Schizophrenia risk polymorphisms in the *TCF4* gene interact with smoking in the modulation of auditory sensory gating

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Several polymorphisms of the transcription factor 4 (*TCF4*) have been shown to increase the risk for schizophrenia, particularly *TCF4* rs9960767. This polymorphism is associated with impaired sensorimotor gating measured by prepulse inhibition—an established endophenotype of schizophrenia. We therefore investigated whether *TCF4* polymorphisms also affect another proposed endophenotype of schizophrenia, namely sensory gating assessed by P50 suppression of the auditory evoked potential. Although sensorimotor gating and sensory gating are not identical, recent data suggest that they share genetic fundamentals. In a multicenter study at six academic institutions throughout Germany, we applied an auditory P50 suppression paradigm to 1,821 subjects (1,023 never-smokers, 798 smokers) randomly selected from the general population. Samples were genotyped for 21 *TCF4* polymorphisms. Given that smoking is highly prevalent in schizophrenia and affects sensory gating, we also assessed smoking behavior, cotinine plasma concentrations, exhaled carbon monoxide, and the Fagerström Test (FTND). P50 suppression was significantly decreased in carriers of schizophrenia risk alleles of the *TCF4* polymorphisms rs9960767, rs10401120rs, rs17597926, and 17512836 ($P < 0.0002$–0.00005). These gene effects were modulated by smoking behavior as indicated by significant interactions of *TCF4* genotype and smoking status; heavy smokers (FTND score ≥4) showed stronger gene effects on P50 suppression than light smokers and never-smokers. Our finding suggests that sensory gating is modulated by an interaction of *TCF4* genotype with smoking, and both factors may play a role in early information processing deficits also in schizophrenia. Consequently, considering smoking behavior may facilitate the search for genetic risk factors for schizophrenia.

single nucleotide polymorphism | intermediate phenotype | nicotine | gene–environment interaction

Recent large genomewide association studies (GWAS) identified and consistently confirmed that common variants of the transcription factor 4 (*TCF4*) gene contribute to the risk of schizophrenia (1–3). In these analyses, two single nucleotide polymorphisms (SNPs) located in the intron of the *TCF4* gene on chromosome 18q21.1 (rs9960767, rs17512836) and an intragenic SNP near the *TCF4* gene (rs4309482) have shown the strongest association with the disease (1–3). *TCF4* is a class I basic helix–loop–helix (bHLH) protein involved in the control of neuronal and glial progenitor cells, which are important for the development of the mammalian central nervous system (CNS) (4, 5). The exact role of *TCF4* in the brain and the functional activity of these nonsynonymous *TCF4* variants on the level of gene expression are not yet fully understood (4, 6). A recent postmortem study suggested that the rs9960767 SNP is neither functional nor affects mRNA expression in the adult human brain, indicating that *TCF4* mutations may exert their effects on expression through posttranscriptional effects or exclusively in a developmental context (e.g., by gene–environment interactions) (7).

In a translational animal study, it was initially shown that transgenic mice moderately overexpressing *TCF4* in the postnatal brain display profound reductions in sensorimotor gating as measured by prepulse inhibition (PPI) of the acoustic startle response (8), which is an established translational endophenotype of schizophrenia (9). Consequently, we recently investigated the impact of the *TCF4* rs9960767 SNP on PPI in humans and found that the schizophrenia risk allele C of this SNP was strongly associated with reduced PPI in two independent samples of healthy volunteers and schizophrenia spectrum patients (10).

Auditory sensory gating, i.e., P50 suppression of the auditory evoked potential (AEP) is another measure of gating function. P50 gating is regarded as a useful endophenotype of schizophrenia and is conceptually related to, albeit not equivalent with, sensorimotor gating as measured by PPI (9). In the classical auditory conditioning-testing P50 paradigm (11), pairs of identical auditory stimuli (conditioning stimulus S1 and test stimulus S2) are presented at an interstimulus interval (ISI) of 500 ms, whereas the cortical response to these stimuli is assessed via electroencephalography (EEG). Normal subjects usually have a suppressed P50 response to the second stimulus. Source localization studies and intracerebral electrophysiological recordings suggest that the generators of sensory gating are in the hippocampus, insula,

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lateral temporal, temporo-parietal, and prefrontal cortex (PFC) (12–14). Auditory sensory gating has been conceptualized as an important preattentive filter function that protects cognitive processes from potentially interfering and irrelevant information (15). Accordingly, P50 suppression has been related to attentional performance, working memory, and behavioral inhibition (16–19).

Schizophrenia patients display diminished P50 suppression (20–25), even if some studies failed to find differences between patients and controls (26–29). However, meta-analyses confirmed moderate to large effects of sensory gating deficits in schizophrenia across studies (30–32). The P50 suppression was suggested as an endophenotype of schizophrenia (9) because it is heritable (33–35), it is reduced in subjects with schizotypal personality disorder and in unaffected relatives of schizophrenia patients (36–38), and decreased P50 suppression levels are present in early stages of schizophrenia (39, 40). Moreover, P50 suppression deficits have been repeatedly associated to SNPs of the α7 nicotinic acetylcholine receptor subunit gene (*CHRNA7*) (41), whereas the initially reported impact of catechol-*O*-methyltransferase (*COMT*) gene variations on P50 suppression (42) was not replicated in three following studies (43–45). Despite the fact that schizophrenia patients display both sensorimotor and sensory gating abnormalities, and although both measures are procedurally similar, they are usually not correlated and might be regulated by distinct neuronal pathways (46–49). However, a recent analysis from the Consortium on the Genetics of Schizophrenia (COGS) suggested that both gating measures likely share some genetic basis: PPI and P50 suppression were both influenced by variations in *CHRNA7*, the neuregulin-1 (*NRG1*) gene, and the ionotropic glutamate receptor δ2-subunit gene (*GRID2*) (45, 50, 51). In the COGS study, the *TCF4* gene was not investigated and, thus, the impact of the *TCF4* SNPs on schizophrenia risk and sensorimotor gating (PPI) warrants also an investigation of its influence on P50 suppression.

Because recent GWAS findings identified several *TCF4* polymorphisms as genetic risk factors for schizophrenia (1–3), human carriers of the *TCF4* rs9960767 risk allele C showed reduced gating abilities in the PPI paradigm (10), and auditory sensory gating is an established endophenotype of schizophrenia (9), we hypothesized that healthy carriers of *TCF4* schizophrenia risk polymorphisms would also display reduced auditory sensory gating as reflected in a decreased P50 suppression. Given that risk gene variants for schizophrenia (including those identified in *TCF4*) are common in the general population and because heritable variation of endophenotype measures is also present in subjects not affected with schizophrenia (33–35), one can study the possible association of risk variants with putative endophenotypes in healthy subjects. This approach offers a number of advantages over classical case-control studies: (*i*) confounding effects of illness and treatment are ruled out, (*ii*) larger samples stratified for the presence or absence of possible environmental moderators can be studied in a limited time, and (*iii*) independence of conventional, phenotypic diagnostic criteria. Thus, positive findings for single gene variants in healthy controls can help to discover biologically valid knowledge about previously unknown mechanisms linked to this gene (52). To test our hypothesis, we assessed a large and genetically homogeneous sample of 1,821 volunteers exclusively of German ancestry with an established auditory sensory gating paradigm in the frame of a multicenter study at six academic institutions throughout Germany. All participants were genotyped for the 20 most significant *TCF4* SNPs from the recent GWAS of Ripke et al. (ref. 1; http://www.broadinstitute.org/mpg/ricopili/; window 1.5Mb, threshold $P < 1.0 \times 10^{-4}$) and for the most significant *TCF4* SNP rs9960767 reported by Stefansson et al. (2) and Steinberg et al. (3). Given that smoking is a critical confounding factor with regard to the P50 suppression (53) and because schizophrenia patients are frequently heavy smokers (54), we additionally investigated the impact of smoking severity as a possible mediating factor on *TCF4* gene effects on P50 suppression.

Results

One of the 21 *TCF4* SNPs was monomorphic (rs17509991) and, therefore, excluded from further analyses. All other SNPs were in Hardy–Weinberg equilibrium (HWE) (Table S1) and partly in strong linkage disequilibrium (LD) (Fig. S1).

Unless otherwise stated, we compared SNP risk allele carriers against noncarriers throughout (10). Percent P50 suppression was significantly affected by 4 of the 20 *TCF4* SNPs (Bonferroni-corrected threshold $P < 0.0024$ for 21 markers) and smoking status, whereas there was also a significant genotype–smoking interaction in each marker (Table 1). Given that the rs17597926 and the rs17512836 SNP showed almost perfect linkage disequilibrium (LD, $r^2 = 0.99$), only data of the rs17597926 SNP are further presented. Remaining pairwise relationships showed moderate LD between SNPs (Fig. 1). In each of the significant SNPs, carriers of the respective schizophrenia risk alleles displayed reduced auditory sensory gating (Fig. 2*A*). The significant interactions of the factors genotype and smoking status indicated that the genotype effects were different among never, light, and heavy smokers (Fig. 2*B*). Heavy smokers [Fagerström Test of Nicotine Dependence (FTND) score ≥ 4] consistently showed stronger *TCF4* gene effects on P50 suppression (Cohen's d = 0.63–1.04) than light smokers (d = 0.27–0.43) and never-smokers (d = −0.04 to 0.06]. Bonferroni post hoc tests of the main effect of smoking status replicated the finding shown in an overlapping sample of the present population (53): In all tests, heavy smokers displayed lower P50 suppression levels than light smokers ($P = 0.014–0.006$; d = 0.34–0.59) and never-smokers ($P = 4.4 \times 10^{-5}$ to 5.5×10^{-6}; d = 0.55–0.76), whereas light smokers and never-smokers did not significantly differ ($P = 0.070–0.482$; d = 0.17–0.21). The effect for the repeated factor electrode position alone was not significant (all tests: F < 1.2), but it interacted with genotype in the analyses of rs9960767 [$F_{(1,1812)} = 7.10$; $P = 0.008$; $\eta_p^2 = 0.004$] and rs10401120 [$F_{(1,1799)} = 6.94$; $P = 0.008$; $\eta_p^2 = 0.004$], indicating that the *TCF4* genotype effect was more evident at the frontal electrode (Fz) (Fig. S2). Further interactions between factors and covariates were not significant. Finally, the covariate age ($P = 4.1 \times 10^{-14}$ to 7.7×10^{-15}, $\eta_p^2 = 0.033–0.035$) revealed a significant effect, whereas the impact of sex, study center, cotinine plasma level, and longitude/latitude of study center was not significant (all tests: F < 1.25). As shown before (53), sensory gating increases with age (see also correlation analyses in *SI Discussion*). Longitude and latitude were included in the analysis to control for subtle population differences between the sampling sites. Because only SNP rs9960767 presents a reasonable number of rare homozygotes ($n > 10$), the analysis of this marker was repeated in a threefold genotype factor design. However, the genotype effects and genotype–smoking interactions remained largely the same (Fig. S3).

The genotype groups of the significant SNPs did not differ regarding any demographic data, smoking parameters, or psychometric scales (Table S2). Thus, the effects observed because of risk allele carrier-ship could not be explained by demographical stratification effects.

As shown in Table S3, the four *TCF4* SNPs showing significant association with P50 suppression did not significantly affect the amplitude of the P50 AEP neither on the response to the first click (S1) nor to the second click (S2). Analyses of the difference and the ratio of P50 amplitudes as further measures of auditory sensory gating revealed the same effects as in the analysis of percent P50 suppression. Again, the *TCF4* genotype effect on P50 suppression was most pronounced at the Fz electrode. Correlations of P50 parameters with demographic and smoking variables are shown in *SI Discussion* and Table S4.

To assess the association between the reduced levels of P50 suppression and *TCF4* risk allele carrier-ship, odds ratios (OR) were calculated. If clinical criteria of one and two SDs were applied to define low P50 suppression phenotypes, all of these phenotypes showed significant associations with the respective

Table 1. The effects of *TCF4* SNP risk allele carrier-ship and smoking status on percent P50 suppression of the auditory evoked potential (averaged across electrode positions Fz and Cz)

TCF4 genotype	Position on chromosome 18q21.1	Allele frequency number, %			Total *n*	Schizohrenia risk allele*	Genetic model†	Effect on P50 suppression		
		Common homocygotes	Heterozygotes	Rare homocygeotes				Factor genotype (df = 1)	Factor Smoking (df = 2)	Interaction smoking–genotype (df = 2)
rs9960767‡	Intron 53155002	AA 1,610, 88.4%	AC 199, 10.9%	CC 12, 0.7%	1,821	C	AA vs. AC+CC	$F = 16.7$; $P = 4.5 \times 10^{-5}$; $\eta_p^2 = 0.010$	$F = 11.7$; $P = 8.7 \times 10^{-6}$; $\eta_p^2 = 0.013$	$F = 7.1$; $P = 0.001$; $\eta_p^2 = 0.008$
rs10401120	Intron 53192498	CC 1,647, 90.9%	CT 156, 8.6%	TT 9, 0.5%	1,812	T	CC vs. CT+TT	$F = 15.9$; $P = 6.9 \times 10^{-5}$; $\eta_p^2 = 0.009$	$F = 10.9$; $P = 1.9 \times 10^{-5}$; $\eta_p^2 = 0.013$	$F = 5.8$; $P = 0.003$; $\eta_p^2 = 0.007$
rs17597926§	Intron 53205938	GG 1,723, 94.6%	GA 95, 5.2%	AA 3, 0.2%	1,821	A	GG vs. GA+AA	$F = 14.5$; $P = 1.4 \times 10^{-4}$; $\eta_p^2 = 0.008$	$F = 9.5$; $P = 7.9 \times 10^{-5}$; $\eta_p^2 = 0.011$	$F = 4.9$; $P = 0.008$; $\eta_p^2 = 0.006$
rs17512836¶	Intron 53194961	TT 1,721, 94.6%	TC 95, 5.2%	CC 3, 0.2%	1,819	C	TT vs. TC+CC	$F = 13.9$; $P = 2.0 \times 10^{-4}$; $\eta_p^2 = 0.008$	$F = 9.2$; $P = 1.1 \times 10^{-4}$; $\eta_p^2 = 0.011$	$F = 4.4$; $P = 0.013$; $\eta_p^2 = 0.005$

*According to Ripke et al. (1) and Stefansson et al. (2).
†ANCOVA with genotype (twofold), and smoking status (threefold) as fixed factors, electrode position (twofold) as repeated factor, and age, sex, study cite, cotinine plasma level, and longitude and latitude of the study centers as covariates.
‡Most significant *TCF4* SNP in the schizophrenia GWAS of Stefansson et al. (2).
§Second most significant *TCF4* SNP in the schizophrenia GWAS of Ripke et al. (2).
¶Most significant *TCF4* SNP in the schizophrenia GWAS of Ripke et al. (1).

four *TCF4* risk-alleles within the total sample (range OR = 1.81–2.58) and within heavy smokers (range OR = 3.21–7.60) but not within the never-smokers (range OR = 1.23–1.75) (Table S5). At each criterion, the odds ratios were highest in heavy smokers and lowest in never-smokers, whereas light smokers were intermediate (range OR = 2.44–2.78). Moreover, there was no association of the four significant *TCF4* SNPs with the smoking phenotype (Table S6).

Discussion

In an endophenotype-based association study approach, we investigated the impact of *TCF4* schizophrenia risk SNPs on an established psychophysiological endophenotype of schizophrenia. We could demonstrate that carrier-ship of risk alleles at four *TCF4* SNPs (rs9960767, rs17512836, rs17597926, and rs10401120) were significantly associated with reduced auditory sensory gating as measured by P50 suppression of the AEP. Noteworthy, two of these SNPs (rs9960767, rs17512836) were among the top markers associated with schizophrenia with genomewide significance in recent large GWAS meta-analyses (1, 2), whereas SNP rs17597926 was the marker in the *TCF4* gene showing the second smallest *P* value in one of these studies (1). Markers rs17512836 and rs17597926 were in strong LD ($r^2 = 0.99$), thus representing essentially the same association signal. Further pairwise LD relationships were moderate ($r^2 = 0.38$–0.64). Interestingly, the genotypic effects of each SNP were strongly modulated by smoking behavior with only smokers showing reliable *TCF4*-P50 suppression associations, whereas the genetic effect was small or not present in never-smokers. Moreover, genotype–smoking interactions were dose-related because *TCF4* SNP genotype effects amplified with increasing smoking severity.

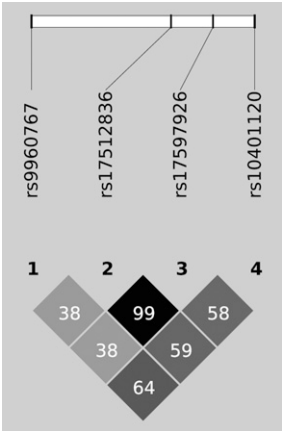

Fig. 1. Pairwise linkage disequilibrium (LD), as measured by r^2, of four *TCF4* SNPs that were significantly associated with percent P50 suppression.

TCF4 belongs to a subfamily of *bHLH* transcriptional regulators that recognizes the Ephrussi-box (E-box) binding site on the DNA that usually lies upstream of a gene in a promoter region (55). At early developmental stages, E-box transcription factors such as *TCFE2a, TCF12*, and *TCF4* show widespread 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 (8, 56). *TCF4*-null knockout mice die in the first 24 h after birth and display brainstem abnormalities (4, 57). Haploinsufficiency of the *TCF4* gene in humans causes the Pitt-Hopkins syndrome—an autosomal-dominant neurodevelopmental disorder characterized by severe mental and motor retardation, microcephaly, epilepsy, facial dysmorphisms, and intermittent hyperventilation—reflecting that *TCF4* is critical for the development of the mammalian CNS (6, 55). Thus, the respective *TCF4* polymorphisms might have a subtle impact on brain development that contributes to gating abnormalities and that increases the risk for schizophrenia.

The present results are in line with our previous findings that the schizophrenia risk allele C of the *TCF4* rs9960767 SNP is associated with diminished sensorimotor gating as measured by PPI (8, 10). Although PPI and P50 suppression are not correlated and might be regulated—at least in part—by distinct neuronal mechanisms (46–49), recent work suggests that they nevertheless might share some common genetic pathways (41, 45, 50, 51). However, the modifying influence of smoking on the effect of *TCF4* shown here was not present in our previous investigation

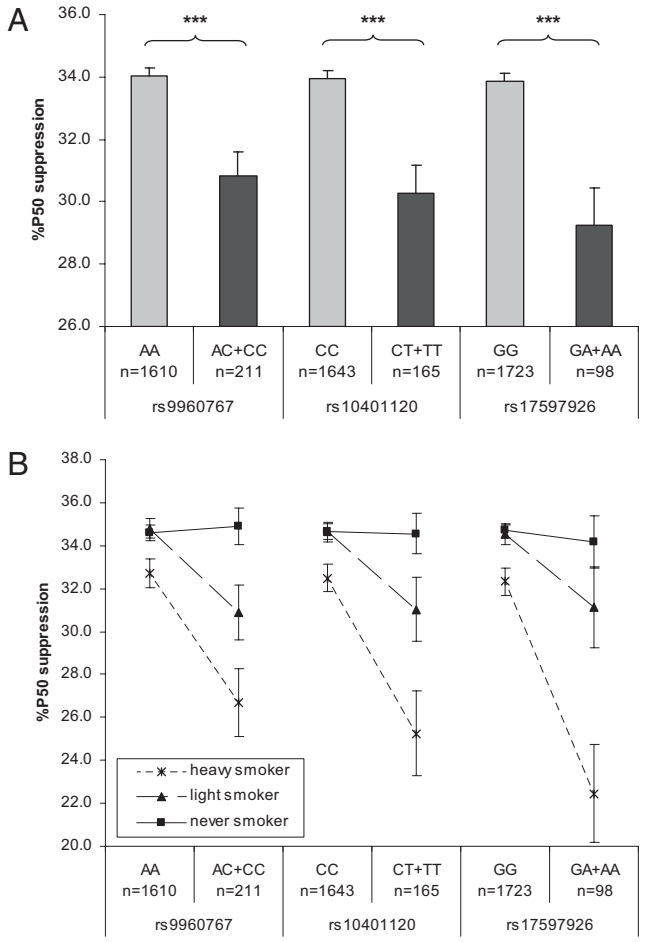

Fig. 2. The effects of three *TCF4* SNPs (rs9960767, rs10401120, and rs17597926, which was in almost complete linkage disequilibrium with rs17512836) on percent P50 suppression of the auditory evoked potential (means and SEM; adjusted for age, sex, study site, cotinine plasma level, and longitude and latitude of the study center) averaged across the electrode positions Fz and Cz (*A*), and stratified according to smoking behavior (never-smokers: n = 1,023, light smokers: n = 466, and heavy smokers n = 332) (*B*). ***$P < 2.0 \times 10^{-4}$.

on *TCF4* gene effects on PPI (10). The previously investigated samples may have been too small and underpowered to reliably examine the effects of smoking as a mediating factor on the *TCF4* gene effects on PPI. The potentially modifying effect of smoking on *TCF4* gene effects on PPI (and other schizophrenia endophenotypes) should therefore be investigated in larger samples. Finally, the *TCF4* rs9960767 genotypic effect on PPI displayed a much stronger effect size (Cohen's d = 0.90) than the mean effect on P50 suppression (d = 0.18–0.20). This difference may be explained either by the "winner's curse"—which means that estimations of the genetic effect based on novel association findings tend to be upwardly biased (58)—by the fact that P50 suppression usually displays a less beneficial retest reliability compared with PPI (21, 59), or by a differential impact of *TCF4* on the underlying neural systems (or a mixture of these factors).

Interestingly, *TCF4* genotype effects on P50 suppression were more evident at the Fz than on the vertex electrode (Cz). Previous studies reported that the PFC substantially contributes either to the sensory gating process per se (13) or at least to the generation of the P50 amplitude (14). 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 PFC (60). Thus, *TCF4* mutations (in combination with smoking) might have a specific impact on PFC function in schizophrenia.

How can the unexpected smoking–genotype interaction regarding P50 suppression be elucidated? There are at least two possible scenarios: The first is a hidden gene–gene interaction: In this model, *TCF4* SNPs interact 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, whereas the *TCF4* SNPs were exclusively associated with P50 suppression but not with smoking. 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 were reliably associated with smoking behavior (61–63), and also with sensorimotor gating (PPI) (64) and cognitive performance (65). SNPs of the *CHRNA7* would also be interesting candidates because of the reported associations with P50 suppression and smoking behavior (41, 66, 67).

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. (7) that the *TCF4* schizophrenia risk alleles may exert their effects on expression exclusively in a developmental context because postmortem data of this study suggested that SNP rs9960767 is neither functional nor affects mRNA expression in the adult human brain. Furthermore, evidence accumulates that risk SNPs might have significant expression effects on other genes but not on the one in which they are located (68). However, at this point, we can only hypothesize which neurobiological mechanisms might underlie this smoking–TCF4 interaction on P50 suppression: (*i*) Smoking-induced plasticity of brainstem nAChR (69–72) in concert with subtle neurodevelopmental changes in pontine nuclei induced by *TCF4* gene variations (4) may affect P50 suppression. (*ii*) *TCF4*-induced changes in the noradrenaline system (6, 55) might interact with nicotine-induced changes of α7 nAChR function (73) when modulating P50 suppression. (*iii*) Nicotine may be involved in the methylation of DNA sequences leading to an epigenetic change of the expression of the *TCF4* gene (or other genes interacting with *TCF4*) with functional consequences on early information processing (74, 75).

Several studies have demonstrated that *CHRNA7* promoter variations are associated with schizophrenia and affect P50 suppression (41). The present study adds *TCF4* mutations as complementary genetic factors (or more exactly a *TCF4*–smoking interaction) to the population variation in P50 suppression and it has been shown that also *TCF4* gene variations increase the risk for schizophrenia (1–3). However, it should be noticed that the explained variances of P50 suppression by the factors *TCF4* genotype (0.8–1.0%), smoking (1.1–1.3%), and age (3.3–3.5%) as well as of the *TCF4*–smoking interaction (0.07–0.08%) were rather small, reflecting that either many other genetic factors might be involved or that P50 suppression may also have a strong state-dependent part as it was suggested (33).

The question arises whether effects of population stratification might have influenced our results because we have not typed a standard panel of ancestry-informative SNPs to control for stratification effects. However, we aimed to build a genetically highly homogeneous sample of subjects with an ancestry exclusively from Germany, and we randomly selected our participants from the general population to avoid possible stratification effects. Additionally, the European autosomal gene pool was recently found to be rather small, especially in northern and middle Europe subpopulations (76). Nevertheless, this study has shown a continent-wide correlation between geographic and genetic distance along the north-south axis and, to a lesser extent, also along the east-west axis (76). Therefore, we included longitude and latitude of the study centers as covariates in our analyses to control for

even subtle population stratification, which did not change our results. We also explored regional differences in *TCF4* SNP genotype frequencies between study centers located in three areas of Germany (North, Midwest, and South), but we found no significant differences between these regions (Fig. S4). Lastly, the *TCF4* SNP allele frequencies of the present sample fitted with the frequencies of the European HapMap data (CEU), and none of the investigated SNPs deviated from the HWE. Thus, the strong association of *TCF4* with auditory sensory gating is likely not explainable by population stratification effects.

In conclusion, our results suggest that the schizophrenia risk alleles of *TCF4* variants interact with smoking behavior with regard to auditory sensory gating, which is an established endophenotype of schizophrenia. We hypothesized that this finding could be interpreted as a gene–environment interaction with plausible neurobiological explanations. If smoking behavior strongly modulates the *TCF4* SNP effects on a proposed endophenotype of schizophrenia, it might also modulate the risk for schizophrenia itself. We therefore suggest the investigation of potential moderating effects of dimensional and binary measures of smoking behavior on genetic risk factors of schizophrenia. In case-control association studies, stratification for smoking behavior may add power to yield 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 increases the risk for psychosis (77, 78). Finally, an extended endophenotype including electrophysiological gating measures such as PPI or P50 suppression, smoking behavior, and risk genes such as *TCF4* may be suitable as an early indicator for a developing psychosis.

Materials and Methods

Participants. The study was carried out in the framework of the German multicenter study: *Nicotine: Physiological and Molecular Effect in the CNS* (53, 79). All subjects were randomly selected from the local general population of seven cities across Germany (Aachen, Berlin, Bonn, Düsseldorf, Erlangen, Mainz, and Mannheim) via official residents' registers and contacted by letter with an invitation to participate in the study. Overall, n = 56,350 subjects were contacted, of whom n = 4,760 responded by phone and completed an initial prescreening interview. Healthy subjects of German origin who met the inclusion/exclusion criteria (*SI Discussion*) were invited for a final screening investigation, which included a lifetime smoking history assessment, a medical examination, a standardized psychiatric interview (SCID-I), a drug urine screening, exhaled carbon-monoxide (COHb) measurement, and blood for routine clinical laboratory tests and genotyping. n = 2,442 subjects were finally included in the study. Across study sites, n = 468 subjects had either no electroencephalography (EEG) assessment (e.g., the Mannheim center did not apply EEG) or displayed an insufficient EEG data quality, whereas n = 238 subjects either did not provide blood samples, their DNA was not recoverable, or the genotyping failed, leaving a final sample of n = 1,821 participants (Table S2). The sample consisted of 1,023 never-smokers (lifetime smoking <20 cigarettes), 466 light smokers FTND score <4), and 332 heavy smokers (FTND ≥4).

The study was approved by the ethics committees of each study site's local university and was conducted according to the declaration of Helsinki. Written informed consent was obtained from all subjects.

Study Procedures. Across all study sites, the study was performed on the day of study inclusion according to the same standard operating procedure including regular monitoring with strict adherence to a fixed time table. After inclusion, subjects provided extensive demographical and smoking-related information and answered a battery of questionnaires on smoking-related behavior (for details, see *SI Discussion*), drug/alcohol consumption habits, and personality (79). Afterward, a neuropsychological test battery and an EEG investigation were performed (79). The EEG was obtained between 1:30 AM and 2:30 PM in all subjects. Subsequently, venous blood was obtained to determine cotinine plasma levels and for genotyping. In smoking subjects, the EEG was recorded between 1 and 3 h after the last cigarette smoked ad libitum. Genotyping information is given in the *SI Discussion*.

P50 Auditory Double Click Paradigm. Subjects were instructed to keep their eyes closed throughout the EEG experiment. Five minutes of continuous resting EEG was recorded before the P50 auditory double-click paradigm, which was based on the work of Adler et al. (80). One hundred pairs of two 250 sinus tones (clicks) of 2,000 Hz (50-ms duration including rise and fall time) were administered binaurally via headphone at a 50-dB sound pressure level. A fixed ISI of 500 ms was chosen between paired clicks S1 and S2. Between pairs of clicks, the intervals were (pseudo)randomized varying between 5 and 9 s (mean 7 s). Subjects were instructed to stay awake and to listen to the tones. EEG data acquisition and analysis is described in *SI Discussion*. The amplitudes of the event-related P50 potential (filtered at 10–45 Hz, 12 dB) were calculated across electrodes by automatically locating the most positive peak in the respective time window. The P50 response was the most positive peak between 48 and 68 ms after stimulus onset (both after S1 and S2) (24). The P50 amplitude was measured relative to the preceding negativity. For the analysis of P50 suppression, the percent P50 suppression ($[1 - S2/S1] \times 100$), the difference of P50 amplitudes (S1 − S2), and the ratio of P50 amplitudes (S2/S1) was used. Low value of the percent and the difference suppression as well as high values of the ratio indicate weak inhibition of the second click (i.e., no gating or filtering) or low P50 amplitude after the first click. Fz and Cz electrodes were analyzed because previous studies have shown that P50 suppression measured at these electrodes discriminate best between schizophrenia patients and controls (23, 24, 81). Details of the statistical analysis are given in the *SI Discussion*.

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