REVIEW

# Genetic analysis of psychiatric disorders associated with human chromosome 18

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## Abstract

Current models on the etiology of psychiatric disorders support the idea of a biologic cause as well as interactions of biologic systems with the environment. The elucidation of the genetic etiology is of paramount importance to understand the cause of psychiatric disorders. Human chromosome 18 was identified as one of the first chromosomes to be aberrant in psychiatric patients and has subsequently served as a model to identify the molecular cause. In this article I review a multitude of methodologies that can be used in determining the genetic basis of schizophrenia, affective disorder and autism associated with human chromosome 18. These strategies include the use of chromosome aberrations, linkage and association studies, mouse–human comparative genomics, mutation analysis on candidate genes, trinucleotide repeat expansion studies, search for genes demonstrating parental effects and bioinformatics. Current data from the use of these methods are cited from the literature. Linkage and association studies have suggested at least 2 candidate loci on the short and long arms of chromosome 18 for each of these psychiatric disorders. Some loci are supported by the mapping of chromosome aberrations from psychiatric patients. Mutation analyses of psychiatric patients with 4 candidate genes (*NEDD4L*, *IMPA2*, *PACAP* and *GNAL*) mapping within these loci have been unsuccessful, although an association was found with the *IMPA2* gene in patients with schizophrenia. With these methods and findings, our understanding of the cause of psychiatric disorders associated with human chromosome 18 has improved and will advance, especially with emerging data from the human and rodent genome projects.

## Résumé

Les modèles courants de l'étiologie des troubles psychiatriques appuient le concept d'une cause biologique, ainsi que celui d'interactions entre des systèmes biologiques et l'environnement. Il est primordial de clarifier l'étiologie génétique des troubles psychiatriques si l'on veut en comprendre la cause. On a identifié le chromosome humain 18 comme un des premiers chromosomes à présenter une aberration chez les patients en psychiatrie et on l'a utilisé ensuite comme modèle pour déterminer la cause moléculaire. Dans cet article, je passe en revue une multitude de méthodologies qu'il est possible d'utiliser pour déterminer l'origine génétique de la schizophrénie, du trouble affectif et de l'autisme associés au chromosome humain 18. Ces stratégies comprennent l'utilisation des aberrations chromosomiques, les études de liens et d'associations, la génomique comparative entre souris et humains, l'analyse de mutations de gènes candidats, les études sur l'expansion de la répétition des trinucléotides, la recherche de gènes démontrant des effets parentaux, et la bio-informatique. Les données courantes tirées de l'utilisation de ces méthodes proviennent des publications. Des études de liens et d'associations ont indiqué au moins deux lieux candidats sur les bras court et long du chromosome 18 pour chacun de ces troubles psychiatriques. La cartographie des aberrations chromosomiques chez des patients en psychiatrie appuie certains de ces lieux. Des analyses de mutation chez des patients en psychiatrie présentant quatre gènes candidats (*NEDD4L*, *IMPA2*, *PACAP* et *GNAL*) correspondant à ces lieux n'ont pas porté fruit, même si l'on a trouvé un lien avec le gène *IMPA2* chez des patients atteints de schizophrénie. Ces méthodes et ces constatations ont amélioré notre compréhension de la cause des troubles psychiatriques associés au chromosome humain 18 et la feront progresser, compte tenu spécialement de nouvelles données provenant des projets de cartographie du génome humain et de celui de rongeurs.

| Glossary of terms used in the text | |
|---|---|
| Term | Definition |
| Allele | One of several alternative forms of a gene or marker due to variation in the DNA sequence |
| Complex trait or disease | A trait or disease that involves 2 or more genes and possible interactions between the environment and genes |
| Deletion chromosome | An abnormal chromosome with an internal deletion or a deletion at the end of the chromosome |
| Dominant | One altered copy of a gene is required to form the trait |
| Genetic drift | Random changes in allele frequencies in the population |
| Genetic heterogeneity | Two or more genes produce the same phenotype |
| Genotype | The alleles of genes or markers in an individual |
| Genotyping | Determination of the genotype of selected genes or markers (or both) |
| Inversion chromosome | An abnormal chromosome with an inverted DNA segment |
| Isochromosome | An abnormal chromosome with 2 identical arms |
| Locus, loci | A unique chromosome location defining a gene or DNA sequence that is associated with a phenotype |
| Major gene | A gene with a major role in the formation of a trait |
| Marker | Mostly a polymorphic DNA sequence used in genetic mapping |
| Meiosis | A reductive cell division process that occurs solely in the testis and ovary during the formation of gametes |
| Microsatellite marker | A polymorphic marker comprising usually 2–4 base pairs of tandem DNA repeats |
| Minor (susceptibility) gene | A gene with a minor role in the formation of a trait |
| Mosaic | Two or more genetically different cell lines derived from a single zygote |
| Oligonucleotide | A short piece of DNA |
| Orthologue | A functionally equivalent copy of a gene in a different organism |
| Penetrance | The probability of expressing a phenotype from a particular genotype |
| Phenotype | The manifestation of a trait |
| Polygenes | A combination of genes that contribute to a trait |
| Polymorphic | The existence of 2 or more alleles of a marker or gene at a frequency of at least 1% in the population |
| Recessive | Two altered copies of a gene are required to form the trait |
| Recombination | The exchange of DNA during meiosis |
| Recombination fraction | The frequency of DNA exchange between 2 genetic markers |
| Ring chromosome | An abnormal chromosome that looks like a ring, and is formed by the breakage and union of the extreme ends of the chromosome |
| Single nucleotide polymorphic marker | A polymorphic marker comprising a single base-pair variation in the DNA sequence |
| Translocation chromosome | A chromosome formed by breakage and reunion between 2 nonidentical chromosomes |

Psychiatric disorders were originally proposed to have an environmental etiology.[1] Thus, cranial trauma or an abnormal social environment, particularly in the presence of the parents, are a few examples that may induce behavioural problems. Determination of the underlying cause of psychiatric disorders was and still is challenged by difficulties in phenotype classification and methodologic designs. Subsequent improvements in genetic methods to study human pedigrees segregating with disease phenotypes contributed to evidence suggesting that not only can the environment influence the cause of a psychiatric disorder, but there is also a biologic basis. Genetic studies have suggested the presence of defective major genes and minor (susceptibility) genes as the underlying cause of psychiatric disorders.[1] Models have favoured the hypothesis of multiple genes acting synergistically with each other and the environment in causing psychiatric disorders.[1] The molecular players and the quantification of factors involved in such complex traits are unknown, based on our current understanding of the exact etiology. Genetic studies, mostly within the last decade, have provided evidence for possible loci contributing to the cause of affective disorder on human chromosomes 1, 4, 6, 10, 12, 13, 18, 21, 22 and X,[2] schizophrenia on human chromosomes 1, 6, 8, 10, 13, 15, 18 and 22,[2] and autism on human chromosomes 2, 3, 4, 7, 8, 10, 11, 12, 15, 16, 18 and 20.[3,4] In this paper I review strategies that can be

used to identify chromosome regions and subsequently genes that contribute to schizophrenia, affective disorder and autism, in particular on human chromosome 18. Cytogenetic analysis on mentally ill patients as early as the 1960s reported human chromosome 18 as one of the first chromosomes identified as aberrant or defective in these patients.[5] Subsequently, human chromosome 18 has served as a model to study many different ethnic families affected with psychiatric disorders, in a search for the molecular cause or genes. The generic strategies used to define these intervals on human chromosome 18 and subsequently the genes are outlined in Fig. 1. The underlying evidence of what suggested that these disorders had a genetic basis is proposed, followed by genetic methods that can be used to

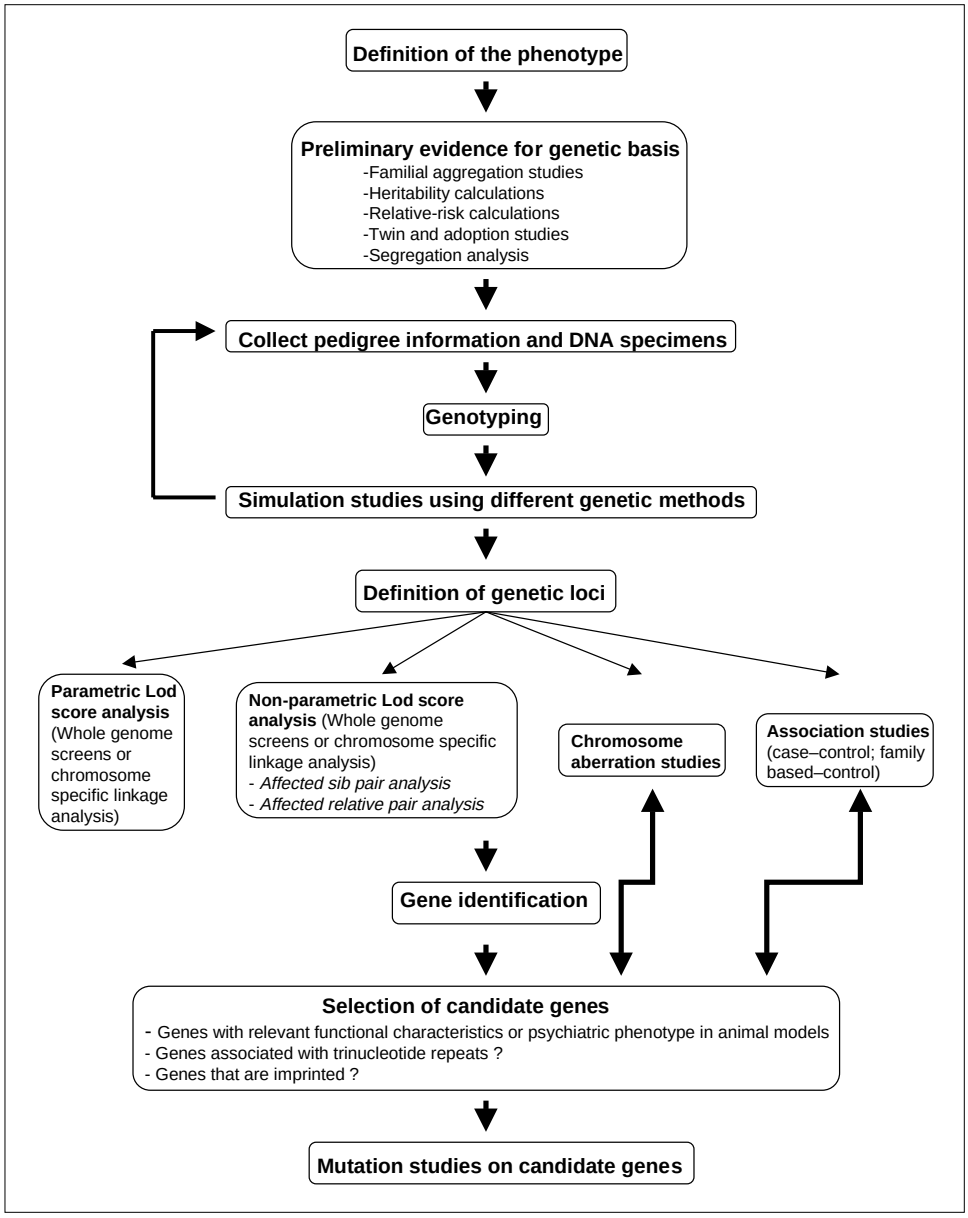


**Fig. 1: Overview of methods used to ascertain the genetic component of a disease and subsequent steps in the identification of chromosomal regions and genes that contribute to the phenotype. Lod = logarithm of the odds.**

identify major or susceptibility genes. Finally, findings reported in the literature obtained from a selection of these methods are described in families and isolated subjects affected with schizophrenia, affective disorder and autism. A review of these methods is important since they are applicable to recent improved genotyping technologies and emerging data from the human and rodent genome projects, in search of genes that contribute to psychiatric disorders on human chromosome 18.

## Definition of the phenotypes

Phenotype definition with respect to the classification is very pertinent in determining whether the etiology is from single or multiple causes, and whether these causes are independent or associated with each other. Misclassification and use of affected subjects who are inappropriately grouped as having the same disease phenotype leads to spurious genetic analysis and subsequently incorrect findings. The phenotypes described below that are associated with schizophrenia, affective disorder and autism are established and cited from the *International Classification of Diseases*, 10th edition,[6] and the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition.[7]

### *Schizophrenia*

A diagnosis is established from the recognition of an assortment of symptoms prevalent for at least 6 months and includes at least 1 month of active-phase symptoms.[6,7] This disorder leads to social and occupational dysfunction, poor personal hygiene and learning problems. Both positive and negative symptoms are observed. The positive symptoms include distortions in thought content (delusion), perception (hallucinations), disorganized speech and grossly disorganized or catatonic motor behaviour (decreased reaction to environmental stimuli and resistance to instructions). The negative symptoms include affective flattening (unchanged facial expression with poor eye contact and reduced body language), algoia (decreased fluency and productivity of thought and speech) and avolition (inability to initiate and persist goal-directed activities). The prevalence is estimated between 0.5% and 1.5% in the population. The usual age of onset is during adolescence, and occurrence before adolescence is rare. The disorder is more common in men between 18 and 25 years of age, with a 5-year lag in onset in women. Late-onset schizophrenia occurs after the age of 45 years, mostly in women (3%–10%). The typical symptoms in women are paranoid delusions, hallucinations and flattening of affect, whereas men usually demonstrate more negative symptoms (affective flattening, avolition, social withdrawal). The prognosis is better in women. However, in general, the overall prognosis is poor, since 20% to 40% of patients attempt suicide over the course of the illness. There is also a greater frequency of violent behaviour among these patients.

### *Affective disorder*

This is classified as bipolar disorder I or II.[6,7] For the diagnosis of bipolar disorder I, 1 or more manic episodes must be followed by 1 or more major depressive episodes. In some adolescents (10%–15%) with recurrent major depressive episodes, bipolar disorder I and mixed episodes (major depressive, manic, hypomanic) will develop. In men, the first episode is more likely to be a manic episode, and in women a major depressive episode. Rapid cycling is more common in women than in men, but the interval between episodes decreases as the person ages. The prevalence is estimated at 0.4% to 0.6% in the population, with men and women equally affected. The age of onset is usually early adolescence. Like those affected with schizophrenia, the prognosis is also poor if the condition remains untreated, since 10% to 15% commit suicide, most likely from depression. Manic episodes may also induce abusive and other violent behaviour. Moreover, learning difficulties and occupational and social dysfunction are also evident. Diagnosis of bipolar disorder II is based on the occurrence of 1 or more major depressive episodes accompanied by at least 1 hypomanic episode. The interval between episodes decreases as the patient ages. A few patients (5%–15%) have multiple mood episodes within a given year. Over 5 years, some of these patients may suffer a manic episode. In men, the number of hypomanic episodes equals or exceeds the number of major depressive

episodes, whereas in women major depressive episodes predominate. The prevalence is about 0.5% in the population. Like bipolar disorder I, the prognosis is poor if untreated; 10% to 15% will commit suicide. Learning difficulties, social, interpersonal and occupational dysfunctions are also evident.

*Autism*

Diagnosis is based on impaired development in social interaction and communication, and very limited activity and interests.[6,7] Impairment of nonverbal behaviour, such as eye contact, body postures, gestures and facial expression, are also noted. In general, there is a delay in the development or total absence of speech. For those who can speak, there is an inability to initiate or sustain a conversation, and the patient will use idiosyncratic language. When speech develops, the pitch, intonation, rate, rhythm or stress may be abnormal. Furthermore, grammatical structure of sentences is immature and includes repetitive use of language. Language comprehension is often delayed, with the inability to understand simple questions or directions. Stereotyped body movements such as clapping, finger flicking and swaying, and abnormalities of posture, such as walking on tiptoe, are also noted. The prevalence is estimated at 0.05% in the population, with an age of onset before 3 years. Some individuals may become autistic during adolescence, whereas others can improve their language skills by this age. In general, the prognosis is poor as a result of failure to develop peer relationships and lack of social or emotional reciprocity.

## Preliminary methods to suggest a genetic basis for psychiatric disorders

*Familial aggregation*

A trait, of either simple or complex type, clustered among multiple members of a family is termed familial aggregation. Evidence for familial aggregation comes predominantly from the analysis of patient chart data from specialty clinics. In general, specialty clinics provide very accurate diagnostic findings on patients and family histories. If affected people from the general population are randomly selected, the incidence of false ascertainment of familial aggregation increases due to inaccurate or incomplete documented family histories. Families of people affected with autism, affective disorder or schizophrenia show increased occurrence of the trait segregating among them.[2,8] This method, however, cannot be used solely as an indicator of the genetic basis of a mental disorder, since the trait can possibly be a result of cultural, diet or environmental causes rather than genetic. To confirm such findings, several tests are used to justify the genetic basis of complex diseases.

*Heritability*

Heritability refers to the total variation in phenotype that is due to the additive effects of genes or the environment, or both. The genetic variance can further be partitioned into major genes and the genetic background (or polygenes). A genetic etiology is proposed when a total variation in phenotype (heritability value) of over 30% is calculated for a trait in affected families. For affective disorders, the variance is calculated as high as 80%,[9] for schizophrenia at least 71%[10] and autism as high as 90%.[11] False calculations of total variation in phenotype can arise from the use of affected siblings with shared environments but with different genetic causes of the trait.

*Relative risk*

Relative risk compares the frequency of a trait in relatives of the affected person to the frequency of the trait in the general population, creating a relative-risk ratio. A ratio greater than 2 suggests that there is an underlying genetic component for the trait. A high relative risk may or may not imply that the trait likely has a genetic basis. In fact, the degree of relationship, population frequency and type of genetic interaction (additive or multiplicative) influence the value of the relative risk. The relative risks for autism, schizophrenia and affective disorder in siblings compared with the normal population exceed the value of 2.[9–11]

*Twin and adoption studies*

These studies determine the extent of genetic and

possible environmental influences on the genesis of the trait. Monozygotic twins are genetically identical, and dizygotic twins are 50% identical. If concordance for a trait is 100% between monozygotic twins, and 25% to 50% between dizygotic twins, the trait is likely to be strictly genetic. Variability in this range implies environmental influence on the trait. Using adoption studies, the trait has a genetic influence if the risk is higher in biological relatives of the adopted subject than in adopted relatives. With use of these strategies, it is possible to provide evidence of genetic or environmental etiology of the trait from the extent of similar traits shared between monozygotic twin pairs, dizygotic twin pairs, monozygotic verses dizygotic twin pairs or adopted children within the family. These studies have suggested a genetic basis for autism, schizophrenia and affective disorder.[9–11]

*Segregation analysis*

Segregation analysis refers to the derivation of hypothetical models, including genetic and nongenetic factors as the cause of the phenotype, using data sets available from affected family members. With this method, many models can be tested and simulated to determine the mode of inheritance and the penetrance. From this information, the most appropriate linkage analysis strategy can be designed. Modes of inheritance include classic Mendelian models (a single major gene acting as recessive or dominant in causing the trait), polygenic models (the trait is due to cumulative effects of several minor genes) or mixed models (the trait is due to both major genes and minor polygenes). The last 2 will require the use of nonparametric linkage analysis. Segregation analyses have primarily suggested polygenic or mixed models of inheritance for autism, schizophrenia and affective disorder.[12–14]

## Genetic methods used to identify chromosomal regions and psychiatric genes on human chromosome 18

Once the genetic basis of a psychiatric disorder has been established by 2 or more of the methods described, efforts are then focused on localizing the position of the genetic etiology to 1 or more human chromosomes. This is followed by identification and mutation testing of a possible disease-causing gene, or genes. To initiate these experiments, pedigrees with at least 1 affected subject manifesting the trait of interest are obtained. Choice of these pedigrees is based on fulfilling the diagnostic criteria for the trait studied. DNA specimens are collected from the pedigrees and used for genotyping with a panel of genetic markers.

*Whole genome screens and chromosome 18 specific linkage studies*

The concept of genetic linkage refers to the nonrandom segregation or inheritance of genetic markers or genes from parents to offspring. A disease gene can cosegregate (coinherit) with either allele of a marker. If the disease gene and marker are unlinked, then an affected person has an equal chance of inheriting either allele of a marker. If the disease gene and marker are linked, then an affected person has a biased chance of inheriting only 1 allele of a marker. There are several types of genetic markers that are used for linkage analysis. These markers must be polymorphic in order to detect variation among subjects. Generally for linkage analysis, microsatellite and single nucleotide polymorphic (SNP) markers are the most common types used. SNP markers are currently preferred for linkage analysis since they are most abundant in the human genome (about 1 in every 500 base pairs of DNA), the amount of DNA specimen needed for genotyping is very small and genotyping can be done using high-throughput detection methods. Furthermore, SNP markers permit the detection of large numbers of genetic variations among subjects.

The extent of linkage depends on the distance between markers or between a marker and a disease locus. A measure of the estimated genetic distance is determined by the number of recombination events during meiosis. Linkage is established between 2 loci when the recombination fraction is less than 50%, complete linkage is established when there is no recombination and no linkage is established when the recombination fraction is 50%. More than 10 informative recombination events from affected people

are needed to establish linkage between a disease locus and a marker. Linkage analysis in humans uses the same basic principle, except logarithm of the odds (Lod) score analysis is performed. Lod score analysis, a statistical method to calculate linkage, essentially simulates recombination events that can occur in large family datasets to maximize the detection of linkage between markers or a marker and a disease locus. Simulation of recombination events are used in this method, since there is an absence of large human pedigrees with numerous offspring. The term Lod score refers to the $Log_{10}$ likelihood of the odds for linkage of a marker and a disease locus versus odds for no linkage between a marker and a disease locus in the same family. Lod scores can be calculated from either extended multigeneration families or small families manifesting the disease phenotype. The Lod scores can be compiled from each of the families or omitted from a family in order to obtain a maximum Lod score. A Lod score of 3 or greater suggests at least a 1000:1 odds ratio for linkage.[15] A Lod score of –2 or less suggests at least 100:1 odds ratio against linkage[15] and can be used to exclude loci not associated with the phenotype studied. When linkage analysis is performed with a defined genetic model (i.e., parametric linkage analysis), parameters such as the Mendelian mode of inheritance, allele frequencies and penetrance are specified. Parametric linkage analysis is typically used to identify major genes in the cause of the phenotype. With complex traits such as psychiatric disorders, there is no plausible Mendelian mode of inheritance; therefore, the power to detect linkage is low. A major gene may be acting in conjunction with several susceptibility genes or the environment (or both) in the cause of the phenotype. Lod score analysis can still be performed but assumes the genetic model is mostly unknown (nonparametric).

For complex traits, the segregation information is unclear. Two methods of nonparametric linkage

**Table 1: A selection of parametric and nonparametric linkage findings for autism, affective disorder and schizophrenia susceptibility loci on chromosome 18**

| Type of screening | Disorder | Method used | Population studied | Possible susceptibility loci | Reference |
|---|---|---|---|---|---|
| Chromosome 18 specific linkage | Affective disorder | Affected sib pair | American | 18q12.3, 18q21.3 | 17 |
| | | Affected sib pair | American | 18q21, 18q22-q23 | 18 |
| | | Parametric | Belgian | 18q21–q23 | 17 |
| | | Parametric, affected relative pair | Bulgarian | 18p11.2 | 17 |
| | | Parametric, affected sib pair | German | 18p11.2, 18q22–q23 | 19 |
| | | Parametric, affected sib pair | Ashkenazi Jews, Belgian | 18q21.3–q22.3, 18q23 | 20 |
| | | Parametric | Denmark | 18q12 | 21 |
| | Schizophrenia | Parametric, affected relative pair, affected sib pair | German, Israeli, Arabic | 18p11.2 | 17 |
| Whole genome screening | Affective disorder | Affected sib pair | British, Irish | 18p11.3 | 9 |
| | | Parametric | American | 18q22–q23 | 22 |
| | | Parametric, affected relative pair and sib pair | Amish | 18p11.3–18q12.1 | 17 |
| | | Affected sib pair | American | 18q22.3 | 17 |
| | | Parametric, affected sib pair | Amish | 18q11, 18p11.3, 18q21 | 23 |
| | | Parametric | Costa Rican | 18q22–q23 | 24 |
| | | Parametric, affected sib pair | American | 18p11.2, 18q21 | 25 |
| | | Parametric | Swedish | 18p11.2, 18q12 | 26 |
| | Schizophrenia | Affected sib pair | British | 18q21.1 | 27 |
| | Autism | Affected sib pair | American | 18p11.2 | 28 |
| | | Affected sib pair | Worldwide | 18q22.1 | |

sib = sibling.

analysis are used.[16] The first, sib pair analysis, relies on the fact that each sibling is 50% identical genetically. Therefore, for any locus each sibling has a 50% chance of inheriting the same allele from a parent. For affected siblings, if a locus has a significant contribution to the phenotype, then the locus has greater than 50% chance of being inherited from the transmitting parent. The number of siblings needed to achieve a high Lod score depends on the population allele frequency and high risk of the allele for the phenotype over other alleles. This method is used if nuclear families are available with multiple affected siblings, and the parental genotypes are known. The second method, affected relative pair analysis, determines shared allele frequencies in pairs of affected pedigree members, including siblings. This method is used if extended family members or mixtures of relative pairs are available. With both methods, the Mendelian mode of inheritance is not required to be specified and as a result reduces the risk of generating spurious linkage through the use of a misspecified model. In addition, there is increased sensitivity in the detection of genes with minor effect on the phenotype, no need for stringent phenotype ascertainment and nonpenetrant people are avoided for analysis. The main disadvantage is that these methods are less powerful than classic linkage analysis in detecting major genes.

Before linkage analysis is performed on families demonstrating the phenotype of interest, simulation studies are warranted. Simulation studies are performed to determine whether the experimental design is sufficient to provide evidence for linkage, and calculate predicted Lod scores based on the dataset used. Simulation studies utilize data such as the phenotype, number of people genotyped, the number and type of genetic markers, hypothetical penetrance values, hypothetical modes of inheritance and type of linkage algorithm. Findings from simulation studies can suggest which people, experimental designs and linkage

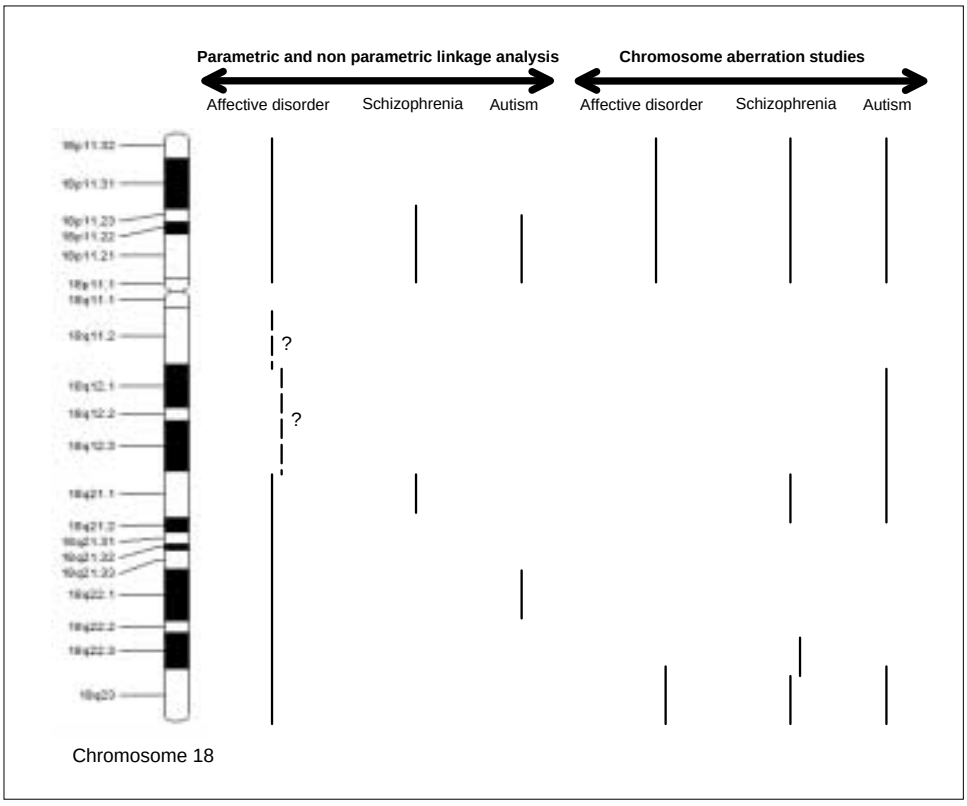


**Fig. 2: Summary of possible map positions for susceptibility genes on human chromosome 18 that contribute to affective disorder, schizophrenia or autism. Findings from both linkage analyses and chromosome aberrations studies are shown.**

methods best provide evidence for linkage.

Genome screens can be performed by linkage analysis to identify shared regions of the genome by descent, and therefore influence on the phenotype. Genome screens are brute force methods to identify several chromosomal regions associated with a trait. Genome screens have suggested 2 possible susceptibility loci on chromosome 18 for autism, 1 for schizophrenia and at least 4 for affective disorder (Table 1,[9,17–28] Fig. 2). Once genome screens have identified susceptibility loci on chromosome 18, a denser panel of chromosome 18 markers can be used and retested on the original genome screen dataset, or on additional affected individuals, using staged linkage methods. This confirms the genome screen findings and localizes the locus more precisely. Several studies reported possible susceptibility loci on chromosome 18, using chromosome 18 specific linkage analyses (Table 1[17–21]). Susceptibility genes were proposed rather than major genes, since the calculated Lod scores were not significant for linkage (Lod score < 3).[15] Some studies failed to find linkage to chromosome 18 for schizophrenia[17] and affective disorder.[17] These contradictory findings could be a consequence of genetic heterogeneity whereby a specific ethnic population segregates with a different susceptibility psychiatric gene. Differences in phenotype ascertainment and methodologic designs can also account for these findings.

*Candidate-gene studies*

Candidate genes are primarily proposed in chromosomal regions defined by linkage analysis or by the mapping of chromosome aberrations. The criteria for candidate genes contributing to psychiatric disorder include the following: expression in brain, particularly in regions that are functionally affected; characterized function implicated in the phenotype; and orthologues that manifest a psychiatric disorder phenotype in a model organism, if mutant. The search for mutations in a selection of chromosome

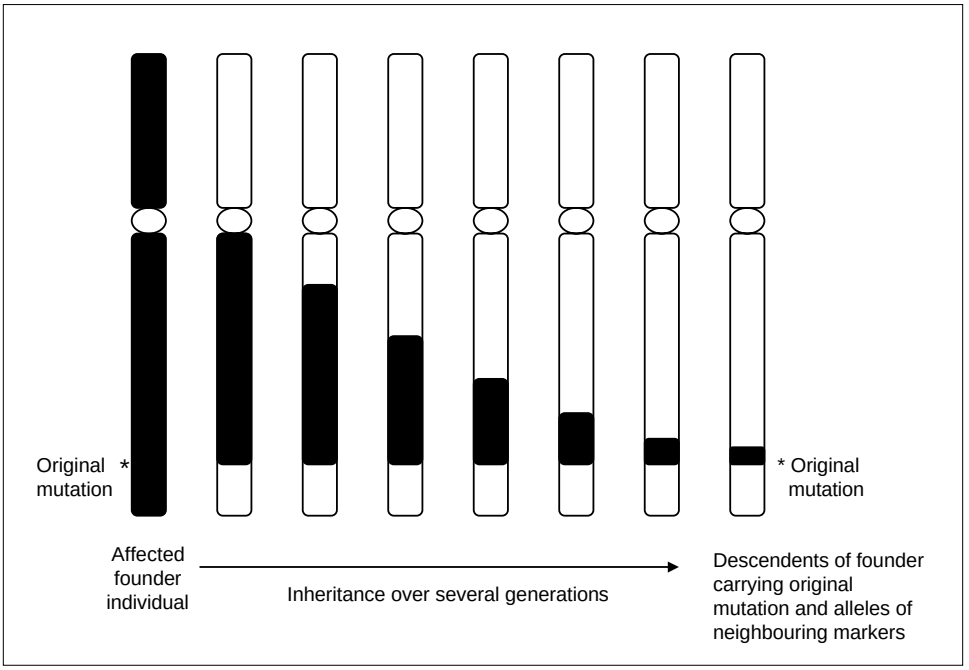


**Fig. 3: Concept of linkage disequilibrium mapping in association studies. The asterisks represent the mutation in the founder affected individual who has a set of marker alleles along the entire chromosome. On inheritance of the mutation, genetic drift and recombination during meiosis results in loss of marker alleles that are associated with the disease chromosome. The black shaded region represents the marker alleles that are conserved over several generations from the founder.**

18 candidate genes among affective disorder and schizophrenia patients has been unsuccessful. Specifically, affective disorder patients were examined for mutations in the *NEDD4L* (neural precursor cells expressed developmentally down-regulated) gene mapping at chromosome 18q21.[29] This gene may function in neural sodium homeostasis.[29] In patients with schizophrenia and affective disorder, mutations were examined in the *IMPA2* (myoinositol monophosphatase 2) gene mapping at chromosome 18p11.2.[29,30] The IMPA2 protein is involved in phospholipase C signalling through which the functions of neurotransmitters and hormones are controlled.[29,30] This pathway is also inhibited by lithium, which is used as a mood stabilizer in the treatment of patients with affective disorder.[29,30] In schizophrenia and affective disorder patients, mutation analyses were done on the *PACAP* (pituitary adenylcyclase activating peptide) gene mapping at chromosome 18p11.[31] The PACAP protein may function to induce catecholamine enzymes, protein kinase A and phospholipid-dependent protein kinase C that is a target of lithium treatment.[31] Interestingly, protein kinase A increases the dopamine beta-hydroxylase activity of the dopaminergic pathway[31] that is implicated in schizophrenia. Finally, mutation studies were reported in schizophrenia patients in the *GNAL* (olfactory G-protein alpha) gene, mapping at chromosome 18p11.2.[32] The GNAL protein functions in transmembrane signalling, which includes the coupling of the dopamine $D_1$ receptors to adenyl cyclase of the dopaminergic pathway.[32] Mutation analysis on chromosome 18 candidate genes for autism is yet to be reported.

*Association–linkage disequilibrium studies*

Association studies are either case–control studies on unrelated persons or family based–control studies.[33] Association studies are important because they supplement linkage analysis by providing supportive evidence for linkage and a more precise definition of the locus, although genome-wide or candidate interval association studies are an independent alternative to genetic linkage analysis. Moreover, association studies are important because they provide increased sensitivity to detect disease associated chromosomal regions in complex traits. Linkage disequilibrium (LD) is allelic association maintained by strong linkage. To perform case–control studies, the allele frequencies are compared in unrelated affected individuals and appropriately matched control subjects with respect to factors such as age, gender and ethnicity. Family based–control studies are done by comparing the allele frequencies transmitted to the affected offspring with those not transmitted. Association or LD studies rely on the assumption that allele frequencies of markers close to or within a disease locus are higher in affected people than in the family or population controls. This method (Fig. 3) is based on the premise that a single or few founder-affected individual(s) possessed a chromosome with the disease gene that had neighbouring genetic markers with particular alleles. Over successive generations, the

**Table 2: A selection of chromosome 18 aberrations reported in patients with autism, affective disorder or schizophrenia**

| Disorder | Chromosome findings | Possible map position of locus | Reference |
|---|---|---|---|
| Autism | 46,XY/46,XY,r(18) [mosaic] | 18p11.3, 18q23 | 40 |
| | 46,XX/46,XY,r(18) [mosaic] | 18p11.3, 18q23 | 40 |
| | 46,XX,del(18)(p11.3)/44,XX,i(18q) [mosaic] | 18p1 | 41 |
| | 46,XY,del(18)(q12.2q21.1) | 18q12.2-q21.1 | 42 |
| | 46,XY,del(18q21.2-qter) | 18q21.2-qter | 43 |
| Affective disorder | r(18) [familial] | 18p11-pter, 18q23-qter | 44 |
| | t(2;18)(q21q23) [familial] | 18q23 | 44 |
| Schizophrenia | 46,XX,del (18)(p1) | 18p1 | 44 |
| | inv(18)(p11.3q21.1) [familial] | 18p11.3, 18q21.1 | 45 |
| | t(15;18)(q13.3q22.3) [familial] | 18q22.3 | 46 |

r = ring chromosome; del = deletion chromosome; i = isochromosome; t = translocation chromosome; inv = inversion chromosome.

alleles of neighbouring genetic markers lose association with the disease locus as a result of recombination during meiosis and genetic drift. The final result is only a few markers that share alleles identical by descent with the disease locus. Success with this method depends on genetic markers that are tightly linked or very close in physical distances so that the alleles of markers always cosegregate with the disease locus. Certain factors such as allele frequencies, mutation rates, genetic heterogeneity, marker density and the population size, growth rate, structure and age, challenge the accuracy of association studies. Case–control association studies are most effective in geographically isolated populations, since the problem of genetic heterogeneity is absent or minimal. However, the accuracy of the diagnostic criteria, proper selection of matched controls, and choice of methodologic design and statistical analysis are sources of potential problems that can arise with population-based association studies.[33]

Initial reports have shown association of markers mapping to chromosome 18p11 in German and Israeli patients with affective disorder or schizophrenia.[17,34,35] These findings suggested susceptibility regions for these disorders on chromosome 18. Subsequent reports of several LD studies with candidate genes on chromosome 18, however, demonstrated both positive and negative results. For instance, LD studies failed to find an association with markers mapping within the *GOLF* gene at chromosome 18p11 in German patients with schizophrenia[36] and in North American patients with affective disorder.[37] No association was found with markers mapping within the *PACAP* gene at chromosome 18p11 in affective disorder and schizophrenic patients of Japanese descent.[31] However, LD studies with the markers mapping within the *IMPA2* gene at chromosome 18p11.2 showed an association in Japanese patients with schizophrenia.[30] LD studies using a Costa Rican population with a diagnosis of affective disorder also showed statistically significant association with chromosome 18 markers mapping to chromosome 18q22–q23, 18q12.3 and 18pter,[38] but not to chromosome 18q23 in a Faroe Island population (predominantly Norwegian).[39] These contradictory findings are possibly a result of genetic heterogeneity or differences in clinical ascertainment, population type, marker density and experimental methods used.

*Chromosome aberration studies*

Table 2[40–46] provides a list of human chromosome 18 aberrations that are associated with affective disorder, schizophrenia and autism. Chromosome aberrations provide supportive evidence for putative loci defined by linkage or association–LD studies. The use of chromosome aberrations have assisted in the identification of disease genes typically by altering the expression of flanking genes or by disrupting the coding segment of a gene. A phenotype associated with a chromosome abnormality can be a valid association or a coincidence. An association of a psychiatric disorder with a cytogenetic region ascertained by means of a chromosome abnormality is supported by the presence of multiple chromosome abnormalities that involve the same cytogenetic interval and psychiatric disorder, by supplemental evidence, by linkage or by LD mapping of a psychiatric disorder to a cytogenetic region, which co-localizes with a chromosome abnormality, and to familial segregation of a chromosome abnormality with a psychiatric disorder.

The earliest evidence for psychiatric disorders associated with human chromosome 18 arose from studies of Ring chromosome 18 and deletions of the long (q) or short arm (p) of chromosome 18. Patients with deletions of the long arm of chromosome 18 manifest features of the "18q- deletion syndrome."[47] In addition to dysmorphic features, these patients had neurologic signs such as mild to severe mental retardation, psychotic behaviour, language difficulties and autism. An "18p- syndrome" was proposed based on subjects with deletions of the short arm of chromosome 18 and having dysmorphic features, developmental delay, mental retardation and speech anomalies.[48] "Ring chromosome 18 syndrome" was proposed and included dysmorphic features, speech defect and mental retardation, and behavioural defects such as irritable, aggressive and social isolation.[49] These chromosome aberrations have supported studies by linkage analysis that suggested susceptibility genes for autism, affective disorder or schizophrenia on chromosome 18.

## Alternative supplemental genetic methods

*Use of bioinformatics*

Bioinformatics involves the storage, retrieval and analysis of biologic data using computational methods.[50] Initially, for the genetic analysis of simple and complex traits, the focus was on the development of improved computational algorithms for use in methods such as segregation analysis, simulation studies

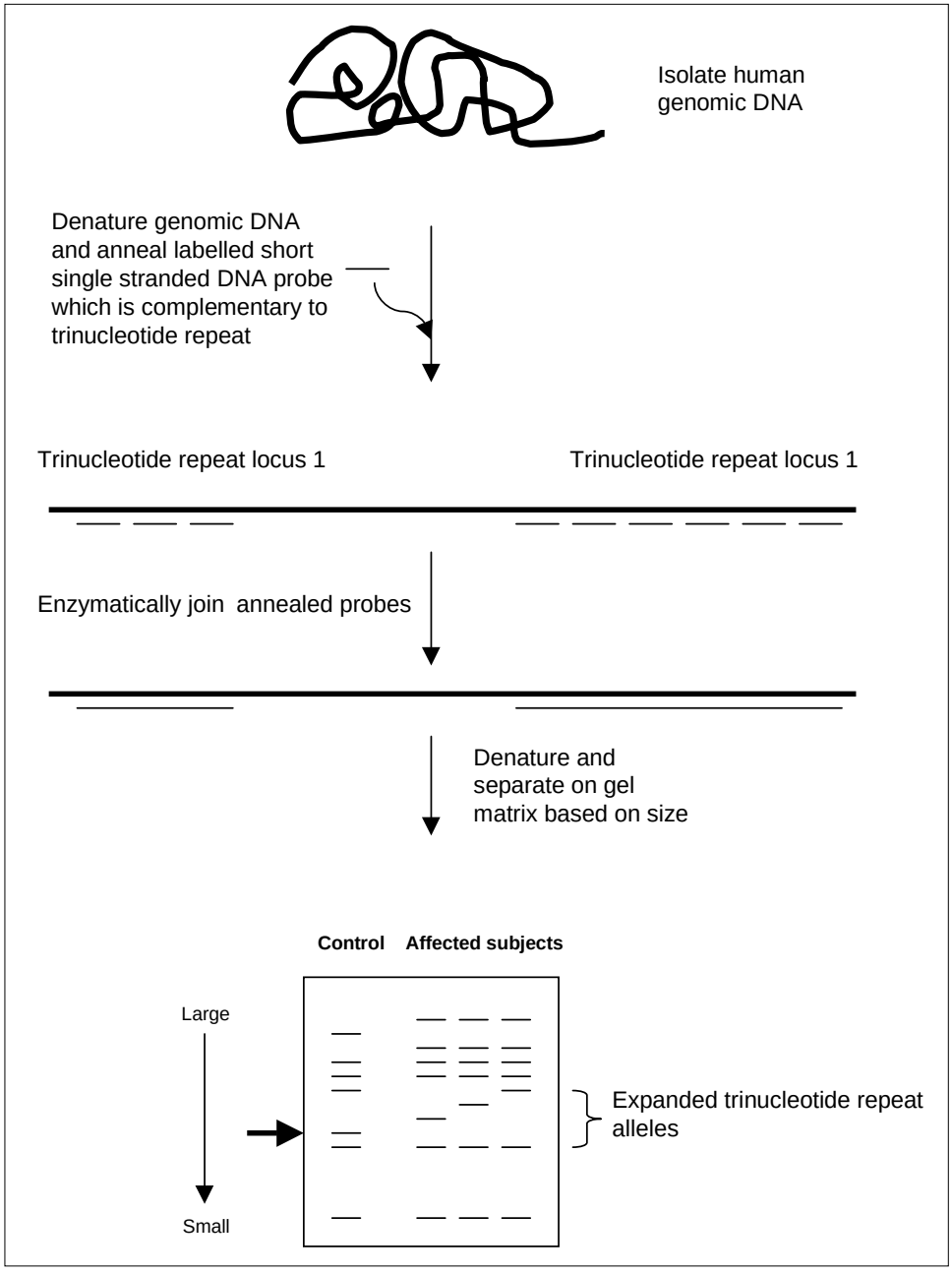


**Fig. 4: The repeat expansion detection (RED) method. In this illustration, expansion of a trinucleotide repeat locus is noted in affected persons when compared with a control subject. The shift in the migration of RED products is due to changes in expanded or increased size of the products.**

and linkage. The field of bioinformatics has evolved from statistical informatics, and can be applied to confine disease-associated chromosome regions and subsequently identify disease genes. The Human Genome Project has made available large amounts of data, including gene sequences and new genetic markers. These data can be stored and manipulated using computational algorithms designed within commercial software or Web-based tools. An excellent example is BLAST (basic local alignment search tool), which can query a DNA sequence against a database of DNA sequences to determine its identity, to determine any related sequences among organisms and to discern possible functional and structural motifs. With the use of bioinformatics, candidate intervals containing genes for autism, affective disorder and schizophrenia can be obtained by searching for related chromosome regions in the human genome that harbour a family of related psychiatric genes. Furthermore, human candidate genes or genes isolated in animal models with phenotypes manifesting these disorders, or even sequence-specific regulatory motifs of psychiatric genes can be mapped by sequence alignment to ascertain whether localization is on chromosome 18. The genomic sequence is also an asset for the identification of a denser map of genetic markers that will provide more reliable calculations in association–LD studies with candidate genes mapping to selected intervals for autism, schizophrenia or affective disorder, using case–control or family based–control studies.

*Repeat expansion studies*

Psychiatric illnesses commonly tend to exhibit the phenomenon of "anticipation." In successive generations, the severity and age of onset of the illness increases. Anticipation is attributed to the instability of trinucleotide repeats residing in a major or susceptible locus of the phenotype. Trinucleotide repeats are repeats of 3 core nucleotides that are usually unstable due to expansions from DNA replication errors. These repeats can map within the coding or noncoding regions of a gene. It is proposed that instability of these repeats affects the expression of a gene or the function of the encoded protein. Genome wide screening for trinucleotide repeats in affective disorder and schizophrenia were performed using a method known as repeat extension detection (RED).[51] This method (Fig. 4) detects a repeat expansion independent of the location. Genomic DNA is used as a template in a 2-step cycle process. The ability of a single oligonucleotide to hybridize to its complementary strand in genomic DNA is the basis of this technique. A thermostable ligase is used to covalently link adjacent oligonucleotides that produce larger single-stranded fragments. Repeated cycles of denaturation and annealing followed by ligation results in the formation of detectable DNA fragments and are seen as a ladder of bands.

Once trinucleotide repeat expansions are detected using the RED method, candidate trinucleotide repeat loci can then be examined for instability. With the availability of the chromosome 18 genomic sequence from the Human Genome Project, the sequence can be searched for genes associated with trinucleotide repeats, then subsequently assayed for repeat instability. The CTG18.1 trinucleotide repeat locus mapping at chromosome 18q21.1 and 8 other novel trinucleotide repeats mapping at chromosome 18q21.33–q23 were examined by several studies that reported no changes in expanded alleles between control subjects and those with affective disorder in European and North American families.[52,53] However, other studies have reported some correlations between expansions of the CTG18.1 locus and affective disorder in the Caucasian population.[54] In several ethnic groups affected with early onset childhood schizophrenia, a weak correlation was found with expansions of the CTG18.1 locus in affected males[51] but not North American families.[52] The CTG18.1 repeats are located within the *SEF2-1* gene that encodes a protein that regulates the expression of other genes. This gene possibly functions in muscle, brain and liver. These findings of trinucleotide repeat expansions are controversial since they could be coincidences independent in the cause of the phenotype. However, this assertion occurs with candidate genes in general.

*Use of animal models: mouse–human comparative genomics*

The mouse has served as an excellent model for per-

forming psychiatric analysis on strains that are carriers of a particular genetic background and genetic abnormalities suspected of contributing to mental illness. The availability of the mouse genomic sequence (Rodent Genome Project) as a spin-off of the Human Genome Project has allowed for direct comparative genomics between human chromosome 18 and mouse chromosomes. Human chromosome 18 shares evolutionary conserved regions of common gene content with mouse chromosomes 1, 17 and 18 and possibly 2, 5 and 7. The use of a mouse model can allow the manipulation of genes that are suspected candidates of psychiatric disorders, or be used to verify such genes found mutated in psychiatric patients. Although the mouse is not a perfect model since it lacks the higher cognitive function and language seen in humans, certain tests such as the "hanging wire" test can be used to assess motor deficits in autism, and the "Morris water maze" and "five-arm maze" tests can be used to examine impaired learning and memory in schizophrenia.[55] So far, no mouse models for autism and affective disorder have been created for genes that have orthologues mapping to these defined loci on human chromosome 18. A recent report of the synaptotagmin IV (SytIV) mouse model described these mice with deficits in fine motor coordination and memory, reminiscent of the schizophrenia spectrum.[56] The *SYTIV* gene maps to human chromosome 18q12.3, a region implicated in the susceptibility of affective disorder and schizophrenia. The SytIV protein may be a transmembrane protein that can be induced by neuronal depolarization, and can regulate synaptic function.[56] It is also expressed in dopaminergic nerve innervations, suggesting that SytIV may regulate dopamine homeostasis that is abnormal in schizophrenic patients.

*Parent of origin studies*

Psychiatric illnesses are speculated to be associated with the phenomenon of imprinted effects or genomic imprinting.[57] In this manner, the phenotype is due to a specific parental sex who has transmitted the mutant gene. Only 1 allele of a gene therefore contributes to the phenotype. No robust evidence for imprinting effects are associated with chromosome 18 based on current findings from clinical correlations with chromosome aberrations.[57] However, linkage studies with chromosome 18 genetic markers suggest possible findings of parental bias in the transmission of affective disorder. Biased or excessive maternal transmission of affective disorder at chromosome 18p11.2 were reported by several studies.[17,25,58] Some cases of biased or excessive paternal transmission of affective disorder at chromosome 18q21 were also noted.[18] Other than imprinting effects, these differences can arise as a result of how the mode of inheritance is defined for linkage analysis.[19] The mouse *Impact* gene, shown to be imprinted in the mouse by paternal specific expression, maps to human chromosome 18q11.2–q12.1, which is possibly another susceptibility locus for psychiatric disorders.[59] The *Impact* gene has unknown function but is highly expressed in the brain. However, the human *IMPACT* gene is shown not to be imprinted. Chromosome 18 specific linkage analysis with a European population affected with schizophrenia has shown no parental origin effects thus far.[17] Likewise, no parental origin bias has been reported in autistic patients demonstrating linkage to chromosome 18.[3,28]

## Comment and conclusions

In general, evidence from familial aggregation and segregation analysis of affected pedigrees are most common and sufficient to provide preliminary support for a genetic basis of a trait. Calculations of heritability, relative risk, and twin and adoption studies together provide more robust evidence for such a genetic basis but are limited to available ample subjects for analysis. Once the genetic basis of a trait is established, affected pedigrees are sought based on fulfilling the diagnostic criteria for the trait. DNA specimens are collected from these pedigrees and used for genotyping. Simulation studies are mandatory in determining the best experimental design to provide evidence for linkage, and can suggest acquiring or omitting additional subjects or genetic markers, or both, for analysis. Depending on findings from simulation studies and other factors such as resources used for genotyping and the type of sample available (for instance sibling pairs, number of pedigrees and number of familial generations), parametric or nonparametric

whole genome or chromosome specific linkage analysis is performed to define regions of the human genome that contribute to the trait. Linkage analysis by genome screening is important to identify several possible disease associated chromosomal regions. Staged or chromosome specific linkage analysis provides more robust evidence for linkage to a chromosomal region; however, this method can be done independent of the genome screening data. In fact, the finding of frequent disease associated aberrations of a chromosome from patients manifesting the trait of interest can justify the use of chromosome specific linkage analysis. Confirmation of linkage to candidate chromosomal regions are by association studies with a denser panel of genetic markers, some of which are usually designed within candidate genes, and by the analysis of chromosome aberrations from affected patients. Association studies are more sensitive to detecting disease-associated chromosomal regions in psychiatric disorders and can be done independent of genome-wide or chromosome specific linkage analysis. However, this is currently an unpopular strategy because it is tedious and very costly. With respect to chromosome 18, candidate genes for schizophrenia, bipolar disorder and autism can be identified by the use of bioinformatics, mouse–human comparative genomics, trinucleotide repeat expansion studies, parent-specific gene expression studies and mapping of chromosome aberrations in search of disrupted genes or genes that map close to chromosome aberration breakpoint junctions. The choice depends on the level of technical expertise and available resources to perform the experimental analysis. Candidate genes are then subjected to mutation analysis to identify causative or susceptibility genes for the studied trait. Nondefinitive results do arise from the strategies discussed in this article. Examples include: low positive Lod score values (< 1.5) from linkage analysis, or conflicting linkage reports by several groups to a chromosomal region. Nondefinitive results can be resolved by replication studies, which can use different ethnic populations, population age group or experimental designs to produce informative results. In addition, a change in the choice of subjects used, based on more stratified diagnostic criteria, and type, chromosome location and number of genetic markers used for genotyping, can resolve nondefinitive results.

Linkage, association and chromosome aberration studies have suggested intervals on both the short arm (p) and long arm (q) of chromosome 18 that may contain genes for psychiatric disorders. Candidate regions that are proposed based on more than one genetic mapping method or independent confirmation by more than 1 research group are chromosome regions that warrant further analysis. In general, the findings from linkage, association and mapping of chromosome aberration studies suggest controversial loci for autism, schizophrenia and affective disorder on chromosome 18. The findings from independent linkage analysis,[9,17–20] association studies[38] and mapping of chromosome aberrations[44] suggest that chromosome 18p11 and 18q21–q23 are candidate regions for affective disorder. Independent linkage analysis,[17,27] association studies[30] and mapping of chromosome aberrations[45] suggest that chromosome 18p11.2 and 18q21.1 are candidate regions for schizophrenia. The findings from linkage studies[28] and the mapping of chromosome aberrations[41,43] suggest that chromosome 18p11.2 and 18q22.1 are candidate regions for autism. In many instances, the use of these genetic methods report overlapping candidate regions on chromosome 18 for 2 or all 3 disorders. An example is chromosome 18p11.2, which is a common candidate cytogenetic region for all 3 disorders. These findings suggest that the genetic bases for these disorders are etiologically identical,[60] at least for harbouring a candidate cytogenetic interval at chromosome 18p11.2. If this is a likely cause, then differences in the psychiatric phenotype are possibly the result of different genetic backgrounds or environmental influences.[60] However, a more likely explanation is that human chromosome 18p11.2, for instance, contains many genes that contribute to the cause of these psychiatric disorders. Coincidentally, these genes map to common regions on chromosome 18 (e.g., chromosome 18p11.2) and have distinct psychiatric phenotypes if mutant. Spurious reported loci for psychiatric disorders associated with chromosome 18 is likely a result of inappropriate experimental design.

Genes proposed thus far as the cause of autism, affective disorder and schizophrenia on chromosome 18 are likely designated as susceptibility genes. This originates from the findings of Lod score values cal-

culated below the statistical significance for linkage, using genetic markers mapping to the candidate intervals. These genes have a minor role that contributes to the phenotype. The interactions of these genes with other minor genes, major genes and the environment result in a psychiatric phenotype. Mutation studies have been unsuccessful on 4 candidate genes mapping to possible disease-associated chromosome 18 intervals among patients with schizophrenia and affective disorder,[17,29–32] although an association was found with the *IMPA2* gene in Japanese schizophrenia patients.[30] Moreover, trinucleotide repeat expansion and imprinting studies have failed to suggest promising candidate psychiatric genes on chromosome 18. These results suggest that confirmation of these possible chromosomal regions harbouring susceptibility genes is warranted and can be achieved by the mapping of additional chromosome aberrations, especially those that are inherited in families with psychiatric disorders. The mapping and characterization of chromosome aberrations will determine whether any genes are disrupted or have altered expression. Furthermore, genomic sequence data from the Human Genome Project will assist in the identification of novel genes and additional genetic markers. The Rodent Genome Project (mouse and rat) can also provide a list of candidate psychiatric genes, since phenotypic and functional analyses of genes are typically established in rodents. Based on mouse–human comparative genomics, the *SYTIV* gene in humans is a promising candidate for schizophrenia. These resources will be valuable for association and mutation studies in humans on possible susceptibility genes.

Thorough characterization of gene function is achieved by the construction of animal models. These findings can be supplemented by in vitro methods to discern properties of the protein with respect to functional motifs, method of action by interacting with other proteins or biologic pathways, and the structure. The use of this information is relevant to the design of specific treatment by means of enhancing or inhibiting protein function. The ultimate drug treatment will have little or no side effects and completely eliminate symptoms. The targeting of a single protein is a possible strategy in drug design. However, a more likely strategy is the design of a cocktail of drugs that interact with more than 1 protein found to be implicated in the cause of affective disorder, autism or schizophrenia.

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