CMLS, Cell. Mol. Life Sci. 59 (2002) 331–348
1420-682X/02/020331-18 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Recent advances in the genetics of schizophrenia

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Received 11 May 2001; received after revision: 20 July 2001; accepted 18 September 2001

Abstract. The genetic etiology of schizophrenia, a common and debilitating psychiatric disorder, is supported by a wealth of data. Review of the current findings suggests that considerable progress has been made in recent years, with a number of chromosomal regions consistently implicated by linkage analysis. Three groups have shown linkage to 1q21–22 using similar models, with HLOD scores of 6.5, 3.2, and 2.4. Other replicated loci include 13q32 that has been implicated by two independent groups with significant HLOD scores (4.42) or NPL values (4.18), and 5p14.1–13.1, 5q21–33, 8p21–22, and 10p11–15, each of which have been reported as suggestive by at least three separate groups. Different studies have also replicated evidence for a modest number of candidate genes that were not ascertained through linkage. Of these, the greatest support exists for the DRD3 (3q13.3), HTR2A (13q14.2), and CHRNA7 (15q13–q14) genes. The refinement of phenotypes, the use of endophenotypes, reduction of heterogeneity, and extensive genetic mapping have all contributed to this progress. The rapid expansion of information from the human genome project will likely further accelerate this progress and assist in the discovery of susceptibility genes for schizophrenia. A greater understanding of disease mechanisms and the application of pharmacogenetics should also lead to improvements in therapeutic interventions.

Key words. Linkage; association; epidemiology; endophenotype; PANSS.

What is schizophrenia?

Schizophrenia is a complex and variable disorder characterized by cognitive, social, and affective impairment, and by the presence of psychotic symptoms. Common features include delusions, auditory hallucinations, disordered thinking, unusual speech or behavior, and social withdrawal. Suicide is the single largest cause of death in schizophrenic individuals. In the Chestnut Lodge follow-up study, 40% reported suicidal ideation, 23% reported suicide attempts, and 6.4% died from suicide [1]. Attempts have been made to clearly define schizophrenia for the purposes of optimizing diagnosis and treatment, and to create a discrete disease entity amenable to etiological studies. Some consensus has been reached in the universal adoption of structured interview criteria for diagnosis, such as the DSM-III-R [2, 3] and the ICD-10 [4], though the DSM-III-R criteria are more commonly used in linkage studies. Although the DSM-IV has been available since 1994 [5], it is rarely found in currently published studies due to the number of years typically required to collect large samples of schizophrenic families.

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The DSM-IV differs from the previous DSM-III-R classification in that more weight is given to negative symptoms. A narrow definition of schizophrenia usually includes schizophrenia and chronic schizoaffective disorder, a broad definition commonly includes the spectrum disorders such as schizotypal or paranoid personality disorders. Subjects with schizophrenia spectrum disorders exhibit similar traits to the narrow definition, but do not have the requisite number and configuration of symptoms for a diagnosis of the core syndrome. Subjects with schizophrenia spectrum disorders are frequently found in families ascertained for schizophrenia.

Core symptoms may be more sensitive to genetic risk than the DSM-III-R classification of schizophrenia [6]. The Positive and Negative Syndrome Scale (PANSS) [7], for example, provides quantitative measures on core symptoms and includes 7 positive symptoms that are related to psychosis and thought disorder, 7 negative symptoms that are related to deficits in affect and behavior, and 16 general psychopathology items that are related to the general severity of illness (e.g., anxiety). In longitudinal studies, these symptom clusters were found to be stable during remission phases of schizophrenia over periods of 1–10 years [8]. Factor analyses of the PANSS items have consistently generated five factors: negative, positive, excitement, cognitive, and depression/anxiety [9, 10].

How common is schizophrenia?

Epidemiological studies of this disorder have shown that schizophrenia occurs in all populations, with a prevalence of 1.4–4.6 per 1000 and an incidence of 0.16–0.42 per 1000 [11]. It is thought to affect about 1.3% of the adult population of the United States. While there is some variability in prevalence, the differences between populations are not nearly so great as for other common complex disorders such as heart disease and diabetes. Furthermore, prevalence does not differ appreciably between men and women, and there is no evidence of sex difference with regard to the symptom profile of schizophrenia. However, women tend to have a later age of onset, better premorbid functioning, and a higher percentage of remitting course than men [12]. The high incidence of schizophrenia in the population and the poor prognosis and outcome of patients represents a major health burden in both developed and developing countries.

Evidence for a genetic basis

Family, twin, and adoption studies have clearly shown that genes play an important role in determining susceptibility to schizophrenia. Twin studies are commonly used to estimate the extent of the genetic component of any trait or disease, which can be calculated from the relative concordance rates between monozygotic (MZ) and dizygotic (DZ) twins. In simple terms, the greater the MZ concordance compared to the DZ concordance, the greater the inherited component. Five new studies since 1995 [reviewed in ref. 13] agree with previous findings, showing an MZ probandwise concordance rate of 41–65% and a DZ concordance rate of 0–28%, generating heritability estimates of approximately 80–85%. The MZ twin concordance rate is substantially higher than the population prevalence of 1% and is also higher than the concordance rate in DZ twin controls, thus suggesting a significant genetic component. Twin studies have also been used in biometric studies to determine the most likely disease model. Cardno and colleagues performed such an analysis on pooled twin data and found that a model containing additive genetic and environmental effects gave the best fit, with a heritability of 83% (95% CI 74–90) and specific environmental effects of 17% (95% CI 10–26) [13]. Discordant MZ twin studies have also shown that the children of both affected and unaffected MZ twins have similar risks of schizophrenia-like psychosis [14]. This indicates that reduced penetrance is present, as they have inherited the susceptibility genes but have not expressed the disorder. Reduced penetrance is also indicated by the less than 100% concordance in MZ twins. This may be due to interacting genetic or environmental factors, epigenetic mechanisms such as imprinting, X chromosome inactivation, or random events.

Adoption studies have been used to show that a shared genetic component and not shared familial environment contributes to disease susceptibility. Pooled samples from a Danish-American adoption study showed a highly significant concentration of schizophrenia (5.0 vs 0.4% in controls, $p = 0.0013$) and latent schizophrenia, a non-psychotic schizophrenia-like syndrome (10.8 vs 1.7% in controls, $p = 0.00002$), among the biological relatives of chronic schizophrenic adoptees [reviewed in ref. 15].

Segregation of the syndrome in families has also been investigated to predict the most likely disease model. The lambda risk ratio, which is basically the fall-off in concordance rates of disease between first-, second- and third-degree relatives, can give an indication of the most likely model. Using this measure in families with schizophrenia, Risch [16] reported that three to four interacting loci (a multiplicative model) were most likely involved in determining risk, rather than an additive model. However, these and other biometrical methods were designed for simple mendelian disorders and are therefore of limited use for polygenic traits, where multiple genetic effects may be aggregated into what appear to be simple mendelian patterns. Nevertheless, the presence of epistasis is likely and complicates the discovery of susceptibility loci considerably, as epistatic loci investigated indi-

vidually may show little or no effect. Loci with additive effects are easier to detect, because each locus will show a distinct effect by itself. Thus, analytical methods need to be tailored to account for this possibility.

Endophenotypes of schizophrenia

Some researchers have suggested that endophenotypes for schizophrenia may be more useful than categorical diagnoses for determining susceptibility genes. The theory is that the endophenotypes may reflect more proximal effects of genes that contribute to the overt schizophrenia phenotype. Some of these traits are often found in healthy biological relatives in pedigrees containing schizophrenic probands, and are often observed to be inherited in a mendelian manner. A variety of neurophysiologic measures have been explored as endophenotypes. Defects in smooth pursuit eye movements (SPEMs) are caused by insufficient inhibition of reflexive saccades (small jerky eye movements). SPEM is present in 50–80% of patients with schizophrenia, in 40% of their first-degree relatives, and in 8% of the normal population [17]. Attentional deficits and diminished cognitive flexibility are also found, with slowed information processing by reduced habituation of the P50 EEG wave and by reduced amplitude and increased latency of the P300 wave upon auditory stimulation and by discriminative visual tasks. Decreased P50 inhibition is found in about 50% of patients and in 10% of subjects in the normal population [18]. P50 (auditory gating) is measured as a decremented response to the second of two auditory stimuli. The amplitude of response in normal subjects is around 40% of the conditioning response, but in schizophrenics, the response is usually > 50% and sometimes exceeds the conditioning response. Furthermore, the P50 deficit may be inherited as a dominant trait in some schizophrenic families. P300 has been associated with a wide array of psychiatric illnesses including alcoholism, and is not thought to be specific to schizophrenia. Reduced memory function, especially spatial working memory and verbal long-term recall, has also been observed in patients and their relatives [reviewed in ref. 19] and may be another possible endophenotype.

An additional attentional impairment as measured by the Continuous Performance Test (CPT) has also been found in siblings of schizophrenic probands [20]. A recent report described how at-risk offspring who later developed schizophrenia spectrum disorder as young adults displayed attentional deficits as young as 12 years old [21]. This trait has been found to be heritable (39% for verbal and 49% for spatial attention) and stable and is thought to reflect biological susceptibility to schizophrenia [22]. Results of genetic studies using these endophenotypes will be discussed later.

Environmental influences

The genetic models indicate a role for environmental factors in the pathogenesis of the syndrome. However, these have yet to be clarified and a variety of theories exist. Of these, obstetric complications (OC), intrauterine growth abnormalities, and perinatal brain damage are the most prominent. A meta-analysis of 854 patients (47.8% with OC) from 11 European research groups showed a significant association between an earlier age of onset of schizophrenia and the presence of OCs, with an odds ratio of 1.52 (95% CI 1.04–2.22) [23]. A further meta-analysis on data obtained from 12 studies of 700 schizophrenic subjects and 835 controls also showed significant associations between schizophrenia and premature rupture of membranes, gestational age less than 37 weeks, and the use of either resuscitation or an incubator [24]. In addition, the North Finland Birth Cohort, a study of 11,017 individuals, has shown a sevenfold excess of perinatal brain defects in subjects with schizophrenia. Another Finnish study found association between indicators of intrauterine and childhood undernutrition and lifetime risk of schizophrenia [25]. However, several recent studies have found no evidence for an association between OCs and risk for schizophrenia [26, 27].

Associations have also been found between schizophrenia and seasonality of birth, with an excess of winter births in patients (January–March in the northern hemisphere, July–September in the southern hemisphere), particularly in urban areas [28]. This was found to be consistent in a review of 250 studies, which showed a 5–8% excess of schizophrenia in winter births [29]. This association has been postulated to be due to the greater likelihood of contracting infections in winter; however, an extremely large study from Georgia (schizophrenics n = 11,736 and controls n = 734,879) found confirmation of the seasonal excess, but no association with the presence of either measles or influenza [30]. Unfortunately, information on OC is often unavailable, and thus unable to contribute to our understanding of etiological models for schizophrenia.

How do we find susceptibility genes for schizophrenia?

Thus, given that schizophrenia is a disorder with a complex etiology, how can we best uncover susceptibility genes? Whatever method is used, the primary requirement is a reasonably powered data set with reliable diagnoses. Underpowered data sets have been the Achilles heel of schizophrenia genetics research, and only recently have moves been made toward pooling of subjects and some consensus in methodology. An underpowered data set will increase the probability of a false-negative result

and decrease the chance of obtaining positive results when linkage or association to a genetic marker is present. Furthermore, known, rare causes of schizophrenia such as chromosomal abnormalities need to be excluded from the data set, to avoid inflating the phenocopy rate. One example of this is the 22q deletion syndrome, in which a high prevalence of psychiatric illnesses has been found. For a comprehensive review of chromosomal abnormalities in schizophrenia, see Bassett et al. [31]. Schizophrenic pedigrees that contain balanced translocations can also indicate the position of putative susceptibility genes by mapping the breakpoints on both chromosomes.

The most common approaches used in genetic studies are linkage and association methods and both methods and findings will be explained in detail below. However, the advent of chip technology has also led to the inception of a functional genomics approach. Advantages of the latter approach are that no a priori knowledge of the genes involved in the disorder is required and the sheer number of genes that can be tested simultaneously for differential expression. The major disadvantage of this approach is that the primary causal gene may not be detected due to low copy number, but many more abundantly represented genes downstream of the primary gene defect may be found. There may also be difficulties in replicating results from these experiments. However, these can be invaluable in elucidating novel biochemical pathways and pathogenic mechanisms. For example, one such study used methamphetamine treatment of rats (an animal model for psychotic mania) and then compared gene expression of specific brain regions with those of untreated animals [32]. The data were then cross-matched against human genomic loci thought to be associated with either schizophrenia or bipolar disorder. Several novel candidate genes were found, one of which, G-protein coupled receptor kinase 3 (GRK3), was subsequently validated by the finding of a decrease of the corresponding protein in lymphoblastoid cell lines derived from patients with bipolar disorder. This gene maps close to a region implicated by linkage studies on chromosome 22q11. Another study used microarray expression profiling of prefrontal cortex from schizophrenics and controls and found that transcripts encoding proteins involved in the regulation of presynaptic function were decreased in the schizophrenic subjects [33]. The two most consistently altered transcripts were N-ethylmaleimide-sensitive factor and synapsin II. Thus different approaches may compliment one another and cross-referencing between disciplines may be a useful tool for validation of candidate genes.

Candidate genes for schizophrenia have largely been derived from the dopaminergic and serotonergic systems due to evidence from neuropharmacological studies that these monoamine neurotransmitters play a role in the etiology of schizophrenia. This approach has not been as successful as was initially anticipated, but this may be partly due to the general lack of power in the association studies used to evaluate these variants, and may yet yield positive results. Recent findings and meta-analyses will be discussed later.

Linkage analysis

Linkage analysis is optimal for detecting rare disease alleles of large effect that co-segregate with disease in families, and has been used very successfully in investigations of rare mendelian single-gene disorders. In essence, linkage looks for alleles that have been inherited by affected offspring and not inherited by the unaffected offspring in a family. Recombination events between the marker and the disease can be used to determine the most likely genetic distance between these two entities, because the greater the genetic distance, the more recombinations are expected. The object of linkage analysis is to estimate the recombination fraction (θ) and test whether a deviation from the null hypothesis of no linkage (50% recombination or $\theta = 1/2$) is significant. Likelihood-based tests can be maximized over different values of θ, and a likelihood ratio generated for each position, which is converted to a LOD score by logging (base 10) this ratio. In general, a value of +3 or more is considered significantly positive evidence for linkage and a value of −2 or less is considered significant evidence for exclusion. However, in genome scans, these values are increased to account for multiple testing, but the specific value depends on the analytical method and the family structure employed [34]. For a parametric analysis, one recommendation for significant and suggestive results corresponds to a LOD score of 3.3 and 1.9, respectively. For a non-parametric sib-pair analysis, the corresponding recommended pointwise p values are 0.000022 (LOD of 3.6) for significant and 0.00074 (LOD of 2.2) for suggestive linkage. Alternative guidelines have been suggested by both Morton [35] and Elston [36].

In complex traits, genetic heterogeneity and reduced penetrance can contribute to an inflated recombination fraction, making localization of etiological loci prone to error. The variability in phenotype of schizophrenia and the overlap with related neuropsychiatric disorders has led to a generally held assumption of genetic heterogeneity. This assumption is further substantiated by the replication of specific susceptibility loci on several different chromosomes, though those loci have yet to be confirmed by the elucidation of the gene defect. An allowance for heterogeneity can be incorporated into a LOD score analysis by generating a heterogeneity LOD score (HLOD), which can be maximized over different values of alpha, the proportion of families linked to that locus. This admixture model is implemented in the HOMOG

program [37] and in GENEHUNTER [38], though the statistics are only likely to be accurate when the number of pedigrees is fairly large. Incomplete penetrance, as would be expected for schizophrenia susceptibility loci, further increases substantially the required number of families [37]. These admixture tests are widely used, but their validity has recently been questioned due to the unverifiability of the underlying assumptions such as a low disease allele frequency and the accuracy of the phenocopy rate [39]. The validity of the heterogeneity test in complex traits will be validated only by the discovery of a susceptibility gene in which the locus has been implicated by such an analysis. For schizophrenia, the issue of heterogeneity may be best addressed by using ethnically homogeneous samples and by prior partitioning of the families by a relevant phenotypic category or measure.

As LOD score tests require certain parameters to be specified, such as disease allele frequency and penetrance, non-parametric tests were developed to avoid the need to specify this information, which if incorrect, could generate erroneous results.

Non-parametric programs such as GENEHUNTER [38], which generate non-parametric linkage (NPL) Z scores, were developed for this purpose. However, simulation studies have shown that the LOD score method is more powerful, as long as both a recessive and a dominant model are tested and penetrance is relaxed to allow for heterogeneity [40, 41]. Non-parametric affected sib-pair (ASP) methods have also been employed in genome scans, such as SIBPAIR [42] and MAPMAKER/SIBS [43], but these are not thought to be as powerful as LOD score methods [44], and provide no information with regard to locus heterogeneity. Proponents of ASP methods claim that their findings will be more generally relevant than extended pedigree analysis, which may contain etiological genes relevant only for that family or a particular subgroup of families (or ethnic group). However, given the difficulties in finding etiological genes for schizophrenia, rare or otherwise, it would seem sensible to use the most powerful methods initially to maximize the chance of finding a gene, and subsequently to evaluate that gene in other sample groups.

Linkage continues to be the most likely avenue through which etiological genes for schizophrenia will be discovered. Given the difficulties in determining candidate genes for psychiatric disorders, using a method that requires no a priori knowledge of the genes involved, such as a whole-genome scan, is advantageous. Furthermore, larger, more homogeneous family collections have accelerated the pace of gene discovery by increasing the power and therefore maximizing the chance of detecting positive results and minimizing the chance of false negatives. As few linkage findings for schizophrenia have achieved the status of significant linkage according to present guidelines [34], suggestive linkages may be substantiated by replication in additional data sets [45]. Thus, the 15 published genome scans and additional regional linkage studies of schizophrenia have been compared for possible overlap of positively linked regions [46]. However, difficulties in these comparisons arise because of differences in power, the use of different marker data sets, different analysis methods, and the inaccuracies of linkage as a mapping tool for complex traits.

Estimates of power within a data set can be helpful when evaluating and comparing results between different genome scans. However, only 5 [47–51] of the 15 schizophrenia scans have estimated this quantity and of these, only 2 have calculated power under the hypothesis of heterogeneity [47, 51]. Under homogeneity, the study of Coon et al. [47] had 70% power to detect linkage only if the marker was at the linked gene, and this value dropped to between 18–39% if 20% of families were not linked to this locus. Power to detect linkage in the study of Brzustowicz [51] was >75% under all models when >90% of the families were linked, and 50–75% when 25% of families were not linked. The remaining studies, which estimated power under homogeneity only, reported power as related to λs values (sibling risk). Excellent power (>90%) was reported by Levinson et al. [48] to detect linkage for a λs of 10 and good power with either a λs of 3 ($\theta = 0$) or a λs of 5 ($\theta = 0.05$), but power was poor when λs was less than 3. Williams et al. [50] reported power of >95% for a λs of 3 and 70% power for a λs of 2. Lastly, assuming a θ of 0.05, Shaw et al. [49] reported a power of <60% for a λs of 2, 3, or 4. However, these estimates of power will be greatly reduced in the likely event of locus heterogeneity. In addition, a general estimate of power can be obtained by examining the sample size and study design. The question of how much variation in localization can be tolerated to constitute an overlap between studies is a topic of much debate at the present time.

Association studies in schizophrenia

Association studies have been employed extensively in the investigation of the genetic etiology of schizophrenia. In the year 2000 alone, around 35 such studies were published and a survey of these publications illustrates both the facility and the limitations of this approach. The vast majority of these are case-control studies, which have the advantages of being powerful for determining small genetic effects, relatively easy to collect, and requiring very little statistical knowledge to analyze. A major flaw in case-control studies is that of poorly matched controls, leading to population stratification and, in particular, false-positive results. Population stratification can be determined by typing the sample with random markers, which should not differ appreciably with respect to allele frequency between cases and controls, if the groups are

well matched [52]. However, this relatively simple procedure has yet to be utilized by those in the field, as evidenced by publications in 2000. Only four investigators used linkage disequilibrium mapping techniques [53–56], which are immune to problems of stratification because of the internal nature of the controls [57].

Anecdotally, three of the four linkage disequilibrium mapping papers showed significantly positive results, in contrast to the case-control studies where approximately 81% of findings were negative (with the schizophrenia phenotype). Some (≈ 31%) of these case-control publications achieved significance by testing for association with particular drug-induced side-effect or subtypes of schizophrenia, such as acute akathisia [58], tardive dyskinesia [59], paranoid schizophrenia [60], catatonic schizophrenia [61], and hallucination-delusion syndrome [62]. Analysis of multiple subphenotypes attracts the criticism of multiple testing, and of these six publications, only one contained an adjustment for multiple testing. Of the four case-control studies that obtained significantly positive results with the schizophrenia phenotype, one contained a supporting replication data set [63], and two contained a Bonferroni correction for multiple testing [60, 64], rendering their results more robust. It should be remembered that the standard threshold significance level of $p < 0.05$ will generate chance findings 1/20 times, suggesting that at least a couple of the 35 studies will represent false-positive results.

Of the case-control studies with negative findings for either schizophrenia or particular subtypes, only 55% of studies appear to have reasonable power to detect such an association. In particular, an investigation of the proneurotensin gene had impressive numbers of subjects (cases 362, controls 398) and also contained a power calculation as additional support for the study [65]. As both the number of subjects and the frequency of the genetic variant will affect the power of the study, a power calculation is a useful addition and can help to generate confidence in the findings. One publication had limited numbers of schizophrenic subjects both with ($n = 72$) and without ($n = 78$) acute akathisia, but supported the validity of their findings with a power calculation [58].

A further group of nine publications dealt with the genetic etiology of drug response in schizophrenia (pharmacogenetics), four showing significant association [66–69]. However, numbers in some of these studies, both positive and negative, were very low in some cases (<50), suggesting inadequate power was attained and that chance findings could be present.

A wide range of candidate genes were investigated, a few of which were following up on linked loci from genome scans. This latter group is likely to expand as many genome scans have now been performed for schizophrenia, and association studies will be integral in discovering causal gene defects at these linked loci. In a complex disease like schizophrenia, classic positional cloning methods such as recombination mapping may be of limited use. In summary, care must be taken in interpreting the results of association studies, with attention to issues of power, multiple testing, and significance level. For guidelines in such matters, useful publications can be found by both Risch [70] and Schork et al. [71]. Overall, approximately 50% of association studies published in the year 2000 appear relatively robust, a proportion which could no doubt be improved. The application of statistical rigor and perhaps a coordination of effort could greatly improve the power and validity of such studies.

The current findings

Established and recent results for those loci with the strongest evidence to date, evaluation of candidate genes at each locus and their possible role in disease pathogenesis are described below.

Chromosome 1q

The most significant evidence for linkage to schizophrenia to date is an HLOD score of 6.5 on chromosome 1q21–22 in 22 extended Canadian families [51]. The maximum multipoint HLOD score (between D1S1653 and D1S1679) was obtained under a recessive model with 75% of the families linked to this locus. These findings have recently been replicated in 13 large, extended British and Icelandic pedigrees, where a maximum five-point HLOD of 3.2 was found, also with a recessive mode of inheritance, near marker D1S196 on 1q23.3 (about 15 cM distal to D1S1679) [72]. These findings are consistent with those of Shaw et al. [49], who found an HLOD of 2.4 at D1S196 with 46% of families linked, in 70 pedigrees of European descent. The disease definition was similar in all three reports, that of schizophrenia and schizoaffective disorder (narrow or core definition), though Gurling et al. [72] also included unspecified functional psychosis in their core definition. In addition, an autosomal recessive mode of inheritance was used to generate the maximum HLOD scores in all three studies. Thus, the replication of not only the chromosomal region but also the disease model in three reports provides strong evidence for a susceptibility locus in this region.

An interesting candidate gene that maps to 1q21 has been evaluated in a number of different studies with conflicting results. The *hKCa3/KCNN3* potassium channel gene is thought to play an important role in modulating neuronal firing patterns, and mRNA has been found by in situ hybridization in the substantia nigra and ventral tegmental area and along the distributions of dopaminergic neurons from these regions into the nigrostriatial mesolimbic pathways [73]. This gene contains two poly-

morphic CAG repeat sequences, expansion of which has been considered as a possible cause of schizophrenia, due to the clinical observation of anticipation [74]. Chandy et al. [75] reported an excess of alleles with higher repeat number in schizophrenics as compared to controls, both in a group of French/Alsatian ancestry and when supplemented with a North American sample. However, a family-based study of 193 German parent/offspring trios showed no such association [76]. Another case-control study of Israeli Ashkenazi Jews corroborated the findings of Chandy et al. [75] finding an excess of high-repeat-number alleles in patients [73]. Similar findings were observed in an Indian schizophrenia sample [77] and a British sample [78], but no association was found in either a Chinese case-control sample [79] or a French family group [80]. Thus, evidence for the involvement of this gene in schizophrenia remains equivocal and its influence may be restricted to specific ethnic groups. Larger studies will be required to confirm or refute these findings, especially if the relative risk for schizophrenia associated with these alleles is <2 [81]. No other studies of candidate genes in this region have been reported recently.

Chromosome 2

Evidence for the involvement of this chromosome was first obtained in five families from Iceland using a non-parametric test at D2S135 ($p = 0.000001$), located at 2q12–13, though this locus was not confirmed in a follow-up international collaborative study comprising families from Austria, Canada, Germany, Italy, Scotland, Sweden, Taiwan, and the United States [82]. Modest evidence for linkage was found in 16 families from Palau in Micronesia at 2p13–14, with a maximum LOD score of 1.79 under a dominant model at D2S441 ($\theta = 0.2$) and also from a non-parametric test (APM = 4.87, $p = 0.0006$) [83]. Results from the NIMH consortium in 43 European-American families showed some evidence for linkage at D2S293 on 2q12 using non-parametric linkage analysis (NPL = 2.41, $p = 0.008$) [84], however this result is below the threshold for suggestive linkage. A two-stage study of initially 43 and then 71 Australian and American pedigrees, initially obtained a maximum NPL score of 2.03 ($p < 0.05$) at the more distal location of D2S436, but this dropped to 1.74 ($p < 0.05$) with the addition of the 28 pedigrees. Thus, there is little consensus for linkage on chromosome 2.

Chromosome 5

A maximum HLOD of 3.35 ($p = 0.002$) was found in the Irish families at 5q22–q31 under a narrow diagnostic model and a recessive genetic model [85]. This result was not subsequently replicated in a large multicenter study, the Schizophrenia Linkage Collaborative Group III (SLCG III) which included the Irish cohort [86]. Weakly positive results were seen nearby on 5q 33.1 in a Finnish cohort of 62 multiplex families, with a two-point LOD score of 1.36 at the *CSF1R* locus [87]. This was subsequently supported by a LOD score >3 in an extended group of 237 Finnish families [88], though these results on 5q22–31 were somewhat proximal to the original findings on 5q33.1. Recent results in a British and Icelandic cohort have provided further evidence of a susceptibility locus in this region with a three-point HLOD score of 3.6 ($p = 0.001$) using a broad diagnostic model and a dominant genetic model at D5S422 on 5q32–33 [72]. Thus, three HLODs of >3 have been obtained in and around this telomeric region of chromosome 5q.

There is also some evidence for a susceptibility locus on 5p, from the same British and Icelandic cohort, where a peak five-point HLOD of 2.8 at D5S426 (5p14.1–13.1) was observed, using a combined diagnostic system composed of liability classes and a dominant mode of inheritance [72]. This follows on from earlier findings of a multipoint LOD score of 4.37 at D5S111 (5p14.1–13.1) in one large pedigree from Puerto Rico using the broad diagnosis of schizophrenia and dominant transmission [89], and NPL scores of 2.55 ($p < 0.009$) at D5S426 and 2.49 ($p < 0.008$) at D5S111 in a group of 12 African-American pedigrees also using the broad diagnosis [72]. Therefore, evidence for involvement of this region is supported by the coincidence of peak LOD or NPL scores in the three different studies at the same two markers (D5S426 and D5S111), and a dominant mode of inheritance in two of the studies.

Chromosome 6

There have been extensive studies of the chromosome 6p22–24 region since the report by Straub in 265 Irish families, where a maximum LOD of 3.51 (0.4 cM from D6S296) was obtained using a broad disease definition and with 15–30% of families linked [90]. This region has been neither confirmed nor excluded by these subsequent reports [reviewed in refs. 91, 92]. Some recent reports have added support for this locus [93, 94], but on the whole, most recent genome scans have not detected any linkage to this region [48, 72, 84, 95, 96]. A whole-genome scan in a set of 11 Israeli and 36 German families using GENEHUNTER and ASPEX (multipoint sib-pair analysis) showed two broad positive peaks about 10 cM apart on 6p [94]. Using multipoint sib-pair analysis and a narrow phenotype definition, the first peak was around D6S260/274/1700 (LOD 2.15) and the second around *HLA-DQB1* (LOD 2.15). When the extended pedigrees were used, a maximum NPL score of 3.3 ($p = 0.001$) was obtained at the *HLA* locus. The TDT (as implemented in ASPEX) was also used to test for association in the presence of linkage and found weak evidence

at two markers in both regions, but these did not achieve significance. Another whole-genome scan of five Austrian pedigrees showed some evidence for linkage at 6p24 at marker D6S309 ($p = 0.0047$) [93].

Linkage in this region has also been investigated using subphenotypes of schizophrenia, and while no evidence was obtained for either the broad or narrow clinical phenotype in ten multiply affected Canadian pedigrees, linkage was found with positive-symptom scores analyzed as quantitative traits (in sib-pairs derived from these families) [97]. The marker D6S1960 (6p11–21) was significant (empirical $p < 0.05$) for linkage with the positive-symptom scale only. Linkage has also been found to 6p using the endophenotype of eye-tracking dysfunction (also SPEM) [98]. A maximum multipoint LOD score of 4.02 was obtained between D6S271 and D6S282, which are both centromeric to the *HLA* region. The *SCA1* gene, which contains a triplet repeat, is within the broadly implicated region on 6p and has previously shown some association with schizophrenia. Li et al. [99] searched for linkage disequilibrium in this region in both a Caucasian case-control dataset and in a Han Chinese sample of affected trios, but found no evidence for preferential transmission for any allele.

Given the evidence for linkage at or near the major histocompatability complex on 6p, a dense map of 13 markers covering 1.8 Mb of DNA in this region was tested in 80 British parent-offspring trios using TDT [53]. A haplotype of markers from the *NOTCH4* gene including a single nucleotide polymorphism in the promoter region and a trinucleotide repeat encoding leucine in the signal peptide domain showed the most significant association ($p = 0.0000078$) with schizophrenia. There was no association found 5′ to this gene, but four genes 3′ of *NOTCH4* (*PBX2, AGPAT1, AGER, TNXA*) could not be ruled out, as the most distal repeat, the tetranucleotide repeat in intron 17, was relatively uninformative. This study illustrates the usefulness of TDT as a mapping tool, once a region has been implicated by linkage. However, a Japanese case-control study using similar markers showed no such association [100], suggesting that this locus may not be etiological for schizophrenia in this ethnic group. The *TNFA* gene is also found at this locus (6p21.1–21.3) and a functional SNP (–308G>A) was typed in 84 schizophrenic patients and 138 controls [101]. The frequency of the –308A allele was significantly increased in the patient group ($p = 0.0042$).

Given the intensive study in this region and the general lack of consensus, if there is a susceptibility gene in this region, it may only be important in a limited number of cases or specific populations, or may be related to a specific component of the schizophrenia phenotype only. Much larger association studies will be required to fine map etiological variants at this locus.

Chromosome 8

Pulver et al. [102] first found preliminary evidence for linkage to 8p21–22, with some support from Kendler et al. [103] in 265 Irish pedigrees (LOD = 2.34, dominant model and broad disease definition). Subsequently, a genome scan in 54 American pedigrees achieved an NPL of 3.64 ($p = 0.0001$) at D8S1771 [104]. Recently, these findings have been further refined by removing some of the heterogeneity in these families [105]. First, 6 families of varied non-Caucasian origin were eliminated from the analysis. Then, these families were stratified according to co-segregating phenotypes (in non-schizophrenic relatives who were previously designated unknown) of either schizophrenia spectrum personality disorders (SSPD), or psychotic affective disorders (PAD). The SSPD group (7 families) showed an enhanced linkage to that found previously, with significance at marker D8S1771 using both the narrow (NPL = 5.04, $p = 0.000002$) and broad (NPL = 6.17, $p = 0.0000008$) definition of schizophrenia using GENEHUNTER. The phenotypic component that was largely responsible for this increase in significance was the inclusion of paranoid personality disorder. In a similar attempt to account for heterogeneity, Kendler et al. [106] hypothesized that the clinical variability was due to genetic heterogeneity, and investigated clinical features in families linked to 5q, 6p, 8p, and 10p in their Irish families. They found that affected individuals in families with evidence for linkage to 8p had significantly more affective deterioration, poorer outcome, more thought disorder, and fewer depressive symptoms than individuals in the other families.

In 21 Canadian families, Brzustowicz et al. [107] found a maximum two-point LOD score of 3.49 under a dominant narrow model with D8S136 ($\theta = 0.1$) on 8p21, though multipoint analysis reduced this LOD score to 2.13. There was no strong evidence for heterogeneity in this region. In addition, a recent genome scan in five British and eight Icelandic families has also validated this locus [72]. A maximum five-point HLOD of 3.2 was found at D8S1771 using a core, recessive disease definition, with only a slight reduction under homogeneity (LOD 3.0). Thus, evidence on 8p21–22 is fairly strong, though a broad region has been implicated. Furthermore, the findings of Pulver et al. [105] illustrate the importance of removing heterogeneity where possible for clarifying linkage findings.

Chromosome 10

A number of regions on chromosome 10 have been implicated, though only the 10p11–15 locus has been convincingly replicated. This locus was first found in the genome screen of European-American families from the NIMH consortium [84]. They observed statistically suggestive evidence for linkage at both D10S1423 on 10p13 (NPL = 3.4, $p = 0.0004$) and the nearby D10S582 on

10p12.31 (NPL = 3.2, p = 0.0006) using sib-pairs derived from these families with a narrow disease definition. A consortium that included the NIMH data set and seven other groups (824 independent affected sib-pairs in total) also provided some evidence for excess allele sharing in this region ($p < 0.05$), but only under the caveat of inter-sample heterogeneity ($p < 0.001$) [86].

Further evidence for this locus came from the 265 Irish pedigrees, where the maximum pairwise HLOD of 1.91 ($p = 0.006$) was found with marker D10S2443 also on 10p12.31 using an intermediate phenotypic definition, a recessive model, and segregating in 5–15% of pedigrees [108]. A genome-wide screen in 71 German and Israeli families found a LOD of 2.1 at D10S1714 on 10p13 when analyzed as affected sib-pairs [94]. The complete families were also analyzed using GENEHUNTER and an NPL of 3.13 ($p = 0.0015$) was observed in this region. TDT analysis achieved only nominal significance at D10S211 ($p = 0.03$) on 10p12.32–33, but this was one of only two regions that achieved even this level of significance in this study. Lastly, in part one of a four-stage genome scan in Finnish pedigrees, weak evidence of linkage was obtained at D10S2325 (ASP $Z_{max} = 1.87$) on 10p14, though this region was not followed up in the remaining stages of this study.

Significant transmission ratio distortion has been found at D10S211 in females (NPL = 1.84, p = 0.04), but not in males in 40 CEPH families [109], casting some doubt on the linkage findings. However, this hypothesis was negated in the NIMH sample by using only male affected sibling pairs for analysis, as evidence for linkage remained (D10S582, NPL = 2.9, p = 0.002) [110]. However, checking for segregation distortion in other markers that may be linked or associated to this region will be necessary in the future to eliminate this possibility.

The remarkable aspect of this locus is the small region around which linkage has been found in the four separate groups, which should facilitate the process of gene discovery and evaluation.

Chromosome 13

Significant linkage to 13q32 was obtained using a non-parametric analysis in 54 multiplex pedigrees of mixed ethnicity (majority of European descent) near D13S174 (NPL = 4.18, p = 0.00002) [104]. Parametric analysis of this region favored a recessive model (HLOD = 3.2), with a narrow definition of disease and approximately 48% of families linked to this locus. This linkage was not enhanced by later stratification according to co-segregating phenotypes of SSPD and PAD, as it was present to varying degrees in all three phenotypic subgroups [105]. In 21 Canadian extended pedigrees, a maximum multipoint HLOD of 4.42 was found 0.1 cM centromeric to D13S793 (around 9 cM distal to D13S174), under a recessive model and using a broad diagnostic classification, and with 65% of families linked to this locus. A consortium from eight centers, which included 53 of the 54 multiplex pedigrees of mixed ethnicity described above, failed to find any support for this locus in the rest of the samples [86]. Weaker support for this locus was found in the Irish sample, with a multipoint HLOD of 1.36 under a recessive model and using a narrow definition of disease [85], and also by Shaw et al. [49] with an HLOD of 1.25 under a dominant model and a narrow disease definition (15 cM centromeric to D13S793). Nonetheless, the findings of both Brzustowicz et al. [107] and Blouin et al. [104] are both significant according to the recommended guidelines for linkage [34], the loci are very close together, and maximum LOD scores were found under similar genetic models. This region of chromosome 13 is fairly gene poor and no studies on potential candidates have been thus far reported.

Chromosome 15

In contrast to the other chromosomal locations implicated in schizophrenia thus far described, a chromosome 15 locus has not been detected in any of the genome scans. However, there is evidence for linkage to an endophenotype, the P50 gating deficit. A high incidence of smoking is found in schizophrenics [111] and nicotine has been shown to normalize the P50 inhibitory deficit, suggesting that schizophrenics may be using nicotine to self-medicate this attentional deficit. Pharmacological studies suggested that the α7-nicotinic receptor was most likely responsible for this mechanism, and decreased expression of this receptor in interneurons of the hippocampus has been observed in postmortem brain tissue of schizophrenics [reviewed in ref. 112]. The α7-nicotinic receptor is a ligand-gated ion channel that admits calcium ions into cells, and it has been proposed to have various developmental roles. Freedman et al. [18] isolated a P1-derived artificial chromosome containing the entire α7-nicotinic receptor gene (*CHRNA7*) and a microsatellite marker D13S1360, which is 120 kb upstream from the putative promoter of this gene. This gene was mapped to chromosome 15q13–14 using radiation hybrid mapping. Assuming autosomal dominant transmission and using the P50 gating deficit as a phenotype generated a maximum LOD score of 5.3 at D15S1360 in nine multiply affected European American families. Linkage to schizophrenia was also tested using affected-only analysis, and generated a modest (non-significant) LOD score of 1.33 ($\theta = 0.07$) at D15S1360.

These findings have recently been replicated and extended in parent-child triads from the NIMH sample [113], following on from modest linkage findings with the classical schizophrenia phenotype in this cohort (D15S1360, Z = 1.5, p = 0.002) [112]. Using ETDT, sig-

nificant genotype-wise disequilibrium with the P50 phenotype was found at D15S165 ($p < 0.007$), a marker located within 1 Mb of *CHRNA7*, although linkage disequilibrium was actually observed with a haplotype (D15S165-D15S1360-D15S144). Stratification into African Americans and European Americans showed similar disequilibrium in both groups at D15S165, but D15S144 was significantly responsible for transmission disequilibrium in the European American sample only. When the TDT was repeated using only the oldest affected child in each family (a test of association), significance was retained for both an allele-wise ($p<0.044$) and a genotype-wise ($p < 0.04$) test.

Similar results have been found in a sample of 20 Southern African Bantu-speaking families, but with schizophrenia as the phenotype [54]. Using an extended set of 20 markers in this region, a non-parametric affected-only multipoint analysis generated scores of 1.81 at D15S1043, 1.79 at D15S1360, and 1.80 at D15S1010 ($p = 0.037$ for all). Transmission disequilibrium testing using a restricted allele and haplotype set from D15S1043 and D15S1360 found significant excess of transmission of the 1.2 haplotype to affected offspring ($p = 0.004$).

Thus, the evidence is compelling for a susceptibility gene for the P50 gating deficit commonly found in schizophrenics in this region and lends support to the hypothesis that endophenotypes may be more closely related to the genetic defect than schizophrenia. Complications have arisen in assessing this gene, as it is now known to have a complex genomic structure. The gene has ten exons and is part of a duplicated cassette of expressed sequences. A duplicon, 1 Mb centromeric to *CHRNA7*, is composed of four novel exons and six exons that are copies of *CHRNA7* exons (5–10). This duplication is expressed in both the brain and the peripheral blood cells in normal subjects, but is missing in some schizophrenic subjects [114]. Mutation screening of the coding regions of *CHRNA7* have found no mutations to account for the linkage findings, suggesting the involvement of either promoter polymorphisms, the duplicated upstream gene, or other nearby genes.

Chromosome 22

Chromosome 22q11.2 microdeletions have been strongly associated with schizophrenia. A recent study has shown that 26% of adults with 22q11.2 microdeletions had either schizophrenia or schizoaffective disorder and, conversely, that up to 2% of patients with schizophrenia and 6% of patients with childhood-onset schizophrenia, had 22q11.2 deletions [reviewed in ref. 31].

Linkage findings have tended to cluster in a region of around 4–5 cM on 22q13.1, slightly telomeric to the 22q11.2 locus, with a peak number of positive findings at D22S278 [reviewed in ref. 115]. Since then, linkage disequilibrium analysis has been largely employed in evaluating markers in this region. The schizophrenia collaborative linkage group for chromosome 22 found further support for a susceptibility locus close to D22S278, by using the TDT in 574 multiply affected families with a narrow definition of schizophrenia (allele wise: $p = 0.015$, genotype wise: empirical p value = 0.009). These results followed on from positive sib-pair linkage findings in the same group, with the same marker ($p = 0.001$).

Linkage has also been found at D22S315 on 22q11–12 (LOD 3.55) using a composite endophenotype of P50 auditory sensory gating and antisaccade ocular motor performance in eight Utah families [116]. This region contains the gene for the soluble form of catechol-O-methyl transferase (*COMT*), which degrades catecholamines and inactivates catechol drugs such as L-dopa, methyldopa, and isoproterenol in the central nervous system [reviewed in ref. 117]. Therefore, as its function fits with the monoaminergic hypothesis of schizophrenia, it has been evaluated as a candidate for schizophrenia in a number of studies. A French case-control sample (schizophrenics = 137, controls = 140) tested for association with five exonic SNPs from the *COMT* gene, and found an association with a variant (Pml I, $p = 0.034$, OR = 1.82) that is in complete linkage disequilibrium with a common Met-Val^{158} substitution known to affect enzyme activity [117]. Two dinucleotide repeats and six SNPs at the *COMT* locus were tested for transmission distortion in 198 schizophrenic Chinese family trios, and a significant excess in transmission of a five-marker haplotype was observed (287G : 186C : Val158 : 900insC : ARVCF930C, $p = 0.0006$). Another case-control study found no association between the Met-Val^{158} substitution and schizophrenia in a Chinese sample (198 patients, 188 controls), though a significant difference in age of disease onset between different genotypes was found ($p = 0.005$) [118]. No association was found with SNPs from the *COMT* locus in 49 British trios [119], though this data set may not have had sufficient power to detect an effect of this estimated magnitude. Thus, there is some evidence for a marginal effect of a *COMT* gene variant in schizophrenia, which will require larger studies to map the etiological mutation.

Another variant (VNTR) in a gene near this locus (*YWHAH*) has also shown significant association with schizophrenia ($p < 0.05$) and early-onset schizophrenia ($p < 0.02$) in a Japanese sample of cases ($n = 118$) and controls ($n = 118$) [120]. The *YWHAH* gene maps to 22q12.1–q13 and codes for the 14-3-3 eta chain protein, which is abundant in the mammalian brain and mediates interactions between diverse biological molecules (tyrosine and tryptophan hydroxylases, PKC, Raf-1, and BAD). Bell at al. [121] performed a systematic single-strand conformation polymorphism screen of this gene and found 12 potential variations, including the 5′ VNTR

that was evaluated in the Japanese study. One variant in the untranslated region of exon 1 (408T>G) was found to occur more frequently in schizophrenics than controls ($p = 0.01$), though significance was reduced after fivefold correction for multiple testing. This association remains to be confirmed in additional data sets.

Linkage has also been found to this region for periodic catatonia, a subtype of catatonic schizophrenia. A LOD score of 1.85 ($p = 0.0018$) in a single large German pedigree was obtained for D22S1169 on 22q13.33 [122]. A candidate gene from this region (*WKL1*) that encodes a putative non-selective cation channel, expressed exclusively in the brain, was screened for mutations and a Leu309Met substitution detected [123]. This variant co-segregated with the disorder in this large pedigree, though ten unaffected individuals also carried the variant, indicating that this allele has incomplete penetrance. This allele was not found in any control subjects or in any individuals from three other German families that had also shown evidence of increased sharing in this region. These results would suggest that this variant is likely to be responsible for periodic catatonia in only a small number of subjects.

Summary

Thus, the findings to date using linkage and subsequent association analysis are fairly encouraging, and suggest that the discovery of the first schizophrenia susceptibility gene may be imminent. However, multilocus models will no doubt be required to verify and refine some of these findings, especially where epistatic interactions between loci exist. Under-utilization of these multilocus methods is, in part, due to their inherent limitations, and further development of these methods will be necessary to bring them into common use. Two-locus linkage is currently possible using the classical method of Lathrop and Ott, which is implemented in the LINKAGE package, though this suffers from problems of increased parameterization and computation time. Recently, an additional model-free multilocus linkage test designed for affected relative pairs, in which additive and multiplicative models may be tested, was developed [124]. This test was used to analyze data from type 1 diabetes sib-pair families, and showed increased power over single-locus models to detect additionally linked loci. ETDT has also recently been generalized to include two-locus models [125]. Thus, progress may be, in part, dependent on further development and implementation of these methodologies.

Association studies in schizophrenia

In addition to those association studies that are related to linkage findings, there are investigations of many other candidate genes that have been implicated by either neurobiological findings or alternative strategies. The most prominent group of these includes dopamine and serotonin receptors, and their regulators.

Dopaminergic variants

The role of the dopaminergic system has been suggested by the antipsychotic effects of dopamine D2/D3 receptor blockers [126] and by the psychotic symptoms induced by indirect dopamine agonists, such as amphetamine and cocaine [127]. Over-activity of dopaminergic neurons in limbic areas of the brain has recently been postulated to be responsible for positive symptoms, and under-activity in the frontal cortex accountable for negative symptoms and cognitive impairment in schizophrenia [128]. The *DRD3* gene (chromosome 3q13.3) is selectively expressed in brain regions associated with control of emotions, motivation, and reward, and recognizes most antipsychotic drugs [129]. Two meta-analyses of association studies (both >5000 subjects) of dopamine D3 receptor gene variants, in particular a Ser-Gly variant in exon 1 detected using the *BalI* restriction enzyme, have shown some of the strongest evidence to date for association with schizophrenia [130, 131], although these results are not conclusive [81]. In the first meta-analysis, when all subjects were stratified into groups according to geographical origin, an excess of homozygosity and 1-1 genotype (*BalI* polymorphism) was observed in the African and Caucasian groups only ($p<0.05$). The second study found no significant evidence for heterogeneity between samples, and also found a significant excess of homozygous genotypes ($p = 0.0002$, OR = 1.23, 95% CI = 1.09 – 1.38). The data sets analyzed were similar, except that Dubertret et al. [131] had 3/29 groups not in Williams et al. [130], and Williams et al. [130] had 9/38 groups not in Dubertret et al. [131]. Nonetheless, despite the differences in level of significance, both studies showed positive associations with homozygosity of this *DRD3* variant. The functional significance of this variant is unclear, though the 2-2 genotype has shown higher dopamine binding affinity than either the 1-1 or 1-2 variants. The DRD3 receptor may function as a dimer as do other G-protein coupled receptors, and the mutation may interfere with dimerization. Also possible is that the Ser9Gly variant is not the etiological mutation, but is in linkage disequilibrium with another variant in this region. A recent report described a screen of the 5′ leader region for polymorphisms, and evaluated a haplotype of these variants in an English case-control sample that had been previously reported to lack association with the Ser9Gly variant [132]. A significant association was found with the frequency distribution of these four variants and disease ($p = 0.0042$), with an excess of a heterozygous genotype in patients. This is in contrast to the meta-analyses

findings, where an excess of homozygotes was found, suggesting that either these other variants are important or that the results of this small study are prone to sampling error. The 5′ region was also screened by Ishiguro et al. [63], and haplotypes of the −712G>C, −205A>G, and Ser9Gly variants were found to be significantly associated with schizophrenia (corrected p = 0.007) in Japanese patients, whereas there was only a marginal association between disease and the Ser9 allele alone (p = 0.02). Evaluating all these variants in larger, preferably family-based studies will be necessary to clarify the role of these gene variations.

The same *DRD3* variant (Ser9Gly) has also been associated with tardive dyskinesia (TD), a common side effect of antipsychotic drug use. TD is a chronic movement disorder found in around 20% of drug-treated schizophrenics. Following on from the original findings by Steen et al. [133] of an association between TD and the 2-2 genotype in a Norwegian case-control group was a replication of these findings in a case-control study of an Israeli sample. This study found a significant excess of allele 2 in schizophrenics with TD compared to normal controls (p = 0.0007) [134]. However, no evidence for an association of *DRD3* and TD was found in a German sample (n = 157) [135]. Most recently, an association was observed between the 2-2 genotype and acute akathisia, another motor side effect of antipsychotics (p = 0.0223) [58]. Clarification is stilll needed, however, as to whether subjects with the 2-2 genotype are more prone to developing either of these motor side effects, or if this genotype is associated with a subtype of schizophrenia with a high prevalence of these side effects. To test the hypothesis that the *DRD3* 2-2 genotype confers risk to a subtype of schizophrenia with TD, Lovlie et al. [136] investigated a group of drug-naive patients in India and found no significant difference in incidence of the 2-2 genotype between patients with and without TD or between patients and controls, though this study had limited power to detect such effects.

No other dopamine receptor genes have been consistently associated with schizophrenia, or with drug response or side effect in schizophrenia [reviewed in ref. 137]. However, an excess of 172 - to 176-bp alleles of a dinucleotide repeat in the brain-derived neurotrophic factor gene (*BDNF*), found on chromosome 11p13, which promotes and maintains *DRD3* expression, was recently found to be associated with a subset of patients with late-onset schizophrenia, who were neuroleptic responders and non-substance abusers [68]. Previously, no linkage or association was found in the Irish families for the *BDNF* gene variant [138], and these current findings await replication.

Serotonergic variants

As with the dopaminergic system, the serotonergic system has been implicated through its involvement in drug response. Many drugs used for treatment of schizophrenia have serotonin neurons as their principal site of action, such as serotonin re-uptake inhibitors and atypical antipsychotics. Hallucinogenic drugs such as LSD, and euphoriants such as MDMA (ecstasy), are also known to act through the serotonin (5-HT) system. Genes regulating the synthesis (*TPH*), storage (*VMAT2*), membrane uptake (*HTT*), and metabolism (*MAOA*) of 5-HT, as well as many receptors, have been investigated in schizophrenic samples. The mechanisms involved, current findings, and the pharmacogenetics of these genes in neuropsychiatric disorders have recently been reviewed [139]. However, due to the frailties of case-control studies as discussed previously, such studies have been omitted from this review with, instead, a focus on family-based studies.

The most replicated finding between serotonin receptors and schizophrenia has been with an exonic variant (102T>C) in the 5-HT_{2A} receptor gene (*HTR2A*), found on chromosome 13q14.2. The 5-HT_{2A} receptor is a post-synaptic G-protein-linked receptor that activates phosphoinositide hydrolysis, and clozapine and other atypical antipsychotic agents have been shown to specifically antagonize this receptor. This gene is present on chromosome 13q14.1–14.2 and contains a number of variants (−1438G>A, His452Tyr, and 102T>C). Most data are available on the 102T>C variant, and a meta-analysis performed in 1997 with subjects from 15 ethnically diverse studies (1533 patients and 1771 controls) showed an excess of the 102C allele in schizophrenic subjects (p = 0.0009, OR = 1.18, 95% CI = 1.07–1.31) [140]. However, this meta-analysis contained a substantial proportion of data from a European multicenter collaboration (EMASS), previously published by Williams et al. [141], close inspection of which reveals differences in allele frequencies in the control groups between ethnically diverse European countries (Sweden and Italy, p = 0.0032). Thus, caution should be exercised when interpreting these results. Subsequently, this association was replicated in a sample of 63 British parent/offspring trios (p = 0.001), and the 102T>C was shown to be in complete linkage disequilibrium with −1438G>A, a promoter polymorphism [142]. In vitro expression studies showed no differential basal or stimulated promoter activity in HeLa cells, though modest differences are difficult to detect with this system and other cell types may respond differently. Since then, no association has been found in a Japanese case-control study with either −1438G>A or 102T>C and schizophrenia [143], and there was no correlation between −1438G>A variant and density of frontal cortical 5-HT2A receptors in autopsied tissue from 58 schizophrenic and 64 non-schizophrenic subjects [144]. However, polymorphic imprinting has been found in this region in brain tissue [145], suggesting that elucidation of the role of this gene will be more complicated than anticipated.

Pharmacogenetic studies

The number of pharmacogenetic reports being published is on the increase, no doubt because of the important potential clinical relevance of these studies. Recent reports have mostly involved either dopamine receptors or cytochrome P450 (*CYP*) enzyme genes (genes that affect drug metabolism). This subject has been comprehensively reviewed recently [146]. Genetic variants in the enzymes *CYP2D6*, *CYPC19*, and *CYP2C9* have been shown to influence the metabolism of neuroleptic drugs. For each of these enzymes, there are extensive (EM) and poor (PM) metabolizers. Approximately 5–10% of Europeans, 2% of Asians, and 7–8% of Africans are PMs of debrisoquine (similar to neuroleptics), as they produce a functionally abnormal or inactive *CYP2D6* enzyme. Atypical antipsychotics such as clozapine exhibit large inter-individual variability in bioavailability, plasma concentrations, and clearance, and are metabolized by both *CYP2D6* and *CYP2C19*. *CYP2D6* has six mutant alleles that code for inactive enzymes: PMs carry two of these alleles and intermediate metabolizers carry one. The prevalence of these alleles was assessed in schizophrenics ($n = 86$) and controls ($n = 145$) and significant differences were found in allele frequency ($p = 0.002$), genotype distribution ($p = 0.016$), and phenotype prevalence ($p = 0.018$) [67]. For allele 4, an OR of 2.54 for schizophrenia and an OR of 5.02 for the PM phenotype were found. As being a PM is related to the development of side effects, patients undergoing long-term psychoactive drug therapy were also investigated. Significant differences between patients with and without side effects were obtained in allele prevalence ($p = 0.002$), genotype ($p = 0.029$) and phenotype ($p = 0.002$) distribution, generating relative risks (RR) of 2.63 for allele 4 and 5.33 for allele 6, and an RR of 7.08 for a PM phenotype.

Drug metabolism is also influenced by diet and, in particular, smoking, whose high prevalence in schizophrenia has already been discussed. Smoking is a potent inducer of the *CYP1A2* enzyme, which also influences antipsychotic drug metabolism. Basile et al. [147] hypothesized that the extent of *CYP1A2* induction may contribute to the development of TD, a common side effect of neuroleptic therapy. TD was measured, using the Abnormal Involuntary Movement Scale (AIMS), in 85 schizophrenics who were typed for an intronic biallelic polymorphism (C>A) associated with reduced inducibility of *CYP1A2*. The mean AIMS score in C-C subjects was 2.7- and 3.4-fold higher than in those with the C-A or A-A genotypes, respectively ($p = 0.0070$). Furthermore, this relationship was exaggerated in the 44 smokers, with higher AIMS scores of 5.4- and 4.7-fold in the C-C homozygotes when compared to heterozygotes and A-A homozygotes, respectively ($p = 0.008$).

Thus, there is tantalizing evidence to warrant further investigations of these genes in schizophrenics.

Conclusions and future directions

The difficulties elucidating cause in complex diseases have created a considerable barrier to progress up to now, and have necessitated the development of statistical and computational tools to address this complexity. Moreover, phenotypic assessment has been scrutinized for its relationship with genetic liability and care taken to account for genetic heterogeneity, boding well for future studies. Recent reports have suggested that detecting genes for schizophrenia, while difficult, is not impossible, with a growing body of positive and replicated evidence being accrued. These studies also support the existence of several major susceptibility genes, which many believe to be additive, though undoubtedly epistasis will exist between certain loci. Evidence also exists for heterogeneity between different ethnic groups, though it should be remembered that differences in allelic frequency of marker alleles between different populations will affect the ability of analytical methods to detect linkage or association, as power is related to allele frequency. Moreover, even under an additive genetic model, some etiological alleles may be so common in a particular population that they are rendered undetectable using linkage methods. For example, the enhanced power of a meta-analysis of 16 family-based association studies investigating type 2 diabetes was required to confirm the influence of a PPARγ variant (Pro12Ala) as it has a frequency of 85% and a modest OR (1.25, $p = 0.002$) [148].

Indeed, much evidence of progress has come from the successful study of other complex diseases, such as type 1/type 2 diabetes and Alzheimer disease, where susceptibility genes have been discovered. Notwithstanding the added complexity of behavioral disorders, these other complex diseases have provided paradigmatic examples that should prove useful in the search for liability genes in psychiatric disorders. Notably, these include an emphasis on the sample size and configuration required to detect modest genetic effects, the use of homogeneous samples, and the combined utility of both linkage and association approaches. In addition, the incorporation of multilocus interactions has proved important. Elucidation of the susceptibility gene for type 2 diabetes, *NIDDM1* (*calpain 10*) was advanced by the inclusion of a gene-gene interaction on chromosome 15 (near *CYP19*) in Mexican American families [149, 150]. In schizophrenia research, these issues are currently being addressed, though the difficulties in collecting sufficiently large samples will take some time to address. However, the accelerated progress of the human genome project should contribute to the en-

tire process by generating a full sequence map onto which markers may be unequivocally mapped and by discovering and mapping the remaining novel genes. In addition, the sheer volume and diversity of research investigating schizophrenia, and the rapid progress of genetics in general, especially over the last 5 years, augur well for the future of psychiatric genetics.

Acknowledgements. Supported in part by the National Institute of Mental Health grants K08 MH01392 and R01 MH62440 and an Independent Investigator Award from the National Alliance for Research on Schizophrenia and Depression (L. M. B.), and the Medical Research Council of Canada grants MT-1225 and MOP-38099, Ontario Mental Health Foundation, and an Independent Investigator Award from the National Alliance for Research on Schizophrenia and Depression (A. S. B.).

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