within gene regulatory programs that are considered to be of particular relevance for schizophrenia. Among the putative *mir-137* targets beyond *TCF4* are several high confidence schizophrenia risk genes (*CSMD1, C10orf26, CACNA1C, and ZNF804A*) and members of schizophrenia-associated glutamatergic, GABAergic, serotonergic, and neuregulin-ErbB signalling pathways (*GRIN2A, GRM5, GABRA1, HTR2C, NRG2, NRG3 ERBB4*) [183]. Although direct target genes of *TCF4* in the brain are not known and putative *mir-137* targets have not been validated in vivo, growing evidence suggests that both factors are crucial players of gene expression networks that may be particularly susceptible to interference by environmental factors. In schizophrenia, multiple genes are thought to cooperate with different environmental factors in unfavourable combinations. Thus, future research should be dedicated to the elucidation of *TCF4*-and *miR-137*-controlled gene regulatory networks that may allow the elucidation of causal gene × gene interactions underlying schizophrenia symptoms.

It has been proposed that the inter-individual phenotypic variability and severity of PTHS may reflect the molecularly divergent mutations that compromise *TCF4* function differentially [11]. Similarly, we hypothesize that a graded level of 'severity' of *TCF4* dysfunction ranging from haploinsufficiency caused by missense mutations in PTHS [134, 135], to chromosomal aberrations [138], while subtle alterations induced by common genetic variants [19, 76, 77] correlate with the 'severity' of the corresponding neurodevelopmental diseases such as mental retardation,

autism-spectrum disorders and schizophrenia. The most obvious common feature of these diseases is the graded intellectual and cognitive impairment. As described above, accumulating data from both human and mouse studies suggest that *TCF4* dysfunction might be particularly important for higher order cognitive processing. Therefore, it may be possible that overlapping mechanisms and/or pathways are affected by *TCF4* which could have implications for the focus of future experiments. Assuming that similar mechanisms are instead quantitatively altered, e.g. in PTHS and schizophrenia (and not categorically qualitatively different), the identification and validation of *TCF4* target genes in PTHS and corresponding loss-of-function mouse models could well be of relevance for schizophrenia. The genetic complexity of schizophrenia per se and the subtle alterations at the gene expression level that one has to assume to occur from the schizophrenia-associated non-coding *TCF4* variants obviously hamper the identification of target genes from patient-derived samples or schizophrenia mouse models. Therefore, the fact that different genetic alterations in *TCF4* are causally associated with several phenotypically overlapping mental disorders could help to guide experimentally feasible attempts to obtain further mechanistic insights into the function of this gene. Future studies on *TCF4* should thus not strictly focus exclusively on models with construct-validity for schizophrenia, which may be out of reach at the moment, but should (as the different types of mutations in the gene) step beyond disorder boundaries by, e.g. analysing genetically defined cellular and animal gain- and loss-of-function models in more depth. In addition, observations from human studies could foster translational studies in model systems approaching gene × environment interactions with relevance for schizophrenia (see below).

By combining electrophysiological measurements with genetics, it has been shown that *TCF4* risk alleles correlate with particular schizophrenia endophenotypes—namely sensory and sensorimotor gating [112–114]. Specifically sensory gating revealed an interesting and unexpected gene × environment interaction: the schizophrenia risk allele C of the *TCF4* rs9960767 SNP was robustly associated with reduced P50 suppression of the AEP. However, this genotype effect was strongly modulated by smoking behaviour given that only smokers showed reliable *TCF4*-sensory gating associations, while the gene effect was not present in never-smokers. Moreover, the genotype × smoking interaction was dose-related, as the *TCF4* genotype effect grows with increasing smoking severity [114]. However, the moderating influence of smoking on the *TCF4* genotype effect was not present in the previous investigation on *TCF4* gene effects on sensorimotor gating measured by PPI [113]. The earlier investigated samples might have been too small and underpowered (healthy sample $n = 98$, schizophrenia spectrum sample $n = 105$) to reliably examine the effects of smoking as a mediating factor on the *TCF4* gene effects on PPI. The potentially moderating effect of smoking on *TCF4* gene effects on PPI (and other schizophrenia endophenotypes) should therefore be investigated in larger samples. Finally, the *TCF4* genotype effect on PPI displayed a much stronger effect size (Cohen's $d = 0.90$) than the mean effect on P50 suppression (mean $d = 0.23$, ranging from 0.03 in never-smokers to 0.69 in heavy smokers), which could be partially explained by a superior reliability of PPI compared to P50 suppression [114].

But how could the unexpected smoking × genotype interaction regarding P50 suppression be elucidated? There are at least two possible explanations: The first is a hidden gene × gene interaction: in this model, *TCF4* interacts with a hidden gene (or genes) so that only the presence of two or more risk alleles is associated with both smoking severity and P50 suppression, while *TCF4* alone was merely associated with P50 suppression but not with smoking. Further studies might investigate possible gene × gene interactions, and promising candidates for the "hidden" SNPs may lie in the *CHRNA3-CHRNA5-CHRNB4* gene cluster coding for α3, α5, and β4 nicotinic acetylcholine receptor (nAChR) subtypes. SNPs from this gene cluster have been reliably associated with smoking behaviour [186–190], and also with sensorimotor gating (PPI) [191] and cognitive performance [192]. The second and maybe more appealing explanation for the present result pattern could be a gene × environment interaction, in which smoking represents a long-lasting and ongoing environmental influence. This interpretation would be in line with the suggestion of Williams et al. [41] that the *TCF4* schizophrenia risk allele may exert its effect on expression exclusively in a developmental context, because their post mortem data suggested that this SNP is neither functional nor affects mRNA expression in the adult human brain. However, at the moment, we can only hypothesise which neurobiological mechanisms might underlie this *TCF4* × smoking interaction on P50 suppression. Using *TCF4* knock-out mice, it was recently shown that *TCF4* plays a unique role in the development of the pontine nuclei [151]. These nuclei are highly interconnected with the cochlear nucleus and neighboring brain stem nuclei that are critically involved in auditory information processing [193, 194]. Moreover, pontine nuclei are also connected to the pedunculopontine nucleus [195], which has been shown to be critically involved in auditory sensory gating and sensorimotor gating in animal studies [196–199]. Most of the auditory pathways within the brain stem are mediated by cholinergic neurotransmission, and the predominant nAChR expressed in the lower auditory brainstem nuclei is the α7 subtype, while α3β4 nAChR

also plays a role but in the development of the auditory brainstem system [200]. Given that repeated exposure to nicotine results in nAChR desensitisation [201, 202] and also to a long-term homeostatic increase of α4β2 and α7 nAChR [203, 204], smoking-induced changes in brainstem nAChR function might interact with developmental changes within pontine nuclei resulting in changes of P50 suppression. Actually, P50 amplitude to S1 was influenced by smoking but not by *TCF4* genotype and, therefore, basic auditory processing was somewhat affected by smoking but not directly influenced by *TCF4*. Moreover, changes in nAChR function induced by chronic nicotine exposure might also impact auditory sensory gating at neocortical or hippocampal levels [205]. Taken together, smoking-induced plasticity of nAChR in concert with neurodevelopmental changes induced by *TCF4* gene variations may have affected P50 suppression in our sample. Alternatively, nicotine may be involved in the methylation of DNA sequences within the *TCF4* gene or other genes interacting with *TCF4*, leading to an epigenetic change of the expression of the corresponding genes. It has previously been shown that nicotine could decrease glutamic acid decarboxylase-67 and DNA methyltransferase-1 via epigenetic mechanisms, which are induced by an activation of nAChRs located on cortical and hippocampal GABAergic interneurons [206]. Additionally, it has recently been demonstrated that smoking affects monoaminooxidase-A (*MAOA*) promoter methylation in DNA prepared from lymphoblasts and whole blood [207]. Interestingly, quitting smoking did not lead to a return to methylation levels found in never-smokers, indicating a long-lasting effect of smoking on DNA methylation. Thus, smoking might exert a sustained impact on central *MAOA* activity (and other genes) via epigenetic mechanisms, leading to changes in noradrenergic function that interact with neurodevelopmental changes caused by *TCF4* gene variations (see above). Eventually, nicotine might also impact the expression of *TCF4* gene directly with functional consequences on early information processing. In summary, the unexpected findings in humans of *TCF4* × smoking interactions has inspired the formulation of several hypotheses linking different biological systems as additional modulators of *TCF4*-associated endophenotypes i.e. information processing and cognition. These, in turn, could be further investigated in *Tcf4tg* mice which display complementary endophenotypes and may offer predictive value for validation and possible pre-clinical studies [112, 119]. Ameliorating the cognitive impairments in schizophrenia, most likely caused by higher order information-processing deficits in dispersed brain circuits, still represents a critical unmet medical need to finally improve the therapeutic options for most schizophrenic patients. The elucidation of dedicated *TCF4* risk variant-associated endophenotypes and corresponding molecular mechanisms certainly represents the first steps towards this goal.

Conclusions

TCF4 is still one of the most promising schizophrenia risk genes, as it is slightly but replicably associated with the illness, is more strongly related to gating endophenotypes of schizophrenia, and seems to be susceptible to environmental impact, as discussed above. Moreover, it obviously plays an important role in brain development and is connected to the function of other genes such as *miR-137*, which are also discussed as schizophrenia risk genes. Taken together, a causal role of *TCF4* for schizophrenia would be in line with neurodevelopmental hypotheses as well as with repeated-hit models and G × E interaction models. Thus, *TCF4* as a schizophrenia risk gene would be versatile model that has the potential to integrate several schizophrenia models previously suggested. However, the small gene effects in large schizophrenia patient populations and the stronger gene effects regarding gating endophenotypes might indicate that there is a subgroup of patients in which TCF4 plays a major role during pathogenesis, while most of the patients have different pathogenic pathways. Thus, future research might accomplish the identification of a specific *TCF4*-lead schizophrenia by combining genotying and (endo)phenotyping. Finally, it is also conceivable that a combination of risk genes ranging from the TCF4 associated bHLH system, including interaction partners and target genes as well as associated regulatory mechanisms such as *miR-137* and possibly Ca^{2+} linked signalling networks, could represent a '*TCF4* gene set' regulating neuronal growth and differentiation in a highly redundant network. These genes might cooperatively be responsible for the pathogenesis within a subgroup of schizophrenia patients. Due to the high functional redundancy in this network, a critical mass of genetically and environmentally induced dysfunction is needed before the systems breaks down. Thus, we might focus on gene sets within the bHLH system and neighbouring regulatory systems to identify patients with a strong genetic and developmental pathogenesis. A postulated TCF4-associated network of risk factors might potentially be suitable as an early indicator for a schizophrenic subtype. When combined with electrophysiological gating measures (such as PPI or P50 suppression), smoking behaviour and cognitive performance, corresponding molecular profiling could guide future stratified sub-population-directed therapies.

Acknowledgments Boris B. Quednow was supported by the Swiss National Science Foundation (Grants No. PP00P1_123516

and PP00P1-146326). Magdalena M. Brzózka and Moritz J. Rossner were supported by grants of the Deutsche Forschungsgemeinschaft (CMPB and KFO 241: RO 4076/1-1). The authors declare no conflict of interests.

References

1. Massari ME, Murre C (2000) Helix-loop-helix proteins: regulators of transcription in eucaryotic organisms. Mol Cell Biol 20:429–440
2. Murre C, Bain G, van Dijk MA et al (1994) Structure and function of helix-loop-helix proteins. Biochim Biophys Acta 1218:129–135
3. Ferré-D'Amaré AR, Prendergast GC, Ziff EB, Burley SK (1993) Recognition by Max of its cognate DNA through a dimeric b/HLH/Z domain. Nature 363:38–45
4. Skinner MK, Rawls A, Wilson-Rawls J, Roalson EH (2010) Basic helix-loop-helix transcription factor gene family phylogenetics and nomenclature. Differentiation 80:1–8
5. Bertrand N, Castro DS, Guillemot F (2002) Proneural genes and the specification of neural cell types. Nat Rev Neurosci 3:517–530
6. Jones S (2004) An overview of the basic helix-loop-helix proteins. Genome Biol 5:226
7. Jin T, Liu L (2008) The Wnt signaling pathway effector TCF7L2 and type 2 diabetes mellitus. Mol Endocrinol 22:2383–2392
8. Ephrussi A, Church GM, Tonegawa S, Gilbert W (1985) B lineage—specific interactions of an immunoglobulin enhancer with cellular factors in vivo. Science 227:134–140
9. De Masi F, Grove CA, Vedenko A et al (2011) Using a structural and logics systems approach to infer bHLH-DNA binding specificity determinants. Nucleic Acids Res 39:4553–4563
10. Sepp M, Kannike K, Eesmaa A et al (2011) Functional diversity of human basic helix-loop-helix transcription factor TCF4 isoforms generated by alternative 5′ exon usage and splicing. PLoS ONE 6:e22138
11. Sepp M, Pruunsild P, Timmusk T (2012) Pitt-Hopkins syndrome-associated mutations in TCF4 lead to variable impairment of the transcription factor function ranging from hypomorphic to dominant-negative effects. Hum Mol Genet 21:2873–2888
12. Chiaramello A, Soosaar A, Neuman T, Zuber MX (1995) Differential expression and distinct DNA-binding specificity of ME1a and ME2 suggest a unique role during differentiation and neuronal plasticity. Brain Res Mol Brain Res 29:107–118
13. Soosaar A, Chiaramello A, Zuber MX, Neuman T (1994) Expression of basic-helix-loop-helix transcription factor ME2 during brain development and in the regions of neuronal plasticity in the adult brain. Brain Res Mol Brain Res 25:176–180
14. Zhuang Y, Kim CG, Bartelmez S et al (1992) Helix-loop-helix transcription factors E12 and E47 are not essential for skeletal or cardiac myogenesis, erythropoiesis, chondrogenesis, or neurogenesis. Proc Natl Acad Sci USA 89:12132–12136
15. Zhuang Y, Barndt RJ, Pan L et al (1998) Functional replacement of the mouse E2A gene with a human HEB cDNA. Mol Cell Biol 18:3340–3349
16. Caudy M, Vässin H, Brand M et al (1988) daughterless, a Drosophila gene essential for both neurogenesis and sex determination, has sequence similarities to myc and the achaete-scute complex. Cell 55:1061–1067
17. Krause M, Park M, Zhang JM et al (1997) A *C. elegans* E/Daughterless bHLH protein marks neuronal but not striated muscle development. Development 124:2179–2189
18. Portman DS, Emmons SW (2000) The basic helix-loop-helix transcription factors LIN-32 and HLH-2 function together in multiple steps of a *C. elegans* neuronal sublineage. Development 127:5415–5426
19. Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium (2011) Genome-wide association study identifies five new schizophrenia loci. Nat Genet 43:969–976
20. Xiang X, Zhuang X, Ju S et al (2011) miR-155 promotes macroscopic tumor formation yet inhibits tumor dissemination from mammary fat pads to the lung by preventing EMT. Oncogene 30:3440–3453
21. Li G, Luna C, Qiu J et al (2011) Role of miR-204 in the regulation of apoptosis, endoplasmic reticulum stress response, and inflammation in human trabecular meshwork cells. Invest Ophthalmol Vis Sci 52:2999–3007
22. Kwon E, Wang W, Tsai L-H (2013) Validation of schizophrenia-associated genes CSMD1, C10orf26, CACNA1C and TCF4 as miR-137 targets. Mol Psychiatry 18(11–12):23
23. Navarrete K, Pedroso I, De Jong S et al (2013) TCF4 (e2-2; ITF2): a schizophrenia-associated gene with pleiotropic effects on human disease. Am J Med Genet B 162:1–16
24. Aronheim A, Shiran R, Rosen A, Walker MD (1993) The E2A gene product contains two separable and functionally distinct transcription activation domains. Proc Natl Acad Sci USA 90:8063–8067
25. Quong MW, Massari ME, Zwart R, Murre C (1993) A new transcriptional-activation motif restricted to a class of helix-loop-helix proteins is functionally conserved in both yeast and mammalian cells. Mol Cell Biol 13:792–800
26. Massari ME, Grant PA, Pray-Grant MG et al (1999) A conserved motif present in a class of helix-loop-helix proteins activates transcription by direct recruitment of the SAGA complex. Mol Cell 4:63–73
27. Qiu Y, Sharma A, Stein R (1998) p300 mediates transcriptional stimulation by the basic helix-loop-helix activators of the insulin gene. Mol Cell Biol 18:2957–2964
28. Loe-Mie Y, Lepagnol-Bestel A-M, Maussion G et al (2010) SMARCA2 and other genome-wide supported schizophrenia-associated genes: regulation by REST/NRSF, network organization and primate-specific evolution. Hum Mol Genet 19:2841–2857
29. Forrest MP, Waite AJ, Martin-Rendon E, Blake DJ (2013) Knockdown of human TCF4 affects multiple signaling pathways involved in cell survival, epithelial to mesenchymal transition and neuronal differentiation. PLoS ONE 8:e73169
30. Tamminga CA, Holcomb HH (2005) Phenotype of schizophrenia: a review and formulation. Mol Psychiatry 10:27–39
31. Goldstein JM, Tsuang MT, Faraone SV (1989) Gender and schizophrenia: implications for understanding the heterogeneity of the illness. Psychiatry Res 28:243–253
32. Faraone SV, Chen WJ, Goldstein JM, Tsuang MT (1994) Gender differences in age at onset of schizophrenia. Br J Psychiatry 164:625–629
33. Bromet EJ, Fennig S (1999) Epidemiology and natural history of schizophrenia. Biol Psychiatry 46:871–881
34. Insel TR (2010) Rethinking schizophrenia. Nature 468:187–193
35. Van Os J, Kapur S (2009) Schizophrenia. Lancet 374:635–645
36. Holzman PS, Matthysse S (1990) The genetics of schizophrenia: a review. Psychol Sci 1:279–286
37. McGue M, Gottesman II (1991) The genetic epidemiology of schizophrenia and the design of linkage studies. Eur Arch Psychiatry Clin Neurosci 240:174–181
38. Harrison PJ, Owen MJ (2003) Genes for schizophrenia? Recent findings and their pathophysiological implications. Lancet 361:417–419

39. Harrison PJ, Weinberger DR (2005) Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry 10:40–68 image 5
40. Ng MYM, Levinson DF, Faraone SV et al (2009) Meta-analysis of 32 genome-wide linkage studies of schizophrenia. Mol Psychiatry 14:774–785
41. Williams HJ, Moskvina V, Smith RL et al (2011) Association between TCF4 and schizophrenia does not exert its effect by common nonsynonymous variation or by influencing cis-acting regulation of mRNA expression in adult human brain. Am J Med Genet B 156B:781–784
42. Williams HJ, Owen MJ, O'Donovan MC (2009) Schizophrenia genetics: new insights from new approaches. Br Med Bull 91:61–74
43. Sullivan PF, Daly MJ, O'Donovan M (2012) Genetic architectures of psychiatric disorders: the emerging picture and its implications. Nat Rev Genet 13:537–551
44. Lee KW, Woon PS, Teo YY, Sim K (2012) Genome wide association studies (GWAS) and copy number variation (CNV) studies of the major psychoses: what have we learnt? Neurosci Biobehav Rev 36:556–571
45. Harrison PJ (1999) The neuropathology of schizophrenia. A critical review of the data and their interpretation. Brain 122(Pt 4):593–624
46. Harrison PJ (2004) The hippocampus in schizophrenia: a review of the neuropathological evidence and its pathophysiological implications. Psychopharmacology 174:151–162
47. Jakob H, Beckmann H (1986) Prenatal developmental disturbances in the limbic allocortex in schizophrenics. J Neural Transm 65:303–326
48. Arnold SE, Hyman BT, Van Hoesen GW, Damasio AR (1991) Some cytoarchitectural abnormalities of the entorhinal cortex in schizophrenia. Arch Gen Psychiatry 48:625–632
49. Falkai P, Schneider-Axmann T, Honer WG (2000) Entorhinal cortex pre-alpha cell clusters in schizophrenia: quantitative evidence of a developmental abnormality. Biol Psychiatry 47:937–943
50. Harrison PJ, Eastwood SL (2001) Neuropathological studies of synaptic connectivity in the hippocampal formation in schizophrenia. Hippocampus 11:508–519
51. Honer WG, Young CE (2004) Presynaptic proteins and schizophrenia. Int Rev Neurobiol 59:175–199
52. Lewis DA (2000) GABAergic local circuit neurons and prefrontal cortical dysfunction in schizophrenia. Brain Res Brain Res Rev 31:270–276
53. Reynolds GP, Beasley CL, Zhang ZJ (2002) Understanding the neurotransmitter pathology of schizophrenia: selective deficits of subtypes of cortical GABAergic neurons. J Neural Transm 109:881–889
54. Bertolino A, Weinberger DR (1999) Proton magnetic resonance spectroscopy in schizophrenia. Eur J Radiol 30:132–141
55. Nudmamud S, Reynolds LM, Reynolds GP (2003) N-acetylaspartate and N-Acetylaspartylglutamate deficits in superior temporal cortex in schizophrenia and bipolar disorder: a postmortem study. Biol Psychiatry 53:1138–1141
56. Weinberger DR, Berman KF, Suddath R, Torrey EF (1992) Evidence of dysfunction of a prefrontal-limbic network in schizophrenia: a magnetic resonance imaging and regional cerebral blood flow study of discordant monozygotic twins. Am J Psychiatry 149:890–897
57. Friston KJ, Frith CD (1995) Schizophrenia: a disconnection syndrome? Clin Neurosci 3:89–97
58. McGlashan TH, Hoffman RE (2000) Schizophrenia as a disorder of developmentally reduced synaptic connectivity. Arch Gen Psychiatry 57:637–648
59. Mirnics K, Middleton FA, Lewis DA, Levitt P (2001) Analysis of complex brain disorders with gene expression microarrays: schizophrenia as a disease of the synapse. Trends Neurosci 24:479–486
60. Frankle WG, Lerma J, Laruelle M (2003) The synaptic hypothesis of schizophrenia. Neuron 39:205–216
61. Marenco S, Weinberger DR (2000) The neurodevelopmental hypothesis of schizophrenia: following a trail of evidence from cradle to grave. Dev Psychopathol 12:501–527
62. Miyamoto S, LaMantia AS, Duncan GE et al (2003) Recent advances in the neurobiology of schizophrenia. Mol Interv 3:27–39
63. Murray RM, O'Callaghan E, Castle DJ, Lewis SW (1992) A neurodevelopmental approach to the classification of schizophrenia. Schizophr Bull 18:319–332
64. Maynard TM, Sikich L, Lieberman JA, LaMantia AS (2001) Neural development, cell–cell signaling, and the "two-hit" hypothesis of schizophrenia. Schizophr Bull 27:457–476
65. Arnold SE (1999) Neurodevelopmental abnormalities in schizophrenia: insights from neuropathology. Dev Psychopathol 11:439–456
66. Impagnatiello F, Guidotti AR, Pesold C et al (1998) A decrease of reelin expression as a putative vulnerability factor in schizophrenia. Proc Natl Acad Sci USA 95:15718–15723
67. Guidotti A, Pesold C, Costa E (2000) New neurochemical markers for psychosis: a working hypothesis of their operation. Neurochem Res 25:1207–1218
68. Van Broeckhoven C, Verheyen G (1999) Report of the chromosome 18 workshop. Am J Med Genet 88:263–270
69. Potash JB, DePaulo JR Jr (2000) Searching high and low: a review of the genetics of bipolar disorder. Bipolar Disord 2:8–26
70. Lewis CM, Levinson DF, Wise LH et al (2003) Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: schizophrenia. Am J Hum Genet 73:34–48
71. Segurado R, Detera-Wadleigh SD, Levinson DF et al (2003) Genome scan meta-analysis of schizophrenia and bipolar disorder, part III: bipolar disorder. Am J Hum Genet 73:49–62
72. Pickard BS, Malloy MP, Clark L et al (2005) Candidate psychiatric illness genes identified in patients with pericentric inversions of chromosome 18. Psychiatr Genet 15:37–44
73. Cross-Disorder Group of the Psychiatric Genomics Consortium, Lee SH, Ripke S et al (2013) Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nat Genet 45:984–994
74. Breschel TS, McInnis MG, Margolis RL et al (1997) A novel, heritable, expanding CTG repeat in an intron of the SEF2-1 gene on chromosome 18q21.1. Hum Mol Genet 6:1855–1863
75. Del-Favero J, Gestel SV, Børglum AD et al (2002) European combined analysis of the CTG18.1 and the ERDA1 CAG/CTG repeats in bipolar disorder. Eur J Hum Genet 10:276–280
76. Stefansson H, Ophoff RA, Steinberg S et al (2009) Common variants conferring risk of schizophrenia. Nature 460:744–747
77. Steinberg S, de Jong S, Irish Schizophrenia Genomics Consortium et al (2011) Common variants at VRK2 and TCF4 conferring risk of schizophrenia. Hum Mol Genet 20:4076–4081
78. Li T, Li Z, Chen P et al (2010) Common variants in major histocompatibility complex region and TCF4 gene are significantly associated with schizophrenia in Han Chinese. Biol Psychiatry 68:671–673
79. Irish Schizophrenia Genomics Consortium and the Wellcome Trust Case Control Consortium 2 (2012) Genome-wide association study implicates HLA-C*01:02 as a risk factor at the major histocompatibility complex locus in schizophrenia. Biol Psychiatry 72:620–628

80. Aberg KA, Liu Y, Bukszár J et al (2013) A comprehensive family-based replication study of schizophrenia genes. JAMA Psychiatry 70:1–9
81. McClellan J, King M-C (2010) Genomic analysis of mental illness: a changing landscape. JAMA 303:2523–2524
82. Papiol S, Malzahn D, Kästner A et al (2011) Dissociation of accumulated genetic risk and disease severity in patients with schizophrenia. Transl Psychiatry 1:e45
83. Wirgenes KV, Sønderby IE, Haukvik UK et al (2012) TCF4 sequence variants and mRNA levels are associated with neurodevelopmental characteristics in psychotic disorders. Transl Psychiatry 2:e112
84. Lennertz L, Quednow BB, Benninghoff J et al (2011) Impact of TCF4 on the genetics of schizophrenia. Eur Arch Psychiatry Clin Neurosci 261(Suppl 2):S161–S165
85. Bleuler E (1911) Dementia praecox oder die Gruppe der Schizophrenien. In: Aschaffenburg G (ed) Handbuch der Psychiatrie, Spezieller Teil, 4. Abteilung, 1. Hälfte. Deutike, Leipzig
86. Kraepelin E (1909) Psychiatrie. Ein Lehrbuch für Studierende und Ärzte
87. Carlsson A (1995) Neurocircuitries and neurotransmitter interactions in schizophrenia. Int Clin Psychopharmacol 10(Suppl 3):21–28
88. Braff D, Stone C, Callaway E et al (1978) Prestimulus effects on human startle reflex in normals and schizophrenics. Psychophysiology 15:339–343
89. Braff DL, Grillon C, Geyer MA (1992) Gating and habituation of the startle reflex in schizophrenic patients. Arch Gen Psychiatry 49:206–215
90. Nuechterlein KH, Dawson ME (1984) Information processing and attentional functioning in the developmental course of schizophrenic disorders. Schizophr Bull 10:160–203
91. Nuechterlein KH, Dawson ME, Green MF (1994) Information-processing abnormalities as neuropsychological vulnerability indicators for schizophrenia. Acta Psychiatr Scand Suppl 384:71–79
92. Gottesman II, Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160:636–645
93. Braff DL, Light GA (2004) Preattentional and attentional cognitive deficits as targets for treating schizophrenia. Psychopharmacology 174:75–85
94. Braff DL, Light GA, Swerdlow NR (2007) Prepulse inhibition and P50 suppression are both deficient but not correlated in schizophrenia patients. Biol Psychiatry 61:1204–1207
95. Cadenhead KS, Carasso BS, Swerdlow NR et al (1999) Prepulse inhibition and habituation of the startle response are stable neurobiological measures in a normal male population. Biol Psychiatry 45:360–364
96. Cadenhead KS, Light GA, Geyer MA, Braff DL (2000) Sensory gating deficits assessed by the P50 event-related potential in subjects with schizotypal personality disorder. Am J Psychiatry 157:55–59
97. Cadenhead KS, Light GA, Shafer KM, Braff DL (2005) P50 suppression in individuals at risk for schizophrenia: the convergence of clinical, familial, and vulnerability marker risk assessment. Biol Psychiatry 57:1504–1509
98. Clementz BA, Geyer MA, Braff DL (1997) P50 suppression among schizophrenia and normal comparison subjects: a methodological analysis. Biol Psychiatry 41:1035–1044
99. Clementz BA, Geyer MA, Braff DL (1998) Poor P50 suppression among schizophrenia patients and their first-degree biological relatives. Am J Psychiatry 155:1691–1694
100. Patterson JV, Hetrick WP, Boutros NN et al (2008) P50 sensory gating ratios in schizophrenics and controls: a review and data analysis. Psychiatry Res 158:226–247
101. Anokhin AP, Golosheykin S, Heath AC (2007) Genetic and environmental influences on emotion-modulated startle reflex: a twin study. Psychophysiology 44:106–112
102. Anokhin AP, Heath AC, Myers E et al (2003) Genetic influences on prepulse inhibition of startle reflex in humans. Neurosci Lett 353:45–48
103. Anokhin AP, Vedeniapin AB, Heath AC et al (2007) Genetic and environmental influences on sensory gating of mid-latency auditory evoked responses: a twin study. Schizophr Res 89:312–319
104. Brockhaus-Dumke A, Schultze-Lutter F, Mueller R et al (2008) Sensory gating in schizophrenia: P50 and N100 gating in antipsychotic-free subjects at risk, first-episode, and chronic patients. Biol Psychiatry 64:376–384
105. Greenwood TA, Braff DL, Light GA et al (2007) Initial heritability analyses of endophenotypic measures for schizophrenia: the consortium on the genetics of schizophrenia. Arch Gen Psychiatry 64:1242–1250
106. Greenwood TA, Lazzeroni LC, Murray SS et al (2011) Analysis of 94 candidate genes and 12 endophenotypes for schizophrenia from the Consortium on the Genetics of Schizophrenia. Am J Psychiatry 168:930–946
107. Quednow BB, Frommann I, Berning J et al (2008) Impaired sensorimotor gating of the acoustic startle response in the prodrome of schizophrenia. Biol Psychiatry 64:766–773
108. Csomor PA, Stadler RR, Feldon J et al (2008) Haloperidol differentially modulates prepulse inhibition and p50 suppression in healthy humans stratified for low and high gating levels. Neuropsychopharmacology 33:497–512
109. Oranje B, Geyer MA, Bocker KBE et al (2006) Prepulse inhibition and P50 suppression: commonalities and dissociations. Psychiatry Res 143:147–158
110. Schwarzkopf SB, Lamberti JS, Smith DA (1993) Concurrent assessment of acoustic startle and auditory P50 evoked potential measures of sensory inhibition. Biol Psychiatry 33: 815–828
111. Allen AJ, Griss ME, Folley BS et al (2009) Endophenotypes in schizophrenia: a selective review. Schizophr Res 109: 24–37
112. Brzózka MM, Radyushkin K, Wichert SP et al (2010) Cognitive and sensorimotor gating impairments in transgenic mice overexpressing the schizophrenia susceptibility gene Tcf4 in the brain. Biol Psychiatry 68:33–40
113. Quednow BB, Ettinger U, Mössner R et al (2011) The schizophrenia risk allele C of the TCF4 rs9960767 polymorphism disrupts sensorimotor gating in schizophrenia spectrum and healthy volunteers. J Neurosci 31:6684–6691
114. Quednow BB, Brinkmeyer J, Mobascher A et al (2012) Schizophrenia risk polymorphisms in the TCF4 gene interact with smoking in the modulation of auditory sensory gating. Proc Natl Acad Sci USA 109:6271–6276
115. Dalack GW, Healy DJ, Meador-Woodruff JH (1998) Nicotine dependence in schizophrenia: clinical phenomena and laboratory findings. Am J Psychiatry 155:1490–1501
116. Kumari V, Postma P (2005) Nicotine use in schizophrenia: the self medication hypotheses. Neurosci Biobehav Rev 29:1021–1034
117. Korzyukov O, Pflieger ME, Wagner M et al (2007) Generators of the intracranial P50 response in auditory sensory gating. Neuroimage 35:814–826
118. Bak N, Glenthoj BY, Rostrup E et al (2011) Source localization of sensory gating: a combined EEG and fMRI study in healthy volunteers. Neuroimage 54:2711–2718
119. Brzózka MM, Rossner MJ (2013) Deficits in trace fear memory in a mouse model of the schizophrenia risk gene TCF4. Behav Brain Res 237:348–356

120. Rössler W, Hengartner MP, Angst J, Ajdacic-Gross V (2012) Linking substance use with symptoms of subclinical psychosis in a community cohort over 30 years. Addiction 107:1174–1184
121. Weiser M, Reichenberg A, Grotto I et al (2004) Higher rates of cigarette smoking in male adolescents before the onset of schizophrenia: a historical-prospective cohort study. Am J Psychiatry 161:1219–1223
122. Gur RE, Calkins ME, Gur RC et al (2007) The consortium on the genetics of schizophrenia: neurocognitive endophenotypes. Schizophr Bull 33:49–68
123. Heinrichs RW, Zakzanis KK (1998) Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. Neuropsychology 12:426–445
124. Cannon TD, Huttunen MO, Lonnqvist J et al (2000) The inheritance of neuropsychological dysfunction in twins discordant for schizophrenia. Am J Hum Genet 67:369–382
125. Van Erp TGM, Therman S, Pirkola T et al (2008) Verbal recall and recognition in twins discordant for schizophrenia. Psychiatry Res 159:271–280
126. Faraone SV, Seidman LJ, Kremen WS et al (2000) Neuropsychologic functioning among the nonpsychotic relatives of schizophrenic patients: the effect of genetic loading. Biol Psychiatry 48:120–126
127. Lennertz L, Rujescu D, Wagner M et al (2011) Novel schizophrenia risk gene TCF4 influences verbal learning and memory functioning in schizophrenia patients. Neuropsychobiology 63:131–136
128. Zhu X, Gu H, Liu Z et al (2013) Associations between TCF4 gene polymorphism and cognitive functions in schizophrenia patients and healthy controls. Neuropsychopharmacology 38:683–689
129. Albanna A, Choudhry Z, Harvey P-O et al (2013) TCF4 gene polymorphism and cognitive performance in patients with first episode psychosis. Schizophr Res. doi:10.1016/j.schres.2013.10.038
130. Kochunov P, Charlesworth J, Winkler A et al (2013) Transcriptomics of cortical gray matter thickness decline during normal aging. Neuroimage 82:273–283
131. Kim S, Cho H, Lee D, Webster MJ (2012) Association between SNPs and gene expression in multiple regions of the human brain. Transl Psychiatry 2:e113
132. Forrest M, Chapman RM, Doyle AM et al (2012) Functional analysis of TCF4 missense mutations that cause Pitt-Hopkins syndrome. Hum Mutat 33:1676–1686
133. De Pontual L, Mathieu Y, Golzio C et al (2009) Mutational, functional, and expression studies of the TCF4 gene in Pitt-Hopkins syndrome. Hum Mutat 30:669–676
134. Sweatt JD (2013) Pitt-Hopkins Syndrome: intellectual disability due to loss of TCF4-regulated gene transcription. Exp Mol Med 45:e21
135. Zweier C, Peippo MM, Hoyer J et al (2007) Haploinsufficiency of TCF4 causes syndromal mental retardation with intermittent hyperventilation (Pitt-Hopkins syndrome). Am J Hum Genet 80:994–1001
136. Takano K, Lyons M, Moyes C et al (2010) Two percent of patients suspected of having Angelman syndrome have TCF4 mutations. Clin Genet 78:282–288
137. Van Balkom IDC, Vuijk PJ, Franssens M et al (2012) Development, cognition, and behaviour in Pitt-Hopkins syndrome. Dev Med Child Neurol 54:925–931
138. Talkowski ME, Rosenfeld JA, Blumenthal I et al (2012) Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. Cell 149:525–537
139. Giurgea I, Missirian C, Cacciagli P et al (2008) TCF4 deletions in Pitt-Hopkins Syndrome. Hum Mutat 29:E242–E251
140. Marangi G, Ricciardi S, Orteschi D et al (2012) Proposal of a clinical score for the molecular test for Pitt-Hopkins syndrome. Am J Med Genet A 158A:1604–1611
141. Lehalle D, Williams C, Siu VM, Clayton-Smith J (2011) Fetal pads as a clue to the diagnosis of Pitt-Hopkins syndrome. Am J Med Genet A 155A:1685–1689
142. Losonczy MF, Song IS, Mohs RC et al (1986) Correlates of lateral ventricular size in chronic schizophrenia, I: behavioral and treatment response measures. Am J Psychiatry 143:976–981
143. Peippo MM, Simola KOJ, Valanne LK et al (2006) Pitt-Hopkins syndrome in two patients and further definition of the phenotype. Clin Dysmorphol 15:47–54
144. Neuman T, Keen A, Knapik E et al (1993) ME1 and GE1: basic helix-loop-helix transcription factors expressed at high levels in the developing nervous system and in morphogenetically active regions. Eur J Neurosci 5:311–318
145. Uittenbogaard M, Chiaramello A (2000) Differential expression patterns of the basic helix-loop-helix transcription factors during aging of the murine brain. Neurosci Lett 280:95–98
146. Ravanpay AC, Olson JM (2008) E protein dosage influences brain development more than family member identity. J Neurosci Res 86:1472–1481
147. Ishibashi M, Moriyoshi K, Sasai Y et al (1994) Persistent expression of helix-loop-helix factor HES-1 prevents mammalian neural differentiation in the central nervous system. EMBO J 13:1799–1805
148. Nakamura Y, Sakakibara S, Miyata T et al (2000) The bHLH gene hes1 as a repressor of the neuronal commitment of CNS stem cells. J Neurosci 20:283–293
149. Ross SE, Greenberg ME, Stiles CD (2003) Basic helix-loop-helix factors in cortical development. Neuron 39:13–25
150. Brockschmidt A, Filippi A, Charbel Issa P et al (2011) Neurologic and ocular phenotype in Pitt-Hopkins syndrome and a zebrafish model. Hum Genet 130:645–655
151. Flora A, Garcia JJ, Thaller C, Zoghbi HY (2007) The E-protein Tcf4 interacts with Math1 to regulate differentiation of a specific subset of neuronal progenitors. Proc Natl Acad Sci USA 104:15382–15387
152. Zhuang Y, Cheng P, Weintraub H (1996) B-lymphocyte development is regulated by the combined dosage of three basic helix-loop-helix genes, E2A, E2-2, and HEB. Mol Cell Biol 16:2898–2905
153. Bergqvist I, Eriksson M, Saarikettu J et al (2000) The basic helix-loop-helix transcription factor E2-2 is involved in T lymphocyte development. Eur J Immunol 30:2857–2863
154. Guella I, Sequeira A, Rollins B et al (2013) Analysis of miR-137 expression and rs1625579 in dorsolateral prefrontal cortex. J Psychiatr Res 47(9):1215–1221
155. Brennand KJ, Simone A, Jou J et al (2011) Modelling schizophrenia using human induced pluripotent stem cells. Nature 473:221–225
156. Quintana J, Wong T, Ortiz-Portillo E et al (2004) Anterior cingulate dysfunction during choice anticipation in schizophrenia. Psychiatry Res 132:117–130
157. Sigurdsson T, Stark KL, Karayiorgou M et al (2010) Impaired hippocampal-prefrontal synchrony in a genetic mouse model of schizophrenia. Nature 464:763–767
158. Meyer-Lindenberg AS, Olsen RK, Kohn PD et al (2005) Regionally specific disturbance of dorsolateral prefrontal-hippocampal functional connectivity in schizophrenia. Arch Gen Psychiatry 62:379–386
159. Wolf RC, Vasic N, Sambataro F et al (2009) Temporally anticorrelated brain networks during working memory performance reveal aberrant prefrontal and hippocampal connectivity in patients with schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 33:1464–1473

160. Braff DL (2011) Gating in schizophrenia: from genes to cognition (to real world function?). Biol Psychiatry 69:395–396
161. Braff DL, Geyer MA (1990) Sensorimotor gating and schizophrenia. Human and animal model studies. Arch Gen Psychiatry 47:181–188
162. Quednow BB, Kühn K-U, Beckmann K et al (2006) Attenuation of the prepulse inhibition of the acoustic startle response within and between sessions. Biol Psychol 71:256–263
163. Guillemot F (2007) Spatial and temporal specification of neural fates by transcription factor codes. Development 134:3771–3780
164. Lin C-H, Hansen S, Wang Z et al (2005) The dosage of the neuroD2 transcription factor regulates amygdala development and emotional learning. Proc Natl Acad Sci USA 102:14877–14882
165. Corneliussen B, Holm M, Waltersson Y et al (1994) Calcium/calmodulin inhibition of basic-helix-loop-helix transcription factor domains. Nature 368:760–764
166. Hauser J, Sveshnikova N, Wallenius A et al (2008) B-cell receptor activation inhibits AID expression through calmodulin inhibition of E-proteins. Proc Natl Acad Sci USA 105:1267–1272
167. Hauser J, Saarikettu J, Grundström T (2008) Calcium regulation of myogenesis by differential calmodulin inhibition of basic helix-loop-helix transcription factors. Mol Biol Cell 19:2509–2519
168. Onions J, Hermann S, Grundström T (1997) Basic helix-loop-helix protein sequences determining differential inhibition by calmodulin and S-100 proteins. J Biol Chem 272:23930–23937
169. Saarikettu J, Sveshnikova N, Grundström T (2004) Calcium/calmodulin inhibition of transcriptional activity of E-proteins by prevention of their binding to DNA. J Biol Chem 279:41004–41011
170. Bading H (2013) Nuclear calcium signalling in the regulation of brain function. Nat Rev Neurosci 14:593–608
171. Greer PL, Greenberg ME (2008) From synapse to nucleus: calcium-dependent gene transcription in the control of synapse development and function. Neuron 59:846–860
172. Cross-Disorder Group of the Psychiatric Genomics Consortium, Smoller JW, Craddock N et al (2013) Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet 381:1371–1379
173. Ferreira MAR, O'Donovan MC, Meng YA et al (2008) Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. Nat Genet 40:1056–1058
174. Psychiatric GWAS Consortium Bipolar Disorder Working Group (2011) Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. Nat Genet 43:977–983
175. Green EK, Grozeva D, Jones I et al (2010) The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. Mol Psychiatry 15:1016–1022
176. Thimm M, Kircher T, Kellermann T et al (2011) Effects of a CACNA1C genotype on attention networks in healthy individuals. Psychol Med 41:1551–1561
177. Erk S, Meyer-Lindenberg A, Schnell K et al (2010) Brain function in carriers of a genome-wide supported bipolar disorder variant. Arch Gen Psychiatry 67:803–811
178. Gomez-Ospina N, Tsuruta F, Barreto-Chang O et al (2006) The C terminus of the L-type voltage-gated calcium channel CaV1.2 encodes a transcription factor. Cell 127:591–606
179. Aizawa H, Hu S-C, Bobb K et al (2004) Dendrite development regulated by CREST, a calcium-regulated transcriptional activator. Science 303:197–202
180. Gaudillière B, Konishi Y, de la Iglesia N et al (2004) A CaMKII-NeuroD signaling pathway specifies dendritic morphogenesis. Neuron 41:229–241
181. Ince-Dunn G, Hall BJ, Hu S-C et al (2006) Regulation of thalamocortical patterning and synaptic maturation by NeuroD2. Neuron 49:683–695
182. Wilke SA, Hall BJ, Antonios JK et al (2012) NeuroD2 regulates the development of hippocampal mossy fiber synapses. Neural Dev 7:9
183. Wright C, Turner JA, Calhoun VD, Perrone-Bizzozero N (2013) Potential impact of miR-137 and its targets in schizophrenia. Front Genet 4:58
184. Smrt RD, Szulwach KE, Pfeiffer RL et al (2010) MicroRNA miR-137 regulates neuronal maturation by targeting ubiquitin ligase mind bomb-1. Stem Cells 28:1060–1070
185. Szulwach KE, Li X, Smrt RD et al (2010) Cross talk between microRNA and epigenetic regulation in adult neurogenesis. J Cell Biol 189:127–141
186. Berrettini W, Yuan X, Tozzi F et al (2008) Alpha-5/alpha-3 nicotinic receptor subunit alleles increase risk for heavy smoking. Mol Psychiatry 13:368–373
187. Bierut LJ, Stitzel JA, Wang JC et al (2008) Variants in nicotinic receptors and risk for nicotine dependence. Am J Psychiatry 165:1163–1171
188. Caporaso N, Gu F, Chatterjee N et al (2009) Genome-wide and candidate gene association study of cigarette smoking behaviors. PLoS ONE 4:e4653
189. Liu JZ, Tozzi F, Waterworth DM et al (2010) Meta-analysis and imputation refines the association of 15q25 with smoking quantity. Nat Genet 42:436–440
190. Saccone NL, Saccone SF, Hinrichs AL et al (2009) Multiple distinct risk loci for nicotine dependence identified by dense coverage of the complete family of nicotinic receptor subunit (CHRN) genes. Am J Med Genet B 150B:453–466
191. Petrovsky N, Ettinger U, Kessler H et al (2013) The effect of nicotine on sensorimotor gating is modulated by a CHRNA3 polymorphism. Psychopharmacology (Berl) 229(1):31–40
192. Winterer G, Mittelstrass K, Giegling I et al (2010) Risk gene variants for nicotine dependence in the CHRNA5-CHRNA3-CHRNB4 cluster are associated with cognitive performance. Am J Med Genet B 153B:1448–1458
193. Ohlrogge M, Doucet JR, Ryugo DK (2001) Projections of the pontine nuclei to the cochlear nucleus in rats. J Comp Neurol 436:290–303
194. Winer JA (2006) Decoding the auditory corticofugal systems. Hear Res 212:1–8
195. Woolf NJ, Butcher LL (1989) Cholinergic systems in the rat brain: IV. Descending projections of the pontomesencephalic tegmentum. Brain Res Bull 23:519–540
196. Erwin RJ, Buchwald JS (1987) Midlatency auditory evoked responses in the human and the cat model. Electroencephalogr Clin Neurophysiol Suppl 40:461–467
197. Fendt M, Li L, Yeomans JS (2001) Brain stem circuits mediating prepulse inhibition of the startle reflex. Psychopharmacology 156:216–224
198. Harrison JB, Woolf NJ, Buchwald JS (1990) Cholinergic neurons of the feline pontomesencephalon. I. Essential role in "Wave A" generation. Brain Res 520:43–54
199. Reese NB, Garcia-Rill E, Skinner RD (1995) Auditory input to the pedunculopontine nucleus: I. Evoked potentials. Brain Res Bull 37:257–264
200. Morley BJ (2005) Nicotinic cholinergic intercellular communication: implications for the developing auditory system. Hear Res 206:74–88
201. Alkondon M, Pereira EF, Almeida LE et al (2000) Nicotine at concentrations found in cigarette smokers activates and desensitizes nicotinic acetylcholine receptors in CA1 interneurons of rat hippocampus. Neuropharmacology 39: 2726–2739

202. Picciotto MR, Caldarone BJ, Brunzell DH et al (2001) Neuronal nicotinic acetylcholine receptor subunit knockout mice: physiological and behavioral phenotypes and possible clinical implications. Pharmacol Ther 92:89–108
203. Besson M, Granon S, Mameli-Engvall M et al (2007) Long-term effects of chronic nicotine exposure on brain nicotinic receptors. Proc Natl Acad Sci USA 104:8155–8160
204. Breese CR, Lee MJ, Adams CE et al (2000) Abnormal regulation of high affinity nicotinic receptors in subjects with schizophrenia. Neuropsychopharmacology 23:351–364
205. Freedman R, Olincy A, Ross RG et al (2003) The genetics of sensory gating deficits in schizophrenia. Curr Psychiatry Rep 5:155–161
206. Satta R, Maloku E, Zhubi A et al (2008) Nicotine decreases DNA methyltransferase 1 expression and glutamic acid decarboxylase 67 promoter methylation in GABAergic interneurons. Proc Natl Acad Sci USA 105:16356–16361
207. Philibert RA, Beach SRH, Gunter TD et al (2010) The effect of smoking on MAOA promoter methylation in DNA prepared from lymphoblasts and whole blood. Am J Med Genet B 153B:619–628
208. Whalen S, Héron D, Gaillon T et al (2012) Novel comprehensive diagnostic strategy in Pitt-Hopkins syndrome: clinical score and further delineation of the TCF4 mutational spectrum. Hum Mutat 33:64–72