Impact of TCF4 on the genetics of schizophrenia

Lennertz, L ; Quednow, Boris B ; Benninghoff, J ; Wagner, M ; Maier, W ; Mössner, R

Abstract: Mutations of the transcription factor 4 (TCF4) gene cause mental retardation with or without associated facial dysmorphisms and intermittent hyperventilation. Subsequently, a polymorphism of TCF4 was shown in a genome-wide association study to slightly increase the risk of schizophrenia. We have further analysed the impact of this TCF4 variant rs9960767 on early information processing and cognitive functions in schizophrenia patients. We have shown in a sample of 401 schizophrenia patients that TCF4 influences verbal memory in the Rey Auditory Verbal Learning Test. Contrary to expectations, carriers of the schizophrenia-associated allele showed better recognition, thus indicating that while TCF4 influences verbal memory, the TCF4-mediated schizophrenia risk is not determined by the influence of TCF4 on verbal memory. TCF4 does not impact on various other cognitive functions belonging to the domains of attention and executive functions. Moreover, in a pharmacogenetic approach, TCF4 does not modulate the improvement of positive or negative schizophrenia symptoms during treatment with antipsychotics. Finally, we have assessed a key electrophysiological endophenotype of schizophrenia, sensorimotor gating. As measured by prepulse inhibition, the schizophrenia risk allele C of TCF4 rs9960767 reduces sensorimotor gating. This indicates that TCF4 influences key mechanisms of information processing, which may contribute to the pathogenesis of schizophrenia.

DOI: https://doi.org/10.1007/s00406-011-0256-9

Impact of TCF4 on the genetics of schizophrenia

Leonhard Lennertz 1*, Boris B. Quednow 2*, Jens Benninghoff 3, Michael Wagner 1, Wolfgang Maier 1, Rainald Mössner 1

* L.L. and B.B.Q. contributed equally to this work

1 Department of Psychiatry and Psychotherapy, University of Bonn, Bonn, Germany
2 University Hospital of Psychiatry, Division of Experimental and Clinical Pharmacopsychology, University of Zurich, Switzerland
3 Department of Psychiatry, University of Duisburg-Essen, Essen, Germany

Corresponding Author:
PD Dr. Rainald Mössner
Department of Psychiatry and Psychotherapy
University of Bonn
Sigmund-Freud-Str. 25
53105 Bonn
Germany
email: rainald.moessner@ukb.uni-bonn.de

Keywords: schizophrenia, TCF4, PPI

Abstract:

Mutations of the transcripton factor 4 (TCF4) gene cause mental retardation with or without associated facial dysmorphisms and intermittent hyperventilation. Subsequently, a polymorphism of TCF4 was shown in a genome-wide association study to slightly increase the risk for schizophrenia. We have further analysed the impact of this TCF4 variant rs9960767 on early information processing and cognitive functions in schizophrenia patients. We have shown in a sample of 401 schizophrenia patients that TCF4 influences verbal memory in the Rey Auditory Verbal Learning Test. Contrary to expectations, carriers of the schizophrenia-associated allele showed better recognition, thus indicating that while TCF4 influences verbal memory, the TCF4-mediated schizophrenia risk is not determined by the influence of TCF4 on verbal memory. TCF4 does not impact on various other cognitive functions belonging to the domains of attention and executive functions. Moreover, in a pharmacogenetic approach, TCF4 does not modulate the improvement of positive or negative schizophrenia symptoms during treatment with antipsychotics. Finally, we have assessed a key electrophysiological endophenotype of schizophrenia, sensorimotor gating. As measured by prepulse inhibition, the schizophrenia risk allele C of TCF4 rs9960767 reduces sensorimotor gating. This indicates that TCF4 influences key mechanisms of information processing which may contribute to the pathogenesis of schizophrenia.

Introduction

The elucidation of the genetic causes of schizophrenia is progressing rapidly. In the present review, we want to focus on the gene named transcription factor 4 (TCF4). In 2007, disruption of the TCF4 gene was shown to cause Pitt-Hopkins syndrome [1, 4, 40]. Pitt-Hopkins syndrome was described in 1978 as a syndrome of mental retardation, microcephaly, facial dysmorphisms, and intermittent hyperventilation [25]. Seizures also frequently occur in the syndrome [33]. In 2008, it was shown that a mutation of TCF4 may also cause mental retardation without the associated features of Pitt-Hopkins syndrome, i.e. without hyperventilation or seizures [18]. This established TCF4 as a cause of non-syndromal mental retardation. In the following year, a large genome-wide association study showed that a common intronic variant of TCF4 (rs9960767) leads to a slight increase in the risk of schizophrenia, with a relative risk of 1.23 [35]. Here, we discuss further findings on cognitive and electrophysiological endophenotypes of schizophrenia to further explore the mechanisms how TCF4 is involved in the development of schizophrenia.

TCF4 and cognitive endophenotypes of schizophrenia

A role of the TCF4 gene in neuropsychological functioning can be assumed following different findings: First, TCF4 may be essential for normal brain development as has been demonstrated in a study conducted by Flora et al. [13]. In this study, an interaction of TCF4 and

MATH1, a proneural protein, on different neural progenitor populations was investigated and disrupted pontine nucleus development was found in TCF4 knock out mice. Second, several studies showed that TCF4 haploinsufficiency contributes to severe neurodevelopmental disorders such as the Pitt-Hopkins syndrome [1, 4, 9, 33, 40]. This autosomal dominant encephalopathy is characterized by severe mental retardation, microcephaly, disrupted motor development, and hyperventilation, as described above [25]. Third, animal studies showed that TCF4 knock-out mice die within 24 hours after birth [13]. However, a recent animal study investigated transgenic mice mildly overexpressing TCF4 in the brain postnatally which allowed studying their behavioural phenotype [5]. As compared to their wildtype littermates, the TCF4 transgenic mice showed less freezing in a fear conditioning paradigm suggesting deficient fear-related learning and memory. Moreover, the mutant mice in this study also showed disrupted PPI (see below). Together these findings in humans and animals argue for a potential role of TCF4 in neurofunctional disruptions ranging from non-viable (as seen in animal TCF4 null mutants) to severe mental retardation (as seen in Pitt Hopkins syndrome caused by TCF4 haploinsufficiency) to mild cognitive impairments (as seen in a girl carrying a de novo translocation with disruption of TCF4 presenting with mild mental retardation) [18].
Partly based on these findings, we sought to examine the role of the common TCF4 schizophrenia risk variant rs9960767 on verbal memory in a sample of schizophrenia patients [21]. Impaired verbal memory is among the most prominent cognitive deficits of schizophrenia [10]. Moreover, studies examining unaffected relatives from multiple affected families (“multiplex families”) and twin studies emphasize the role of verbal memory as a promising endophenotype of schizophrenia. These

highly informative study designs reliably demonstrate an increasing memory deficit along with the increasing genetic load [8, 11, 38].
In our study [21], we investigated a sample of 401 schizophrenia patients, all of whom were genotyped for the rs9960767 variant and completed a neuropsychological verbal memory test. Verbal memory was assessed using the Rey Auditory Verbal Memory Test (RAVLT, [17]). In addition, verbal intelligence as well as age, gender, age at onset, duration of illness, medication type (typical vs. atypical neuroleptics), and DSM-IV schizophrenia subtype were also analysed as control variables. Comparing schizophrenia patients carrying the TCF4 C-Allele (risk allele) against patients with an AA genotype yielded no significant effects for any of the control variables including verbal intelligence (all $p > .20$). No effect of the C-allele on immediate recall and total learning was found. A trend finding for delayed verbal memory emerged, which however indicated better performance in carriers of the risk variant compared to non-carriers. With regard to recognition, schizophrenia patients carrying at least one C-allele significantly recognized more words compared to patients without the risk variant. We also explored functional effects of the TCF4 variant rs9960767 on a comprehensive neuropsychological test battery in a subsample of nearly 200 schizophrenia patients and an additional sample of 205 healthy volunteers (unpublished data). No differences between TCF4 C-allele carriers or subjects with AA-genotype were found with regard to age, gender, and verbal intelligence in both groups. The assessed cognitive functions attention and vigilance, working memory, processing speed, visuo-motor speed and set-shifting, and verbal fluency were all unaffected by rs9960767 in both groups (unpublished data). Of note, although TCF4 mutations are clearly related to severely disrupted intellectual functions, no effect of the rs9960767

polymorphism on verbal intelligence or any other neuropsychological function was evident in our sample [21].
Thus, different arguments emphasize a role of TCF4 in neuropsychological functions. While mutations of this gene lead to severe cognitive deficits, also milder cognitive alterations due to common variants within the TCF4 gene or due to changes at the transcript level were observed. Our study showed that the common TCF4 variant rs9960767 exerts an effect on recognition memory. Brzózka et al. investigated mice mildly over-expressing TCF4 and found reduced fear-related memory and difficulties to unlearn a previously rewarded spatial locus [5]. Both studies differ with regard to investigated subjects (mice versus schizophrenia patients), materials (fear conditioning and spatial memory versus verbal declarative memory), and assessed genetic variability (transgenic mice versus single nucleotide polymorphism) which makes them difficult to relate to each other. However, both studies suggest a role of TCF4 in memory functions. Thus they support the notion that not only TCF4 mutations but also more subtle genetic variation affects neuropsychological functions.

TCF4 and sensorimotor gating

Prepulse inhibition (PPI) of the acoustic startle response (ASR) has been firmly established as an operational measure of sensorimotor gating [3]. PPI is defined as a strong reduction of the amplitude of the startle response that occurs when a distinctive non-startling stimulus is presented 30-500 ms prior to the startling stimulus [15]. It was proposed that the mechanism underlying PPI regulates sensory input by filtering out irrelevant or distracting stimuli in order to prevent sensory information overflow and to allow for selective and efficient processing of relevant

information [36]. Given that PPI was shown for visual, electric, and auditory stimuli (also for a cross modal combination of different stimuli types) and that PPI is measurable in several species ranging from mollusks and fish to higher mammals such as rodents, non-human primates, and humans, it is thought to reflect a fundamental mechanism of preattentional information processing (for review see [26]). Diminished PPI has been consistently demonstrated in patients with schizophrenia [3, 20, 22, 23, 32] and schizotypal personality disorder [6, 7]. The PPI deficit in schizophrenia is supposed to reflect a central abnormality underlying the disease; both neuroanatomical and neurochemical factors have been implicated on the basis of animal studies, which suggest contributions of diverse neurotransmitter systems, and particular functional association with multiple loci in the cortico-striato-pallido-thalamic (CSPT) circuitry, frontal and mediotemporal regions, ventral striatum, ventral pallidum, and pontine regions of the brainstem [12, 37]. Furthermore, PPI is heritable [2, 16], decreased in unaffected relatives of schizophrenia patients [7, 19], affected by SNPs within the dopamine, acetylcholine, and serotonin system [24, 29-31, 34] and already reduced during the prodromal stage of schizophrenia [28, 39], suggesting that PPI is an important and valid candidate as an intermediate or endophenotypic marker in genetic studies of schizophrenia [14].

In two independent samples, we recently demonstrated that the schizophrenia risk allele C of the *TCF4* rs9960767 SNP is strongly associated with reduced sensorimotor gating [27]. In accordance with recent animal data, showing that transgenic mice overexpressing the TCF4 gene in the brain display decreased sensorimotor gating [5], this finding suggests that TCF4 plays an important role in the development of early information deficits in schizophrenia at least in a subgroup of

patients who display diminished PPI. It was recently shown in TCF4 knock-out mice that TCF4 plays a unique role especially in the development of the pontine nuclei [13], which are highly connected with other brain stem nuclei that are critical core regions within the CSPP circuitry processing PPI of ASR [12, 37]. Although the influence of pontine nuclei on PPI has not been directly studied so far, one might assume that developmental changes in these regions caused by TCF4 mutations are possibly associated with functional alterations of interconnections to adjacent brain stem nuclei as well as of the cortico-ponto-cerebellar integration of sensorimotor information. This assumption is also supported by the fact that PPI was strongly affected by TCF4 genotype across the entire range of SOA conditions – from the "preconscious" 30 ms SOA to the "conscious" 120 SOA. This pattern suggests that TCF4 genotype probably influences PPI at an early level of information processing. Finally, given that TCF4 genotype was significantly associated with PPI reduction, a combination of TCF4 genotype and a PPI deficit syndrome might be a promising marker for the early detection of schizophrenia [27].

Pharmacogenetics of TCF4

To assess whether TCF4 influences the antipsychotic drug response in schizophrenia, we have analysed the TCF4 polymorphism rs9960767 in two independent samples of schizophrenia patients comprising more than 200 patients in total. The schizophrenia patients were admitted to hospital due to an exacerbation of psychotic symptoms. TCF4 rs9960767 was genotyped as described previously [21]. We assessed the

improvement of the PANSS scale and subscales during neuroleptic treatment over four weeks. We did not find a significant influence of TCF4 rs9960767 on the improvement of positive symptoms, negative symptoms, general psychopathology, or total PANSS score. Bonferroni correction was employed to control for multiple testing. The findings argue against an important pharmacogenetic role of TCF4 in the antipsychotic drug response of schizophrenia patients.

Conflicts of interest: None

Acknowledgements

This work was supported by the European Union (Grant FP7-Health-F4-2009-242257-ADAMS), by the German Federal Ministry for Education and Research (BMBF) (Grants POSITIVE 01GV0907, NGFN MooDs PNM-01GS08146-3), and by the DFG (Grant WA 737/7). Dr. Quednow is supported by the Swiss National Science Foundation (Grant No. PP00P1 123516). We thank V. Guttenthaler and A. Petruschke for excellent technical assistance.

References

1. Amiel J, Rio M, de Pontual L, Redon R, Malan V, Boddaert N, Plouin P, Carter NP, Lyonnet S, Munnich A, Colleaux L (2007) Mutations in TCF4, encoding a class I basic helix-loop-helix transcription factor, are responsible for Pitt-Hopkins syndrome, a severe epileptic encephalopathy associated with autonomic dysfunction. Am J Hum Genet 80:988-993
2. Anokhin AP, Heath AC, Myers E, Ralano A, Wood S (2003) Genetic influences on prepulse inhibition of startle reflex in humans. Neurosci Lett 353:45-48
3. Braff DL, Grillon C, Geyer MA (1992) Gating and habituation of the startle reflex in schizophrenic patients. Arch Gen Psychiatry 49:206-215

4. Brockschmidt A, et a (2007) Severe mental retardation with breathing abnormalities (Pitt-Hopkins syndrome) is caused by haploinsufficiency of the neuronal bHLH transcription factor TCF4. Hum Mol Genet 16:1488-1494
5. Brzozka MM, Radyushkin K, Wichert SP, Ehrenreich H, Rossner MJ (2010) Cognitive and sensorimotor gating impairments in transgenic mice overexpressing the schizophrenia susceptibility gene Tcf4 in the brain. Biol Psychiatry 68:33-40
6. Cadenhead KS, Geyer MA, Braff DL (1993) Impaired startle prepulse inhibition and habituation in patients with schizotypal personality disorder. Am J Psychiatry 150:1862-1867
7. 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
8. Cannon TD, Huttunen MO, Lonnqvist J, Tuulio-Henriksson A, Pirkola T, Glahn D, Finkelstein J, Hietanen M, Kaprio J, Koskenvuo M (2000) The inheritance of neuropsychological dysfunction in twins discordant for schizophrenia. Am J Hum Genet 67:369-382
9. de Pontual L, et a (2009) Mutational, functional, and expression studies of the TCF4 gene in Pitt-Hopkins syndrome. Hum Mutat 30:669-676
10. Dickinson D, Ramsey ME, Gold JM (2007) Overlooking the obvious: a meta-analytic comparison of digit symbol coding tasks and other cognitive measures in schizophrenia. Arch Gen Psychiatry 64:532-542
11. Faraone SV, Seidman LJ, Kremen WS, Toomey R, Pepple JR, Tsuang MT (2000) Neuropsychologic functioning among the nonpsychotic relatives of schizophrenic patients: the effect of genetic loading. Biol Psychiatry 48:120-126
12. Fendt M, Li L, Yeomans JS (2001) Brain stem circuits mediating prepulse inhibition of the startle reflex. Psychopharmacology (Berl) 156:216-224
13. 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 U S A 104:15382-15387
14. Gottesman, II, Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160:636-645
15. Graham FK (1975) Presidential Address, 1974. The more or less startling effects of weak prestimulation. Psychophysiology 12:238-248
16. Greenwood TA, al. e (2007) Initial heritability analyses of endophenotypic measures for schizophrenia: the consortium on the genetics of schizophrenia. Arch Gen Psychiatry 64:1242-1250
17. Helmstaedter C, Lendt M, Lux S (2001) VLMT - Verbaler Lern- und Merkfähigkeitstest Manual. Beltz, Göttingen
18. Kalscheuer VM, Feenstra I, Van Ravenswaaij-Arts CM, Smeets DF, Menzel C, Ullmann R, Musante L, Ropers HH (2008) Disruption of the TCF4 gene in a girl with mental retardation but without the classical Pitt-Hopkins syndrome. Am J Med Genet A 146A:2053-2059
19. Kumari V, Das M, Zachariah E, Ettinger U, Sharma T (2005) Reduced prepulse inhibition in unaffected siblings of schizophrenia patients. Psychophysiology 42:588-594
20. Kumari V, Soni W, Mathew VM, Sharma T (2000) Prepulse inhibition of the startle response in men with schizophrenia: effects of age of onset of illness, symptoms, and medication. Arch Gen Psychiatry 57:609-614
21. Lennertz L, Rujescu D, Wagner M, Frommann I, Schulze-Rauschenbach S, Schuhmacher A, Landsberg MW, Franke P, Moller HJ, Wolwer W, Gaebel W, Hafner H, Maier W, Mössner R (2011) Novel schizophrenia risk gene TCF4 influences verbal

learning and memory functioning in schizophrenia patients. Neuropsychobiology 63:131-136
22. Ludewig K, Geyer MA, Vollenweider FX (2003) Deficits in prepulse inhibition and habituation in never-medicated, first-episode schizophrenia. Biol Psychiatry 54:121-128
23. Parwani A, Duncan EJ, Bartlett E, Madonick SH, Efferen TR, Rajan R, Sanfilipo M, Chappell PB, Chakravorty S, Gonzenbach S, Ko GN, Rotrosen JP (2000) Impaired prepulse inhibition of acoustic startle in schizophrenia. Biol Psychiatry 47:662-669
24. Petrovsky N, Quednow BB, Ettinger U, Schmechtig A, Mossner R, Collier DA, Kuhn KU, Maier W, Wagner M, Kumari V (2010) Sensorimotor gating is associated with CHRNA3 polymorphisms in schizophrenia and healthy volunteers. Neuropsychopharmacology 35:1429-1439
25. Pitt D, Hopkins I (1978) A syndrome of mental retardation, wide mouth and intermittent overbreathing. Aust Paediatr J 14:182-184
26. Quednow BB (2008) Sensorimotor gating deficits in psychiatric disorders. Zeitschrift für Neuropsychologie 19:139-163
27. Quednow BB, Ettinger U, Mossner R, Rujescu D, Giegling I, Collier DA, Schmechtig A, Kuhn KU, Moller HJ, Maier W, Wagner M, Kumari V (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
28. Quednow BB, Frommann I, Berning J, Kuhn KU, Maier W, Wagner M (2008) Impaired sensorimotor gating of the acoustic startle response in the prodrome of schizophrenia. Biol Psychiatry 64:766-773
29. Quednow BB, Kuhn KU, Mössner R, Schwab SG, Schuhmacher A, Maier W, Wagner M (2008) Sensorimotor gating of schizophrenia patients is influenced by 5-HT2A receptor polymorphisms. Biol Psychiatry 64:434-437
30. Quednow BB, Schmechtig A, Ettinger U, Petrovsky N, Collier DA, Vollenweider FX, Wagner M, Kumari V (2009) Sensorimotor gating depends on polymorphisms of the serotonin-2A receptor and catechol-O-methyltransferase, but not on neuregulin-1 Arg38Gln genotype: a replication study. Biol Psychiatry 66:614-620
31. Quednow BB, Wagner M, Mössner R, Maier W, Kuhn KU (2010) Sensorimotor gating of schizophrenia patients depends on Catechol O-methyltransferase Val158Met polymorphism. Schizophr Bull 36:341-346
32. Quednow BB, Wagner M, Westheide J, Beckmann K, Bliesener N, Maier W, Kuhn KU (2006) Sensorimotor gating and habituation of the startle response in schizophrenic patients randomly treated with amisulpride or olanzapine. Biol Psychiatry 59:536-545
33. Rosenfeld JA, et a (2009) Genotype-phenotype analysis of TCF4 mutations causing Pitt-Hopkins syndrome shows increased seizure activity with missense mutations. Genet Med 11:797-805
34. Roussos P, Giakoumaki SG, Rogdaki M, Pavlakis S, Frangou S, Bitsios P (2008) Prepulse inhibition of the startle reflex depends on the catechol O-methyltransferase Val158Met gene polymorphism. Psychol Med 38:1651-1658
35. Stefansson H, et a (2009) Common variants conferring risk of schizophrenia. Nature 460:744-747
36. Swerdlow NR, Geyer MA (1998) Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. Schizophr Bull 24:285-301
37. Swerdlow NR, Geyer MA, Braff DL (2001) Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. Psychopharmacology (Berl) 156:194-215

38. van Erp TG, Therman S, Pirkola T, Tuulio-Henriksson A, Glahn DC, Bachman P, Huttunen MO, Lonnqvist J, Hietanen M, Kaprio J, Koskenvuo M, Cannon TD (2008) Verbal recall and recognition in twins discordant for schizophrenia. Psychiatry Res 159:271-280
39. Ziermans T, Schothorst P, Magnee M, van Engeland H, Kemner C (2011) Reduced prepulse inhibition in adolescents at risk for psychosis: a 2-year follow-up study. J Psychiatry Neurosci 36:127-134
40. Zweier C, et a (2007) Haploinsufficiency of TCF4 causes syndromal mental retardation with intermittent hyperventilation (Pitt-Hopkins syndrome). Am J Hum Genet 80:994-1001