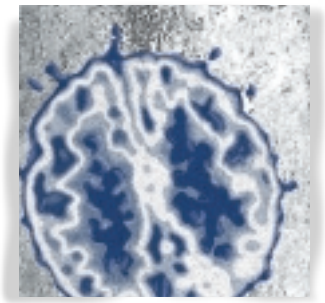
Clinical impact of recently detected susceptibility genes for schizophrenia

Wolfgang Maier, MD; Astrid Zobel, MD; Kai-Uwe Kühn, MD



After years of frustration, the search for genes impacting on schizophrenia is now undergoing some exciting developments. Several proposals of susceptibility genes have been able to be supported by replications. Thus, there are now at least three very strong candidates: the gene for dysbindin (DTNBP1), the gene for neuregulin-1 (NRG1), and a less well-understood gene locus, G72/G30, which are likely to influence manifestations of schizophrenia. Other "hot" candidates such as the disrupted-in-schizophrenia 1 gene (DISC1) and the gene coding for protein kinase B (AKT1) might also prove to be susceptibility genes in the next future. The clinical implications of these findings are not yet fully visible. However, some first insights are possible: most of the genetic findings lack diagnostic specificity, and are also reproduced in bipolar disorder. Strong associations are also obtained on a symptomatic level, not only on a diagnostic level. The pathophysiological role of these hot candidate genes is currently under intensive study.

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All common diseases in the general population are strongly influenced by genetic factors. This is also true for schizophrenia. A long series of family, twin, and adoption studies has clearly demonstrated that heritability is the strongest determinant of schizophrenia. Variance-analysis estimates in twin samples allocate about 80% of the total etiological variance to genetic factors. The underlying genetic mechanism (as evidenced from family and twin data) is clearly not Mendelian; the complexity of patterns of familial aggregation can best be explained by the operation of multiple genes, each with a modest or small effect, and by additional impact of nongenetic, environmental forces. Thus, causal genes are extremely unlikely to explain the vast majority of cases; instead, genes influencing the risk of developing schizophrenia (susceptibility genes) play the major role. Similarly to other common diseases such as hypertension or diabetes, the search for susceptibility genes contributing by DNA-sequence variation to schizophrenia has turned out to be difficult, and the time taken to obtain the first replicable hints was two decades.

Breakthrough in the search for susceptibility genes

In the last 2 years we have experienced a period of excitement in the genetics of schizophrenia, after decades of frustration. Claims of the involvement of genes in the manifestation of schizophrenia were put

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Author affiliations: Department of Psychiatry, University of Bonn, 53105 Bonn, Germany

Address for correspondence: Prof Dr med Wolfgang Maier, Department of Psychiatry, University of Bonn, Sigmund-Freud-Strasse 25, 53105 Bonn, Germany (e-mail: Wolfgang.Maier@ukb.uni-bonn.de)

forward for several genes. These achievements became possible through a genome-wide, hypothesis-free search for genes predisposing to schizophrenia. The successful strategy encompassed two steps: (i) mapping of genes in broad candidate areas on the genome by linkage analysis; (ii) identification of susceptibility genes within this region by either systematic narrowing down or trial and error.

Following this strategy, three genes emerged as strong candidates for susceptibility genes: the dysbindin gene on chromosome 6p (*DTNBP1*),[1,2] the neuregulin-1 gene on chromosome 8p (*NRG1*),[3] and an up-to-now unknown gene locus on chromosome 13q, only expressed in humans (*G72/G30*).[4] Remarkably, for each of these genes a majority of studies have reported significant associations with markers and/or marker combinations (haplotypes). However, the associated markers and haplotypes vary across studies for all three genes.

Caveats to current claims for susceptibility genes for schizophrenia

The confidence in these three claims is, however, limited for the following reasons:

- The fact that the reported at-risk haplotypes in the different studies/samples are not overlapping, and do not include a common denominator allele or core haplotype for any of the claimed susceptibility genes.
- Poor reproducibility of the identical at-risk haplotype in different samples, although for each of the claimed susceptibility genes the vast majority of published inquiries found alleles and haplotypes.
- Absence of demonstrated function of any of the extracted at-risk haplotypes.
- No expressed exonic DNA-sequence variants can explain the reported associations, ie, neither of these claimed susceptibility genes contains DNA-sequence variants that might:
    - result in change of the amino-acid sequence in the expressed protein;
    - account for any of the reported genetic associations with schizophrenia.
- The failure to identify one or more susceptibility variants in any of the claimed susceptibility genes directly influencing the etiology of schizophrenia.

Thus, there is a set of consistencies and inconsistencies which are difficult to understand in combination.

What is the meaning of these findings?

Given the variation of associated markers/haplotypes across studies and small relative risks, the reported findings might reflect false-positives. This possibility, however, is very unlikely. For example, let us look at *NRG1*: The proportion of reports with significant associations in a 300 kb region around the exon 1 is too high to be due to chance (12 out of 14). In addition, the strong association of the originally identified at-risk haplotypes was independently replicated, and several subsequent studies did not use this marker combination; furthermore, this lack of association of original haplotypes occurred in Asian populations, due to its very low frequency, whereas more common variants at the same loci were associated with schizophrenia.[5]

Taking the findings for all the abovementioned genes together, a general pattern can be recognized:

- Several genes impact on the manifestation of schizophrenia; causal genes can be excluded; the absence of strong linkages to any locus across all genome-wide linkage scans.
- All susceptibility genes only contribute by a small or, maximally, moderate effect; the relative risks are small in outbred populations (OR 1.5-2.5).
- The mode of interaction between genes coding for schizophrenia remains obscure.
- The mutants directly influencing the risk of schizophrenia are difficult to find, and still have to be identified; mutations in expressed components (exons) of the genes causing a change in the amino-acid sequence of the protein have not been identified up to now. Instead, several studies report a differential gene expression in cases versus controls in target areas of the brain for *NRG1* and *DTNBP1*; given that associated alleles/haplotypes are located in introns, it can be suggested that the pathogenic mutations induce a regulatory dysfunction.

The meaning of the variation of haplotypes across studies is currently not appropriately understood. Two putative interpretations are possible:

- Different "causal" mutations in the same gene contribute to the emergence of schizophrenia; these mutations are not yet known; different "causal" mutations might predominate in different samples due to "genetic heterogeneity" of schizophrenia; significant associations to different haplotypes may be a consequence.

- Linkage disequilibrium between positional markers is variable across populations and samples; thus, the positional markers in linkage disequilibrium with the same "causal" mutations are different between populations and samples.

It is currently not possible to decide which of both options is true. In any case, it is very unlikely that the disparity between associated haplotypes of the gene reflects a "false positive" finding.

Alternative, successful strategies

Two alternative strategies have also turned out to be successful:

- *Cytogenic analyses in isolated families highly loaded with schizophrenia*: A translocation was detected to cosegregate with the condition status in the family.[6] A specific gene (not previously known) on chromosome 1 was regularly disrupted, and was named *DISC1*. Surprisingly, common mutations in this gene were also found to be associated with schizophrenia in outbred populations.[7]
- *Gene expression might guide to susceptibility genes*: Given the plethora of differentially expressed genes in postmortem brains of patients with schizophrenia, specific hypotheses are required to sharpen the focus to differentially expressed genes for further study. Assuming that synaptic and postsynaptic transmission is a crucial feature of schizophrenia, phosphokinases present as a family of candidate proteins; polymorphic genes coding for these units of intracellular signal transmission thus became "hot" candidate genes. These DNA-sequence variants in the differentially expressed protein kinase B (*AKT1*) were found to be associated with schizophrenia.[8] Several replications of the original finding have now been published.[9]

The detected susceptibility genes throw light on the etiology and the pathophysiology of schizophrenia. None of the abovementioned detected candidate genes has been implicated in the development of schizophrenia before. Therefore, unraveling their role in the core candidate processes underlying schizophrenia, brain maturation, and signal transduction has been the subject of extensive study in the last 2 to 3 years, in in-vitro, animal, and postmortem studies. In addition, the clinical correlates of at-risk haplotypes of the putative susceptibility genes, ie, diagnoses, psychopathological features, course, and neurobiological correlates of schizophrenia, might elucidate the underlying disease process.

Functional and clinical implications

In the absence of the "causal" mutations influencing the etiology and pathogenesis of schizophrenia, conclusive functional and clinical implications can not yet be identified. However, there are emerging patterns:

Diagnostic specificity of claimed susceptibility genes?

Recent cross-nosological twin studies propose sharing of predisposing genetic factors between schizophrenia and bipolar disorder.[10] This observation motivated the test for associations of at-risk haplotypes and at-risk alleles for schizophrenia in bipolar disorder, too: the *G72/G30* gene[11,12] and the *NRG1* gene[13] were also found to be implicated in the etiology of bipolar disorder. Associations between bipolar disorder and variants of the dysbindin gene had not been reported up to now.[14]
Are converging results in favor of a common predisposing susceptibility allele? To answer this question, associations with the same haplotype/marker in schizophrenia and in bipolar disorder in the same population are required. And, indeed, Schumacher et al[12] were able to support this possibility for the G72/G30 gene, and Green et al[13] for the *NRG1* gene. A similar (diagnostically unspecific) pattern is emerging for *DISC1*.[6] A conclusive answer to the question of diagnostic specificity, however, is only possible if the same pathogenic variant impacts in the same direction on the risk for each of both disorders.

Are the two disorders contributing to the observed associations in a global manner or through a specific symptom or symptom pattern?

A refined analysis proposed persecutory delusions to explain the association between the *G72/G30* haplotype and bipolar disorder. This finding was replicated in an independent sample.[15] The association with the neuregulin-1 gene is also largely due to specific symptoms in bipolar cases: mood-incongruent psychotic features proposing a specific effect in this subset of functional psychosis.[13] Thus, the question remains: what is the most appropriate clinical target for an involved susceptibility allele or haplotype? Core symptoms, or diagnoses which are defined through symptom patterns and additional criteria?
It should be kept in mind that complex behaviors such as psychotic and affective disorders are influenced by multiple genes, with each of them influencing multiple

behavioral components at various physiological functions. Against this background it is remarkable that all risk genes identified for schizophrenia and bipolar disorder are involved in glutamatergic transmission[16] or in the development of neurons and glial cells. These observations point to cross-diagnostic communalities in glutamatergic transmission and neurodevelopment.

The impact of individual genetic contributions operates at the level of specific symptoms/symptom patterns but less so on diagnoses

Symptom-based genetic association studies had been–up to now–only rarely conducted for recently proposed susceptibility genes for schizophrenia. Thus, it has been demonstrated that the *DTNBP1* at-risk haplotype is preferentially associated with negative symptoms.[17] Another example is *G72/G30*: the association of the at-risk haplotype with bipolar disorder is exclusively mediated by the symptom "persecutory delusion."[15]

Genetic modification of neurobiological features of schizophrenia might occur independently of the genetic influence on vulnerability

Given the limited empirical work on the recently proposed susceptibility genes for schizophrenia, conclusive evidence is not available. Based on the spatial expression pattern of *NRG1*, *DTNBP1*, and *G72/G30* and interacting genes, an influence on synaptic transmission as a common biochemical pathway has been proposed.[18] Although this hypothesis is attractive given the impact of glutamate on schizophrenia and its treatment, direct genetic evidence is missing. However, the clinical or neurobiological phenotype can also be modulated by polymorphic genes which do not contribute to the vulnerability to the disorder (so-called modulator gene).[19] An extensively studied example of this kind is the catechol-O-methyltransferase (*COMT*) gene. A series of studies reported that the Val/Met polymorphism modifies neurobiological functions associated with schizophrenia as working memory or information processing: the Val-variant being associated with less achievement. On the other hand the Val-variant of the *COMT* gene is not associated with schizophrenia, as evidenced by the recent meta-analysis.[20]

The long-term impact of susceptibility genes of schizophrenia on treatment will be the detection of new targets for new therapeutic agents

Susceptibility genes are beginning to be pinpointed in new circuits involved in the pathophysiology of schizophrenia.[18] The next step will be to uncover the interactions and mechanisms of risk enhancement for schizophrenia. Animal models can be established for this purpose using transgenic techniques. The modification of involved circuits and networks by a variety of drugs can be tested by these means. The pharmaceutical discovery mechanism might help to optimize this process. Appropriate drugs will then hopefully result for use in human drug trials, aiming at a more causal treatment of schizophrenia than is currently available. This hypothesis, however, is under discussion.[21] More refined analyses are needed for each of these genes to uncover their true role in mediating the risk for schizophrenia.

Summary

A series of discoveries has strongly promoted the field of neurobiology of schizophrenia. Several very strong claims for susceptibility genes for schizophrenia are now under ongoing investigation. Although these claims are partly replicable, a series of inconsistencies remains. Despite these still-to-be-resolved issues, the clinical implications are already visible. Currently, the major conclusions are, (i) that most of the claimed susceptibility genes for schizophrenia also reveal genetic associations with bipolar disorder, probably even through the same at-risk haplotypes; thus, a shared genetic vulnerability to both disorders becomes an emerging scenario; (ii) although diagnoses are useful to detect susceptibility genes, the genotype-phenotype relationship might be more symptom- than diagnosis-based. ❑

Repercusión clínica de los genes recientemente detectados que confieren susceptibilidad frente a la esquizofrenia

Después de años de frustración, el estudio de los genes que influyen en la esquizofrenia está dando por fin sus frutos. Se han confirmado algunas propuestas de los genes que confieren susceptibilidad. Así, hoy conocemos como mínimo tres aspirantes sólidos: el gen de la disbindina (DTNBP1), el gen de la neuregulina-1 (NRG1) y un locus génico menos conocido, G72/G30 parecen influir en las manifestaciones de la esquizofrenia. Otros aspirantes "serios," como el gen DISC1 *(disrupted in schizophrenia 1) y el gen cifrador de la proteincinasa B (AKT1), podrían convertirse en genes de susceptibilidad en un futuro próximo. Aún no están claras las implicaciones clínicas de estos datos pero cabe augurar algunas prospecciones iniciales: la mayoría de los datos genéticos carece de especificidad diagnóstica y se reproduce también en el trastorno bipolar. Asimismo, se observan asociaciones fuertes en un plano sintomático, no sólo diagnóstico. En estos momentos se examina con intensidad la utilidad fisiopatológica de estos genes, firmes aspirantes.*

Impact clinique des gènes de susceptibilité détectés récemment dans la schizophrénie

Après des années de recherche sans résultat concluante, le domaine de recherche génetique dans la schizophrénie est entré aujourd'hui dans une phase passionnante. La réplication des résultats de plusieurs études a permis d'étayer l'importance potentielle de gènes de susceptibilité proposés. Actuellement, au moins trois gènes sont considérés comme candidats très sérieux: le gène dysbindin (DTNBP1), le gène neuroregulin-1 (NRG1) et un locus moins bien caractérisé, le G72/G30, qui sont probablement impliqués dans la pathogenèse de la schizophrénie D'autres candidats tels que le "disrupted in schizophrenia 1 gene" (DISC 1), et l'AKT1, codant pour la protéine kinase B, pourraient s'avérer des gènes de susceptibilité dans un futur proche. Les implications cliniques de ces résultats ne sont cependant pas encore clairement établis. Quelques conclusions préliminaires peuvent être tirées: la plupart des résultats ne sont pas spécifiques pour la schizophrénie et se retrouvent également dans les trouble bipolaires. Des associations existent aussi bien au niveau diagnostique que symptomatique. Le rôle physiopathologique de ces gènes candidats est actuellement en cours d'études approfondies.

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