Jenny Ekholm

Molecular Genetics of Bipolar Disorder and Related Traits

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Department of Molecular Medicine
National Public Health Institute Helsinki, Finlandand
and
Department of Genetics, University of Helsinki, Finland

Jenny Ekholm

MOLECULAR GENETICS OF BIPOLAR DISRDER AND RELATED TRAITS

A C A D E M I C D I S S E R T A T I O N

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Kansanterveyslaitos (KTL)
Mannerheimintie 166
00300 Helsinki
Puh. vaihde (09) 474 41, telefax (09) 4744 8408

Folkhälsoinstitutet
Mannerheimvägen 166
00300 Helsingfors
Tel. växel (09) 474 41, telefax (09) 4744 8408

National Public Health Institute
Mannerheimintie 166
FIN-00300 Helsinki, Finland
Telephone +358 9 474 41, telefax +358 9 4744 8408

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Supervised by

Academy Professor Leena Peltonen-Palotie
National Public Health Institute
Helsinki, Finland

Professor Jouko Lönnqvist
National Public Health Institute
Helsinki, Finland

Reviewed by

Assistant Professor Danielle Dick
Washington University
St.Louis, USA

Docent Pekka Uimari
JuriLab
Kuopio, Finland

Opponent

Assistant Professor Brien Riley
Virginia Commonwealth University
Richmond, USA

Vaelsin metsään koska tahdoin elää tietoisesti.
Tahdoin elää pohjia myöten ja imeä elämän koko ytimen.
Tahdoin torjua kaiken mikä ei ollut elämää,
jotta kuollessani en joutuisi toteamaan etten ollut elänyt.
Carpe diem!

Till famo

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ABSTRACT

The unraveling of the underlying genetic basis in diseases has accelerated rapidly as a result of dramatic advances in the field of molecular genetics, and will hopefully in due course lead to innovative, effective treatments for many human genetic diseases. Statistical genetics plays an important role in nearly every aspect of modern genetics, whether one is attempting to find genes responsible for rare monogenic disorders or complex genetic conditions, create a genomic map, or establish the interactions of inheritance and environment in the development of common diseases. Molecular genetic research is fundamentally important to the field of medicine and to the elucidation of the basic mechanisms of biological systems. In this thesis different applications of statistical methods have been used to explore the genetic component of bipolar disorder and related traits.

Here, 41 families affected with bipolar disorder have been ascertained from the isolated population of Finland, presuming that the presence of stronger genetic and environmental homogeneity in this population than in many other populations is an advantage in gene localization. A genome-wide scan was performed identifying possible genomic susceptibility regions for bipolar disorder. The strongest evidence of linkage was provided by a locus on 16p12 in the two-point analysis (Z_{max} = 3.4) and on 4q32 in the three-point analysis (Z_{max} = 3.6). Encouraging results were also observed on chromosomes 12q23 and Xq25 (lod score > 2.0). The latter locus was already identified in a previous study and hence, a denser marker map was constructed covering 13cM of the critical region. The earlier linkage was found in one extended pedigree displaying bipolar disorder in several generations. In order to test the general significance of this linkage finding, as well as to narrow down the linked area, the genome-wide scan family sample consisting of 40 additional families was utilized in this analysis. The X-chromosomal linkage finding was replicated in the other pedigrees, however only with modest statistical significance. The linked region was consequently somewhat narrowed, and it was established in the genome-wide search that Xq25 represents the major linked region in the one extended family.

In order to extract all possible information of the genome-wide scan performed, different statistical strategies were utilized to cluster and re-analyze the results and the study sample. In one line of study, the linkage results of all the available worldwide genome scans in bipolar disorder were re-analyzed in a meta-analysis. From this study it could be

concluded that the strong linkage detected in the individual study samples could not be replicated when combining the linkage results from all studies worldwide. However, weaker linkage findings that seemed to be quite consistent across studies now stood out given the stronger statistical power.

In a different type of study, the genotype data of our bipolar genome-wide scan and a genome-wide scan conducted in schizophrenia also using a Finnish study sample were combined. Instead of assessing linkage of the disease entities as defined in DSM-IV, shared traits of the two disorders such as psychotic and affective symptoms were examined. This study revealed a distinct locus for affective symptoms on chromosome 6p21 and a distinct locus for psychotic symptoms on chromosome 5q14.

Keywords: Bipolar disorder, Genome-wide scan, Linkage analysis

CONTENTS

Abbreviations

ASP	affected sibpair
ATP	adenotriphosphate
AO	affected only
bp	basepair
BPD	bipolar disorder
BPD-I	bipolar disorder, type I
BPD-II	bipolar disorder, type II
BPD-NOS	bipolar disorder, not otherwise specified
cM	centimorgan
cAMP	cyclic adenosine monophosphate
DNA	deoxyribonucleic acid
DSM	diagnostic and statistical manual of mental disorders
DZ	dizygotic
GABA	gamma-aminobutyric acid
HHRR	haplotype-based haplotype relative risk
IBD	identical by descent
IBS	identical by state
L	likelihood
LD	linkage disequilibrium
lod	logarithm of odds
Mb	megabase
MLS	maximum likelihood score
MRI	magnetic resonance imaging
MZ	monozygotic
n	number
NPL	non-parametric linkage
PET	positron emission tomography
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
SNP	single nucleotide polymorphism
STRP	short tandem repeat polymorphism
SZ	schizophrenia
SZ-AFF	schizoaffective disorder
TDT	transmission disequilibrium test
θ	theta, recombination fraction
VNTR	variable number of tandem repeats
Z	lod score
Z_{max}	maximum lod score

List of original publications

This thesis is based on the following original articles, referred to in the text by their Roman numerals. In addition, some unpublished data are presented.

I. **Ekholm J M**, Pekkarinen P, Pajukanta P, Kieseppä T, Partonen T, Paunio T, Varilo T, Perola M, Lönnqvist J, Peltonen L. Analysis of the Bipolar Disorder Susceptibility Region on Xq24-q28 in Finnish Families; Mol Psychiatry 2002; 7(5): 453-9.

II. **Ekholm JM**, Kieseppä T, Hiekkalinna T, Partonen T, Paunio T, Perola M, Ekelund J, Lönnqvist J, Pekkarinen-Ijäs P, Peltonen L. Evidence of susceptibility loci on 4q32 and 16p12 for bipolar disorder. Hum Mol Genet. 2003; 12(15):1907-15.

III. Segurado R, Detera-Wadleigh SD, Levinson DF, Lewis CM, Gill M, Nurnberger JI Jr, Craddock N, DePaulo JR, Baron M, Gershon ES, **Ekholm J**, Cichon S, Turecki G, Claes S, Kelsoe JR, Schofield PR, Badenhop RF, Morissette J, Coon H, Blackwood D, McInnes LA, Foroud T, Edenberg HJ, Reich T, Rice JP, Goate A, McInnis MG, McMahon FJ, Badner JA, Goldin LR, Bennett P, Willour VL, Zandi PP, Liu J, Gilliam C, Juo SH, Berrettini WH, Yoshikawa T, Peltonen L, Lonnqvist J, Nothen MM, Schumacher J, Windemuth C, Rietschel M, Propping P, Maier W, Alda M, Grof P, Rouleau GA, Del-Favero J, Van Broeckhoven C, Mendlewicz J, Adolfsson R, Spence MA, Luebbert H, Adams LJ, Donald JA, Mitchell PB, Barden N, Shink E, Byerley W, Muir W, Visscher PM, Macgregor S, Gurling H, Kalsi G, McQuillin A, Escamilla MA, Reus VI, Leon P, Freimer NB, Ewald H, Kruse TA, Mors O, Radhakrishna U, Blouin JL, Antonarakis SE, Akarsu N. Genome scan meta-analysis of schizophrenia and bipolar disorder, part III: Bipolar disorder. Am J Hum Genet. 2003;73(1):49-62.

IV. **Jenny Ekholm**, Jesper Ekelund, Tuula Kieseppä, Annamari Tuulio-Henriksson, Timo Partonen, Tiina Paunio, Jouko Lönnqvist, Leena Peltonen. Combined analysis of genome-wide scans in bipolar disorder and schizophrenia families reveals locus for mood disorder on 6p21 and for psychotic disorder on 5q14. (submitted Human Molecular Genetics)

1. Introduction

Bipolar disorder is a severe mental disorder affecting approximately 1% of the worldwide population, and it seems to be equally frequent in both sexes and all ethnicities (1). Epidemiological studies, such as family, twin and adoption studies together with genome-wide scans and targeted DNA-studies indicate strong genetic predisposition in bipolar disorder. A strong genetic contribution to causation has been confirmed by both a large twin study, which estimated a heritability rate of about 85% (2), and a Danish population sample of several million, which reported a 13-fold increased risk in first-degree relatives (3).

The process of identifying genes underlying mental disorders faces many difficulties due to complex patterns of inheritance, lack of diagnostic biological tests and strong confounding or interacting influence of environmental factors. Successful genetic studies of complex disorders like bipolar disorder will require very large samples drawn from diverse populations, and/or samples drawn from genetically isolated populations. The Finnish population originates from a limited number of founders and has remained relatively isolated, resulting in increased genetic homogeneity. In addition, the molecular information can be combined with exceptional genealogical data available only in a few countries offered by a well established church record system which dates back to 1640, containing detailed information on births, deaths, marriages and movements of the majority of the population. This provides excellent opportunities for special study designs for the identification not only of rare disease genes but also of major loci, which contribute to complex diseases. The genome-wide scan strategy using family material from the isolated Finnish population has already been a working strategy in identifying predisposing loci in several complex diseases such as multiple sclerosis, familial combined hyperlipidemia, type 2 diabetes, schizophrenia, distal interphalangeal arthritis and migraine with aura. Hence, it could hopefully also shed some light on the underlying biological factors in bipolar disorder.

Through a variety of research approaches, including neuroscience studies, basic science approaches to brain and behavior, genetic investigations, epidemiological studies, and clinical research, better treatments are achieved, and hopefully ways to prevent and cure the illness will eventually be found.

2. Review of literature

2.1 The role of genetics in the etiology of disease

In 1977 Frederick Sanger invented a new method for determining the order of nucleotide sequence of deoksiribonucleicacid (DNA) (4, 5) and subsequently the first human gene, Chorionic Somatomammotropin Hormone, was isolated and sequenced (6). Since then, the understanding of genes and their role in the etiology of disease has substantially increased, especially now that the draft sequence of the human genome is available (7, 8).

The human genome consists of 46 chromosomes; 22 pairs of autosomal chromosomes and 2 sex chromosomes. These are built up of ~3.3 billion base pairs of DNA that in turn code for about 20,000 - 25,000 protein-coding genes, which is only twice as many as in the worm or fly (7, 8). Prior to the sequencing of the human genome, the number of genes in the human genome was predicted to be much greater (50 000 - 140 000). However, the draft sequence revealed that less than 5% of the genome is made up of coding regions, with differing gene densities between different chromosomal regions (7, 8). Even though the human genome has a considerably lower number of genes than initially thought, the differences in the phenotypic complexity observed between the human and the fly or the worm is evident. In 1937, J.B.S Haldane was the first to suggest a theory concerning maximum genetic load of an organism, which illustrates that all genes have a certain low rate of mutations to a deleterious state. He argued that when the number of genes becomes too large, each zygote carries so many new delirious mutations that the population simply cannot be maintained. Hence, the lower number of genes might be compensated by combinatorial diversity generated at the levels of protein architecture, transcriptional and translational control, post-transcriptional modification of proteins, and post-transcriptional regulation. Extensive domain shuffling to increase or alter combinatorial diversity can provide an exponential increase in the ability to mediate protein-protein interactions without dramatically increasing the absolute size of the protein complement (9). The fact that nearly 40% of the genes seem to be alternatively spliced in the human genome (10) further suggests that these mechanisms of combinatorial diversity account for the greater phenotypic diversity found in humans compared with other species.

The sequencing of the human genome has revolutionized the field of genetics. Among other things, it has enabled development of a genome-wide gene map covering previously and newly identified genes, and their internal order to each other. The next big challenge will be to identify function and interaction of the genes with each other, and to eventually identify their respective roles in different disorders. To date, over 1400 genes have been linked to about 1200 Mendelian traits (11).

The role of genetic factors in the etiology of disease varies considerably; some like cystic fibrosis are mostly genetically determined, while others, like post-accidental traumas might be almost entirely induced by environmental factors. Genetic disorders that are a

direct result of a mutation in one gene are called monogenic disorders, but are also known as Mendelian or single-gene disorders. These pose Mendelian patterns of inheritance; X-linked, autosomal recessive or dominant inheritance. The majority of the monogenic diseases are rare, the most common being red-green color blindness affecting approximately 8-10 % of most of the worldwide male population (12). In addition to the ~1200 traits or disorders that are known to portray single gene Mendelian inheritance in humans (11), there are an even larger number of disorders which do not follow the classical patterns of Mendelian inheritance. In recent years several different mechanisms have been recognized accounting for these phenomena, including anticipation, mosaicism, uniparental disomy, genomic imprinting and mitocondrial inheritance (13). The greatest challenge has been met in the identification of disease genes predisposing to complex disorders, also known as multifactorial disorders. Multifactorial diseases are caused by multiple genes interacting with each other and environmental factors. They display a complex pattern of inheritance, with incomplete penetrance, often delayed age of onset and substantial locus heterogeneity. These phenotypically similar diseases do not show any Mendelian pattern of inheritance. It is likely that more than one mutation is required to produce disease manifestation, and the susceptibility to disease may be further mediated by environmental factors in addition to the subtle contribution of a number of genes. Complex disorders like diabetes, asthma, cancer and mental illnesses are common, especially in western populations. In all of these cases, no one gene has been found to manifest the disease. Unraveling the underlying genetic networks as well as the effects of their interactions on disease expression will undoubtedly be a challenge for some time to come, and will be largely assisted by the ongoing efforts to sequence different species as well as the revolutionizing contributions of novel high-throughput technology.

2.2 Strategies for mapping genes predisposing to common complex disorders

Genetic dissection of even simple Mendelian traits has been sufficiently challenging. In recent years, it has become increasingly evident that complex disorders pose a unique challenge that requires more imaginative approaches than ever before. Common complex disorders can generally be divided into different classes such as psychiatric disorders (e.g. bipolar disorder, schizophrenia, unipolar depression), metabolic disorders (e.g. type 2 diabetes, obesity), and autoimmune and inflammatory disorders (thyroiditis, systemic lupus erythematosus, multiple sclerosis, type 1 diabetes, asthma, atopic disorders) (14).

It appears that genes with large effect size in complex disorders are relatively rare. Instead, the genetic make-up seems to be more of an oligogenic (i.e., a few genes each with moderate effect size) or even polygenic nature (i.e., many genes, each with small effect size), in addition to influencing epigenetic factors. To search for the underlying disease variants by examining the genome base-by-base is not yet a reality, thus alternative approaches are utilized. The most widely used methods are linkage analysis, association analysis, allele-sharing methods and animal models (15).

2.2.1 Statistical methods

The two main statistical approaches are linkage and association studies. While linkage studies seek to identify loci that co-segregate with the trait within families, association studies seek to identify particular variants that are associated with the phenotype at the population level. These are complementary methods that together provide the means to probe the genome and describe the genetic etiology of complex human traits, potentially elucidating the mechanisms leading to some of the most important contemporary disease mechanisms.

2.2.1.1 Linkage analysis

Parametric lod score methods

The most commonly used statistic is the logaritm of the odds ratio (lod score). This statistic assesses the probability of two loci on a chromosome being linked, and therefore not being transmitted independently from parent to offspring. Traditionally the distance from disease gene to marker is determined by the recombination fraction, theta (θ), that ranges from θ = 0 (no distance) to θ = 0.5 (no linkage). In 1955 Morton described this statistic in the following formula, which assesses the probability that the co-segregation of illness and marker allele has occurred randomly, versus the probability that the co-segregation of illness and a marker allele has occurred because the marker allele is located near a disease gene on the same chromosome, such that the two are transmitted together more often than expected by chance (=50%):

$$Z(\theta) = \log_{10} [L(\theta) / L(0.5)]$$

in which L is the likelihood function and θ is the recombination fraction. The overall likelihood of a pedigree is determined, for a range of θ values (θ = 0 to θ = 0.5), and the ratio of the likelihoods gives the odds of linkage and the logarithm of odds (Z = lod score) at every θ. In a set of independent families, the overall probability of linkage is the product of likelihoods of possibilities in each individual family, and the lod scores can then be added up across families. For complex disorders the Lod score is maximized over θ (Z_{max}), which is normally the statistic of interest, since the value of θ as a measure of distance is very limited due to confounding by imperfect genotype-phenotype correlation (16).

This statistical method has been successfully applied to localize genes for numerous Mendelian disorders. It is considered to be a model-based (parametric) method, since the values for several variables have to be specified prior to the analysis. These variables include mode of inheritance, frequency, and penetrance of the disease allele (i.e., the probability of being ill given one carries the allele).

Nonparametric methods
The traditional lod score method is a very powerful approach, especially in monogenic disorders where the required parameters are generally known. However, that is seldom the case in complex disorders particularly in diseases that might not develop until later in life. This dilemma is most commonly circumvented by utilizing a so called model-free (nonparametric) method. Nonparametric linkage analysis does not require specification of a genetic model, since all meioses are considered to be independent and equally informative for the disease locus.

One nonparametric method is based on identification of alleles or chromosomal segments that are shared by affected individuals, hence termed allele-sharing method. This particular method can be used either within nuclear families (sibpair analysis) or within extended families or even in whole populations. In sibpair analysis, segments that are identical by decent (IBD) and those identical by state (IBS) are distinguished (17). Identical by state alleles look the same and may even be the same DNA sequence, but they do not derive from a common known ancestor. Identical by descent alleles, on the other hand, are copies of the same ancestral allele. The shared segment analysis can be conducted using either IBS or IBD data. Affected sibling pair analysis (ASP) is a commonly used approach derived from the allele-sharing method that assumes that sibling pairs randomly share 50% of their alleles (18). Therefore, if the alleles of a DNA marker are located near a disease gene which contributes to the disease in the affected siblings, they will tend to share alleles to a greater extent then by chance alone.

Linkage analysis, parametric or nonparametric, can be conducted either by comparing co-segregation of one marker and the disease phenotype (two-point linkage analysis), or by extracting the information from numerous markers simultaneously (multipoint analysis).

2.2.1.2 Association analysis

Linkage analysis is a very robust way to localize a linked genomic region, however even at its best its genetic resolution is limited to 1cM (19). This is approximately equivalent to 1 000 000 bp of DNA, which means that this region can hold tens of genes. Hence, an alternative strategy is needed in order to further narrow down the original linked region. For this purpose, association analysis that is based on linkage disequilibrium (LD) is utilized. The pattern of LD along a chromosome is shaped by the absence of meiotic recombination, thus genomic segments are inherited in blocks from generation to generation, sharing a single genealogical history across their length. The LD is influenced by evolutionary forces such as drift, migration, admixture, and rapid population expansion, thus, it is strongly dependent on population history. Association at population level between a marker and the phenotype of interest can be observed under either of two circumstances; when the functional variant is studied directly, or when the marker variant is in LD with the functional variant.

Association studies can either be based on case-control samples where frequency of alleles of unrelated affected subjects are compared with alleles of unrelated unaffected subjects, or on family-based studies. A large case-control study sample can feasibly be

obtained, and is also the most stastically powerful in the case of no bias. However, the difficulty in matching the controls adequately for age, sex, and ethnicity frequently creates stratification bias and leads to false positive association (20). In order to circumvent this problem several family based control association methods have been developed. Two of the most frequently used methods are haplotype based haplotype relative risk (HHRR) method (21), and transmission/disequilibrium test (TDT) (21, 22). Both methods use the non-transmitted alleles to the affected subjects from the parents as internal controls, thus matching perfectly for ethnicity (21). The main difference between the two methods is that the TDT test extracts only the allele data from the heterozygous parents, while HHRR method also extracts it from homozygous parents. To date, even more efficient new methods have been developed that enables the analysis of linkage and LD jointly (e.g. Pseudomarker) (23), which opens the door for testing of a whole new range of different types of study samples.

2.3 Genome-wide approach

There are two general strategies for identifying complex trait loci depending on what is known about the trait biologically. If no reasonable hypothesis-based candidate genes can directly be tested, the second strategy, a hypothesis-generating approach is considered instead. In this case, anonymous polymorphisms uniformly distributed throughout the genome (traditionally about 400 microsatellite markers for linkage studies) are tested for presence of linked trait locus at each of the loci. This so called genome-wide scan strategy represents a unique tool for detecting previously unknown trait loci.

Conventionally linkage analysis is used to uncover linked loci throughout the genome in these types of studies, although now marker sets for genome-wide scans based solely on association analysis have been developed. However, for the traditional genome-wide scans, large sample sets of families or affected sibling pairs are required. Linkage analysis uses DNA sequences with high variability in order to increase the power to identify markers that are linked with the disease of interest within families. Earlier linkage studies applied restriction fragment length polymorphisms (RFLPs) (24), whereas for many years now short tandem repeat polymorphisms (STRP), also known as microsatellite markers, have been utilized (25). To date, several sets of microsatellite markers have been published (26), resulting in very dense marker maps with approximately 1cM intervals. These DNA sequences represent characteristically short sequences with 2-4 bp repeats, appearing approximately every 30 kb in the human genome. They show considerable variability among people, but most of the time these sequences have no functional consequences. More recently a new generation of polymorphic markers, single nucleotide polymorphisms (SNPs), have taken over linkage and association studies to track diseases in families. SNPs represent a major contribution to genetic variation accounting for approximately 80% of all known polymorphisms in the human genome. They are relatively evenly spread throughout the human genome (ca. one in every ~ 1250 nucleotides) therefore providing markers within the immediate vicinity or even at the locus of interest. Leading biotechnology companies have devolped

their own platforms for screening thousands of SNPs enabling whole-genome studies using a single array chip. For example, Affymetrix has commercially available a 500K Array Set, which comprises of two arrays both holding 250 000 SNPs. Competitors such as Illumina that already have on the market a chip with over 100 000 SNPs, are also now also releasing a 250K chip. The genotyping technology is developing fast and new more powerful methods are constantly on the rise.

The procedure behind a genome-wide screening is rather straightforward, however great difficulties have been met in the application and the interpretation of the results. Thus, in 1995, Lander and Kruglyak performed a simulation analysis of a fully informative marker map (27). Based on the conclusions drawn from that study statistical guidelines for judging validity of genome-wide scan linkage results in complex disorders were suggested that are still widely used. This simulation analysis showed that the traditional lod score 3.0 as a threshold for significant linkage needed to be increased to 3.3 in order to achieve a true genome-wide significance. In other words, with this threshold statistical evidence of this magnitude would be expected to occur 0.05 times in genome-wide scan by chance alone. It was further recommended that a lod score of 1.9 should be considered as suggestive evidence for linkage, since values of this level are expected to occur randomly about once per genome scan. For study designs based on ASP, the corresponding scores (MLS) are 3.6 and 2.2, respectively (Table 1). Overall, the general conclusion is that until an original significant linkage finding has been confirmed (lod score ≥ 1.2 or $P \leq 0.01$) in an independent study sample, it will be considered a false positive (type I error) (27).

Table 1
Significance thresholds for genome-wide linkage.

Method		Genome-wide suggestive linkage	Genome-wide significant linkage
Lod score	*P* value	1.7×10^{-3}	4.9×10^{-5}
	Lod score	1.9	3.3
ASP-MLS	*P* value	1.7×10^{-4}	2×10^{-5}
	Lod score	2.2	3.6

2.4 Candidate gene approach

Candidate genes are implicated in the genetic basis of a phenotype either on the basis of their location in a genomic region that has been identified by mapping studies (positional candidate) or because they fit into a proposed model of the physiology of a phenotype (model candidate or hypothesis-driven candidate). In this approach the association of potentially disease presiposing genetic variants of the gene of interest can directly be tested, providing a very powerful genetic tool. Various study samples can be utilized in this approach (e.g., case/control-sets, affected sib-pairs and multiplex families). Genetic

markers within or flanking the gene of interest are selected for analysis, and since SNPs are on average spaced one every 1250 nucleotides, they provide a magnificent tool for testing of association. At present, haplotype blocks (i.e., genomic blocks separated by recombination) provide an efficient tool for testing of LD in candidate genes, since fewer SNPs need to be genotyped for the same amount of information, providing a more cost-effective and less time-consuming approach. The recently launched project known as the HapMap project aims to develop a map of human haplotypes, or blocks of DNA that may have been conserved in evolutionary history. Together with today's modern technology this provides the possibility of genome-wide association analysis.

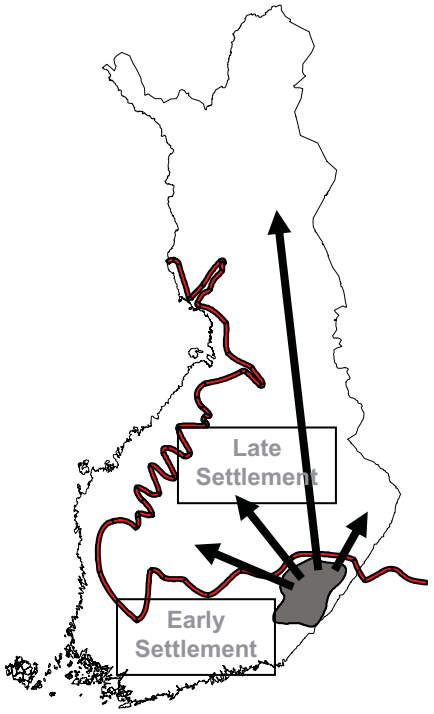
2.5 Advantages of isolated populations in genetic research

One of the most critical phases in a genetic mapping study is the selection of an appropriate study sample. Even though the statistical mapping methods can be very robust, ultimately they will only reflect the quality of the data being analyzed. As previously mentioned, several interacting genes with various penetrances, in addition to environmental factors, are involved in complex diseases. Therefore, it is critical to obtain the most homogeneous genetic sample possible. Contrary to animal samples where breeding, and therefore the genetic background and influencing environmental factors, can be controlled, the paramount option in a human population is the use of genetic isolates. These arise from small and young populations that have grown considerably in size over a short period of time. These usually evolve as a consequence of one or several bottlenecks, after which the population is further genetically shaped by the influences of genetic drift and the founder effect (28), as well as a varying degree of inbreeding that often takes place in most small, isolated populations. All these factors result in the isolated populations becoming genetically more homogeneous and some rare recessive disorders becoming enriched, while again other common Mendelian diseases might be almost non-existent (29, 30). It is believed that because of the genetic homogeneity there would be fewer mutations predisposing to complex disorders compared to more mixed populations. Consequently, the extent of LD will also be greater in a young population, since less recombination events have taken place. This can potentially serve as an advantage in decreasing the number of genetic markers needed to test for linkage.

The Finnish population is among the most studied isolated populations. In fact, genome-wide scans using family material internal isolates of Finland have already been successful in the identification of predisposing loci in in several complex illnesses like type 2 diabetes (31), multiple sclerosis (32), familial combined hyperlipedemia (33), and schizophrenia (34). Briefly, archeological findings conclude that Finland has been inhabited at least 10,000 years (29, 35), suggesting that the first settlers came to Finland when the ice melted after the ice age (36). Genetic studies indicate that the current Finnish population is comprised of small immigrant populations from the south over the Gulf of Finland who arrived in Finland at the beginning of the first millennium (29, 30, 37, 38). A competing model, the dual-origins hypothesis, contends that two different groups settled Finland, identifying the second group to be eastern Uralic speakers who migrated to Finland 4,000 years ago (39). Both models assert that the Finnish population

has remained isolated for 2,000 years, especially in some of the more sparsely populated regions of the country. The relatively large country became sparsely populated by small internal immigrant groups migrating slowly from southwestern parts (early settlement) towards the north and east (late settlement) (Figure1). It should be emphasized that the Finnish population in the late settlement area in the eastern and northern regions of Finland is only 300-400 years, or 15-20 generations, of age. The Saami population occupying the northern parts of Finland, Sweden and Norway is unrelated to the current Finns, and represents an older and more stable population. Archeological findings reveal that the founding population of Finland was very small and did not increase much until the 1700s due to wars, diseases, and famine, resulting in several bottlenecks. Geographical, linguistic and cultural barriers have further sustained Finland's isolation from eastern and western neighboring countries.

Figure 1.
Demonstrates the two settlement waves of Finland.



2.6 Bipolar Disorder

Bipolar disorder (BPD) has been recognized by mankind since the time of the ancient Greeks. However, it was not truly separated as a distinct psychiatric illness until the 20th century when the German psychiatrist Emil Kraepelin made a distinction between manic depression and dementia praecox (later termed schizophrenia). While the acute phase of these distinct illnesses may appear similar due to overlapping symptoms, BPD is characterized by a sudden onset and a fluctuating course in which the individual returns to a relatively normal state between episodes, especially early in the course of the disorder. Schizophrenia, on the other hand, is in general characterized by a long progressive decline without any significant return to the premorbid state.

Unravelling the biological underpinnings of a complex disorder like bipolar disorder is further complicated by the complexity and variation of illness manifestation as well as the elusive linkage between its cyclic nature and its biological substrate.

2.6.1 Clinical and pathophysiological aspects of bipolar disorder

Bipolar disorder, or manic-depressive illness, is one of the most serious and debilitating mental illnesses. The unique hallmark of the illness is mania, which is characterized by prolonged euphoric or irritable mood, racing thoughts, overactivity with a lack of need for sleep, disinhibited speech and sexual activity, an increased optimism to the point of impairment of rational judgement ability, and distractibility. The manic phase is clearly distinct from the patient's normal personality traits and can last for weeks to months, sometimes even years if untreated. The manic phase of BPD commonly cycles with alternative features of severe depression, which symptomatically differs from unipolar major depression and usually consists of atypical symtoms such as prominent fatigue, hypersomnia, and reverse diurnal mood variability (Table 2). Manic-depressive cycles are neither random nor predictable, and as many as 40% of bipolar patients experience a mixed state, which is a condition with either the coexistence or rapid alternation of symptoms of both depression and mania. Many, if not most, patients show a pattern of increasing frequency of cycling over time. The lifetime prevalence of BPD in the general population is approximately 1%, being similar in both sexes and all races (1). There is also an increased tendency for co-morbidity with alcoholism, attention-deficit/hyperactivity disorder, compulsive disorder, drug abuse and eating disorders. Suicide rates for bipolar disorder are alarmingly high, reaching between 10% and 19 %, which is 15 times higher than in the general population (40).

Table 2.
Features of Bipolar Disorder

Feature	Mania	Hypomania	Depression
Severity of disorder	Severity requires hospitalization in most cases, because of major impairment of occupational and social functioning	Does not require hospitalization; unusual demeanor and uncharacteristic behavior noticeable by others, for at least several days	Depressed mood or reduced interest or pleasure in activities, for at least 2 wk; behavior and demeanor different from usual personality, causing noticeable impairment in social, occupational, and other areas of functioning
Symptoms	elevated or irritable mood; feelings of well-being; grandiosity or inflated self-esteem; increased energy; decreased need for sleep; talkativeness; racing thoughts; overoptimistic ideas; distractibility; increased sexual activity and aggressive activity; increased motor activity or agitation; poor judgment and impulsive behavior	Same as mania, but in milder symptoms	depressed mood; loss of interest; reduced self-confidence; reduced energy; insomnia or hypersomnia; decrease or increase in appetite; fatigue; feelings of guilt and worthlessness; poor concentration; ideas or acts of self-harm or suicides; diminished activity

2.6.1.1 Diagnosis

The diagnosis of BPD is based on an evaluation of signs and symptoms in accordance with established diagnostic criteria. The first effort to establish a universal diagnostic system for psychiatric illnesses was made by the World Health Organisation (WHO) in 1948 when they published "International Classification of Diseases" (ICD). Successive versions of the ICD, now undergoing its tenth revision (ICD-10), represent the official diagnostic symtem used by clinicians throughout most of the world. The major exception is the United States, where clinicians use the American Psychiatric Association's (APA) Diagnostic and Statistical Manual (DSM), currently in it's fourth edition. The APA's first diagnostic manual (DSM-I) was published in 1952 and although developed independently from the ICD, they are today generally parallel to each other.

The diagnosis of bipolar disorder is seldom straightforward, sometimes being confused with schizophrenia, and often confounded by comorbid substance abuse. Further, the severity of manic episodes varies among patients. Based on the variation in acuity and duration of manic symptoms, three distinct groups evolve. In addition to the main groups (BPD-I, BPD-II, Cyclothymia), also four subgroups have been distinguished in BPD.

Bipolar Disorder type I (BPD-I)

Estimates of bipolar disorder type I, or classic manic-depressive illness, have ranged from 0.8-1.6% of the population (41). BPD-I represents the most severe form of mania that may display a whole range of the characteristic symptoms, and may include psychotic symptoms such as hallucinations and delusions, with grandiose delusions being the most common. In order to be diagnosed with BPD-I the manic episode must last at least 1 week.

Bipolar disorder I has the highest rate among early onset patients (<15 years of age) (1) and over 90% of the BPD-I experience their first manic episode before the age of 35. There is a trend towards increase in severity of BPD with earlier age of onset. It is thought that most BPD-I cases are treated since the symptoms are behaviorally manifest and observable, and manic behavior is clearly distinguishable from ordinary functioning.

Bipolar Disorder type II (BPD-II)

Bipolar disorder type II is characterized by mild manic episodes without psychotic symptoms, which are accompanied by at least one or more depressive episodes. These hypomanic episodes have to last at least 4 days and are defined by persitantly elevated, expansive mood, with an irritable underpinning. In general, hypomania is not severe enough to cause problems in social activities and work. On the contrary, the increased energy, self-confidence and creativity can be experienced as an advantage. It is important to notice that this episode is still cleary distinguable from the patient's general personality.

The mean age of onset is a bit higher in BPD-II than BPD-I, and the episodes are somewhat shorter, however BPD-II still has a strong tendency to become chronic. It has been reported that BPD-II has a higher prevalence (~ 5%) than BPD-I, and has also been found to be more common among women than men (42, 43). Nevertheless, the exact prevalence is hard to determine since BPD-II does not require hospitalization, therefore allowing many cases to go undetected and undiagnosed.

Cyclothymia

Cyclothymia is a chronic condition of BPD consisting of short periods of mild depression and short periods of hypomania lasting only a few days to a few weeks, again separated by short periods of normal mood. Individuals with cyclothymia are never free of symptoms for more than two months at a time. The prevalence is reported to be about 6% with no significant difference in sexes (44, 45).

Subgroups

Aside from these main groups of BPD four subgroups are also described, namely rapid cycling, ultra rapid cycling, seasonal patterning and post-partum onset. BPD patients with rapid cycling experience more frequent mood episodes than generally seen in BPD-I and BPD-II (4 or more per year). Ultra rapid cyclers often experience 4 or more episodes per week, but can cycle as rapidly as 4 or more cycles per day. In seasonal patterning the onset of the mood episodes can be predicted by a seasonal pattern, and post-partum onset is a mood disturbance that occurs within 4 weeks of childbirth.

Some BPD patients do not fit in any of these descriptions, but clearly present signs of BPD (e.g. overlapping a psychotic disorder, hypomanic episodes with no depressive episodes). These are classified as BPD not otherwise specified (NOS), which usually is the primary diagnosis of an underlying psychiatric disorder that does not presently meet full criteria for any of the classically defined Bipoar Disorders.

Schizoaffective disorder

Schizoaffective disorder is a schizophrenia-bipolar boundary disorder. It is widely debated if it primarily belongs to the affective or the psychotic spectrum, since it displays symptoms from both BPD and schizophrenia. The clinical signs and symptoms include all features of schizophrenia (hallucinations, delusions, disorganized speech, grossly disorganized or catatonic behavior and negative symptoms), mania and depression. The schizophrenic and mood disorder symptoms can appear together as well as independently, varying greatly from person to person.

It is not known how common schizoaffective disorder is, but it is believed to be less common than schizophrenia and mood disorders. Women may also be more often affected than men. To distinguish between bipolar disorder and schizophrenia can be particularly difficult in adolescence, since at that age psychotic features are especially

common during manic periods. Accurate diagnosis is easier once the acute psychotic episode is under control. However, since schizoaffective disorder is so complicated, misdiagnosis is common.

2.6.2 Pathophysiological aspects of BPD

When reviewing the literature of pathophysiological findings in BPD, the complexity of the underlying biological mechanisms is evident, with findings as diverse and contradictory as in genetic studies. Even though the research findings to date provide us with some insights relating to behavior, brain state and intracellular events, no definitive conclusions can be drawn with regard to the individual etiological roles of these factors and even less is known about the underlying inter-connectedness of the complex biological systems involved.

Post-mortem studies are a robust way of glimpsing at the pathophysiological aspect of a disorder. However, in BPD it is difficult to distinguish the nascent brain changes from the ones that are pharmacologically induced by long-term drug treatments. In addition, the BPD patients that die at an advanced age are likely to have petrochemical abnormalities secondary to other brain disorders, including Alzheimer's disease. Conversely, BPD patients that die at a younger age, often by committing suicide during a period of acute stress will induce changes in the brain that are not directly linked to the underlying biological mechanisms of BPD.

The advances in technology have provided us with other tools to measure the anatomy and function of the human brain and its neurochemical abnormalities. These sophisticated neuroimaging techniques have opened whole new possibilities in the research of brain disorders. With computed tomography and magnetic resonance imaging (MRI), structural images of the brain can be attained. Information about brain function has also become accessible through the development of functional MRI and positron-emission tomography (PET) technology. Most of the imaging studies in bipolar disorder thus far have been small, both due to the cost and the difficulty involved in studying patients who are either manic or depressed. Thus, the ability to replicate results has been poor. At present, neither neuroimaging nor neurochemical studies can be used as biologic tests to predict or confirm the diagnosis of BPD.

The fact that no consistent alterations in regional brain functions have been found in BPD is suggestive that BPD is not directly bound to a specific brain region. Although strokes can induce manic behavior, these are more likely syndromic expressions of a final behavioral phenotype that coincidentally resembles the manic state of BPD. At this point, we do not know which is the cause and which is the effect, but it seems clear that any alterations in the function of a complex system like the brain will be manifest throughout the whole system. Unlike schizophrenia, the fact that most bipolar patients return to a state of relative normality, even in the absence of treatment, is strongly suggestive for alterations in a modulatory mechanism.

Thus far alterations in signal transduction appear to offer the most explanatory premise for the range of symptomatology in bipolar disorder. However, the signal transduction alterations typically exhibited by patients with BPD represent endophenotypes rather than causative biological mechanisms. Similarly, early studies examining urine and spinal fluid for abnormalities in metabolites of the chief monoamine neurotransmitters, noradrenalin, serotonin, and dopamine were difficult to replicate and, if replicated, turned out to be a result rather than a cause of the hyperactivity typical of mania and the hypoactivity and weight loss typical of depression. Difficulty discriminating which observed biological phenomena play a role in the etiology of the illness, as opposed to representing biological markers or biological effects of the illness, is the primary hurdle of research in this area.

2.6.3 Treatment of bipolar disorder

In the 1950s the treatment of BPD was revolutionized when lithium was identified to have mood-stabilizing effects, and it also served as a potential window into understanding the disease related alteration at the cellular level. Unlike other medications in psychiatric disorders, lithium is a salt, and consequently, does not have a receptor to which it binds in the brain. It is actively transported into the cell through the sodium channel, and when a neuron depolarizes, the sodium channel opens and both sodium and lithium rush into the cell. The sodium is then actively pumped out, using the sodium-potassium-ATP pump, but lithium remains in the intracellular compartment. Lithium's effect in BPD appears to rely on the modulation of several second messenger systems, including cAMP and phosphoinositol pathways (46). Rather than causing large changes in baseline cellular activity, lithium seems to attenuate responsivity to other neurotransmitters, including serotonin, dopamine, and gamma-aminobutyric acid (GABA) circuits. Its efficacy may possibly be more related to its wide-ranging neurobiological effects rather than to a single mechanism. One possibility is that lithium exerts its effects by resetting the ionic homeostasis in neurons either directly or through its interaction with second messenger systems (47).

The acute phase of mania in BPD is mostly treated with lithium, valproate, and carbamazepine. However, there is a growing consensus that in order to obtain an optimal response in most BPD patients, multiple-drug therapy is required. Bipolar depression generally responds to tricyclic antidepressants, selective serotonin reuptake inhibitors, and monoamine oxidase inhibitors. Nevertheless, the new atypical neuroleptic drugs such as clozapine, olanzapine, risperidone, and ziprasidone have been shown to pose fewer risks of inducing depression than the classic neuroleptic drugs. However, some negative adverse effects of these drugs include weight gain, changes in lipid levels, and abnormalities in glucose tolerance. It usually takes three to six weeks before the full effect of the pharmaceutical treatment can be seen in BPD patients.

Complicating the treatment and the clinical course further is the common co-morbidity of substance abuse among BPD patients. BPD patients in the depressive phase may use alcohol or drugs as self-medication and patients in the manic phase may crave them as

part of the characteristic arousal of this phase. However, the extent of substance abuse in patients with bipolar disorder varies greatly according to culture, country of residence, and socioeconomic class.

2.7 Genetics of bipolar disorder

For more than three decades substantial evidence has suggested that the major psychiatric illnesses of schizophrenia, bipolar disorder, autism, and alcoholism have a strong genetic basis. It has been hoped that identification of these genetic factors might provide the means to learn more about the neural or biochemical mechanisms of these diseases and thus provide a rational basis for the design of effective therapies. About half of the patients diagnosed with BPD have a positive family history of the disorder, and in some families, the disease has traveled across several generations. Although both genetic and non-genetic factors contribute to these diseases, it is clear from the abundant number of family, twin and adoption studies that the etiology of BPD is strongly influenced by genetic factors (see 3.2.1). The inheritance of BPD appears to be multifactorial in nature rather than the result of simple Mendelian transmission, which makes it more difficult to isolate the specific genes or combination of genes that contribute to the manifestation of the illness. Another major challenge in the genetic studies of BPD is to identify the affected phenotype accurately and reliably. Unlike other common medical illnesses, robust biological markers have not yet been identified for BPD, forcing exclusive reliance upon categorical diagnoses. Attempts to replicate linkage findings have also been hampered by genetic heterogeneity, phenocopies, genotyping errors, and the complexities of performing and interpreting statistical analysis (15, 27).

It is recognized that mental disorders arise from complex gene-gene, gene-environment interactions, and that no single gene may be either necessary or sufficient to produce any mental disorder phenotype. Hence, it is not surprising that like most genetically complex medical disorders, mental illnesses exhibit a great deal of clinically significant heterogeneity even within families.

2.7.1 Heritability of bipolar disorder (Family, twin and adoption studies)

A clear heritable component of BPD can already be assumed by observing its clear trend to aggregate in families, such that family members of an affected individual are more often also affected than in comparison to any random person in the general population. To date, this assumption is supported by numerous family, twin and adoption studies that argue strong genetic contribution to the etiology of bipolar disorder. It should be emphasized, though, that within families afflicted with BPD, the nature of inheritance often features a cluster of syndromes, that include in addition to bipolar disorder, alcoholism, non-specified psychosis, schizophrenia and unipolar depression.

While family studies indicate the degree to which diseases aggregate in families, they alone cannot assess the proportion of genetic versus environmental factors involved in

the etiology of a disorder. For this assessment, unusual relationships such as twins, adoptees, or half siblings are required. Twin studies consist of monzygotic twins sharing 100% of their genetic material, as well as dizygotic twins who share on average only 50% of their genetic material, both still sharing a relatively common environment. By monitoring the co-occurrence of a disorder in MZ and DZ twins, a distinction of the genetic and environmental factors involved in the disorder can be made. If there is no genetic component present in the disorder, the concordance rates are similar for both twin types. Thus, in a monogenetic disorder with 100% penetrance, the MZ twin concordance will be 100%, whereas the DZ rate will be equal to that of any other siblings. However, for multifactorial disorders, the rate for MZ twins will typically be less than 100%, but more than for DZ twins. Adoption studies are the most powerful design to test the relative contributions of genetic and environmental factors to etiology of a disease, but these are more demanding to conduct.

Family studies in BPD have shown the approximate lifetime risk for a first-degree relative of a bipolar proband to be 5%-10%. Twin studies have shown that MZ twins have a 75 times higher risk than the general population of contracting BPD if the other twin is affected. Even though fewer adoption studies have been performed, these also demonstrate that biological relatives of BPD patients are substantially more likely to have the disorder than are adoptive relatives.

2.7.2 Previous genetic findings in bipolar disorder

During the past 15 years the vast majority of endeavors to find the genetic loci predisposing mental disorders have utilized the linkage analysis strategy. Despite this effort, a susceptibility gene has not yet been found. The key strategy has been to employ genomic markers and to test for linkage among families with multiple affected members. However, the outcome during the early years (48, 49) was mostly disappointing when the positive linkage findings were not supported by subsequent independent studies (50). Today, it is generally recognized that further advances in unraveling the genetics of BPD will require the analysis of hundreds to thousands of affected individuals and their families. Linkage analysis together with high throughput DNA sequencing, microarrays for whole genome scanning, advances in proteomics, and the development of more sophisticated computer programs for analyzing sequence and association data hold promise of greatly expediting the search for the genetic basis of most mental illnesses, while at the same time providing molecular targets for the development of new and more effective therapies.

The history of genetic studies in BPD might appear as manic depressive as the disorder itself. Yet, the cycle of apparent discovery followed by diasappointment due to lack of replication is a common outcome among most complex disorders. The three main causes for this outcome is locus heterogeneity, inadequate sample sizes and inaccurate phenotype definition, which is especially problematic in psychiatric disorders. In genetics the word heterogeneity refers to two different phenomena, namely locus and allelic heterogeneity. Locus heterogeneity refers to the same or closely similar phenotype

having causal links to mutations in different genes at different chromosomal loci. Allelic heterogeneity describes a state when different mutations in the same gene cause the same phenotypes. Both pose their own set of problems in study designs. In linkage analysis allelic heterogeneity does not create problems, since linkage to the same chromosomal region will still be detected regardless of the specific variation present. Locus heterogeneity, on the other hand, will generally overwhelm linkage analysis because of the presence of several different genes with the same phenotypic outcome. In association analysis, allelic heterogeneity also raises problems as association tests in general depend on cases sharing the same allele. And while locus heterogeneity causes fewer problems for association studies, it still reduces the power of the analysis. It has been shown in a simulation by Suarez et al. (51) that a locus detected in one sample will unlikely be replicated in another sample because different study samples are very likely to contain differing proportions of families linked to particular loci unless the second sample is much larger.

Three general strategies are commonly used in order to overcome the problems caused by locus heterogeneity. First, ascertaining study samples from genetically isolated populations, where the number of different susceptibility genes may be smaller, could help to deal with heterogeneity. Another approach is to focus on familial patterns of the illness, with the assumption that different patterns could point to different susceptibility genes (52-55). And finally, selection of families that are enriched for clinically severe phenotypes might provide an advantage, assuming that these families may be more homogeneous. All of these approaches have one emphasis in common: a constructive ascertainment strategy how families that will be studied are selected and identified. Ascertainment is a fundamental principle of epidemiology, as it should be in the genetic studies of BPD as well. In schizophrenia genetics researchers are on the verge of major breakthroughs, mainly because of the large schizophrenia samples that have been collected based on a population-based ascertainment scheme that systematically samples all families present in a defined region (56, 57). This has subsequently led to identification of strong candidate susceptibility genes (58, 59).

Sample size is still a problem in linkage and association studies in BPD. To date, no genome-wide scan candidate gene study has been based on the kind of sample size known to be necessary for detection of the underlying biological mechanisms in BPD. However, meta-analyses of worldwide genome-wide scans have recently been employed, which have facilitated detection of linkage signals that are weaker in effect but consistent across studies (60) (61).

The lack of specific biological markers in BPD causes a lot of problems in genetic studies, since the same underlying biology may not be shared among BPD patients and therefore the validity of the diagnosis can never ever be guaranteed. One approach that is becoming increasingly popular and that copes with this issue is the use of endophenotypes instead of DSM-based diagnosis. Endophenotypes attempt to go beyond the usual approaches to clinical subtyping based on symptom picture, age at onset, comorbidity, treatment response, etc. To date they have increased substantially our

understanding of the clinical picture in schizophrenia (e.g., prepulse inhibition) (62), panic disorder (e.g., sensitivity to lactate infusion) (63), and alcoholism (e.g., evoked potentials) (64), and are as well thought to be genotypically more homogeneous.

Anticipation is known to involve expansion of segments of the genome consisting of trinucloetide repeats. Thus, it is reasonable that such a mechanism may account for the earlier onset and increased severity of bipolar disorder in subsequent generations of families through which the disorder is segregating. Several studies have shown that there are clinical differences between early and late onset BPD. Early onset of BPD have more psychotic symptoms (65), more manic episodes (66), and have higher levels of comorbidity with conduct disorders, alcohol abuse, drug addiction and eating disorders (67). These patients also respond poorly to lithium (68). Anticipation is the phenomenon whereby a disease becomes more severe and/or presents with earlier onset as it is transmitted down through generations of a family. The only known mechanism for true anticipation is a class of mutations containing repetitive sequences exemplified by the pathogenic trinucleotide repeats. Studies of bipolar disorder (BPD) are consistent with the presence of anticipation and, by inference suggest that trinucleotide repeats contribute to this disorder, although it is possible that these data are the result of methodological problems. On the assumption that anticipation in BPD may be real, several surveys of the genome of BPD probands for large trinucleotide repeats have been conducted, as have studies of many repeat-containing candidate genes.

Genome-wide scans and the most prominent findings

Numerous genome-wide scans have already been completed for bipolar disorder (69-110) and all published genome-wide scans in bipolar disorder are presented in Table 3. Despite difficulties with diagnostic differences between sample populations and the lack of statistical significance in many individual studies, several promising patterns have emerged, suggesting that some true susceptibility loci for bipolar disorder may have been identified. The strongest and most replicated evidence of linkage in genome scans to date are found on chromosomal regions 4p16-p13 (71, 74, 75, 95, 97, 110), 4q21–35 (69-71, 77, 82, 83, 85, 97, 100, 109, 111), 6q14-q24 (69, 71, 74, 80, 81, 83, 85, 103, 104, 107), 8q24 (70, 80, 87, 93, 100, 104), 12q21–q24 (69, 73, 74, 77, 79, 98), 13q12-q14 (70, 73, 87, 89, 93), 16p13–p12 (69, 73, 74, 77, 83, 88, 94) and 18q21–q23 (70, 73, 97, 101, 102). However, methodological issues and non-replications again demonstrate that these findings are not conclusive. Furthermore, no predisposing genetic variant for BPD has yet been found, and like in most complex disorders the linkage peaks are very broad and may contain hundreds or even thousands of genes.

The following chromosomal regions have been implicated in several studies and meet the statistical guidelines for confirmed linkage. Hence, these regions are considered promising candidate regions in BPD.

Chromosome 4p16-p13: Blackwood et al. obtained a two-point lod score of 4.1 (three-point lod score of 4.8 with the neighboring markers) in their genome-wide scan in

one large Scottish pedigree (110). In an effort to replicate the result in 11 smaller pedigrees the old score went down to 2.9, with an alpha-value (α) of 0.35 at theta 0.0 (θ). Lod scores of similar magnitude were reported by Ginns and colleagues in a mental health wellness study of relatives that are at high risk for BPD. In this study they found significant evidence of linkage to the 4p15 chromosomal region, the two-point analysis providing *P*-values less than 3 x 10-5 and the NPL multipoint analysis lod scores of 4.1. Additional support for this locus comes from three independent genome-wide scans (75). First, Ewald et al. obtained a two-point lod score of 2.0, producing an empirical *P*-value of 0.0006 at the same locus in a study of two Danish families (112). Also Detera-Wadleigh et al. detected a parametric lod score of 3.2 in one of the families, however when analyzing their total set of 22 families the signal was slightly weaker ($P = 0.0022$) (95). In the second stage of the Wellcome Trust UK-Irish genome-wide scan Lambert et al. found suggestive evidence of linkage ($Z_{max} = 2.2$) on chromosome 4p14-p13 (71).

Chromosome 4q21-q35: Numerous linkage findings have been reported at chromosome 4q, however the linkage signals are widely scattered reaching from 4q21 to 4q35. In 1999 Adams and colleagues were the first to report significant evidence of linkage to the more telomeric region (4q35: Zmax = 3.6) (109). Also the recent Wellcome Trust UK-Irish genome-wide scan reached statistically significant lod scores (Zmax = 3.3), however the linkage peak being located far more centromeric on 4q12-q21 (71). The same chromosomal region 4q21 was also implicated in a SNP genome-wide scan by Middleton et al. screening Portuguese bipolar families (83). They found nonparametric lod scores approaching significance reaching 3.0 using a marker map with a median intermarker distance of 210kb (83). In the NIMH Genetics Initiative replication study Willour et al. found supporting evidence of linkage to 4q35 reaching a lod score of 2.5. In the same study Willour et al. also reported strong linkage signals merging from another region located approximately 40cM more centromeric, on 4q32 (Zmax = 2.2) (85). In a subsequent genome-wide scan from Finland, a two-point lod score of 2.5 and a significant three-point lod score of 3.6 was obtained with the same exact marker as in the NIMH study (77). Interestingly, Ginns et al. reported in their genome-wide scan of a set of Old Order Amish bipolar pedigrees a locus in the near vicinity at 4q31 linked to mental health wellness (75). The same chromosomal region was implicated in three additional bipolar genome-wide scans. First, in a study by Liu et al., where the lod scores approached significance (Zmax = 3.2) (75), and then by Friddle et al. that found multipoint lod scores of 2.1 in a set of 50 multiplex families (100). Finally, McInnis and colleagues published a genome-wide scan with highly dense microsattelite marker map of 842 markers and found a nonparametric lod score of 2.8 (Zmax = 1.9) on 4q32 and 2.4 on 4q35 (Zmax = 1.2) (70).

Chromosome 6q16-q24: In the third NIMH GI wave 250 bipolar families from the U.S were analyzed, one of the regions that yielded significant linkage was on 6q16 (Zmax = 3.6) (80). Also in a genome-wide scan of 16 extended Portuguese families provided close to significant lod scores resulting in 3.1 (81). However, in another study analyzing an additional 9 Portuguese families highly significant lod scores were

observed. In the preliminary analysis a parametric two-point lod score of 3.6 and a nonparametric lod score of 4.2 were found. When further mapping the critical linkage region with SNPs a nonparametric two-point lod score of 4.4 was observed (Z_{max} = 2.6) (83). In a Swedish bipolar genome-wide scan 9 multigenerational families provided a multipoint lod score of 2.5. However, when further genotyping additional markers in the region a multipoint lod score of 3.3 was achieved (107). Additional support for this region was also provided from the second stage of the Wellcome Trust UK/Irish genome-wide scan (Z_{max} = 2.6) (71), as well as from a bipolar genome-wide scan of two Danish extended pedigrees (Z_{max} = 2.2) (74). McInnis et al. also published a genome-wide scan of 153 U.S. families that obtained a nonparametric lod score of 2.8 using a conditional analysis method (69). Due to the overwhelming linkage support for this chromosomal region an effort between 10 different was made to combine all linkage data for this chromosomal region. This particular chromosomal region, 6q22-q24 has been identified as a region with numerous imprinted genes (113), thus slightly different analyzing strategies were applied. By testing the parent-of-origin effect it showed that the affected siblings share the maternal chromosome more often than the paternal (P = 0.006). In this study lod scores ranging from 2.3 to 5.4 were obtained for 6q when analyzed for each locus conditioned on evidence of linkage to the other. Overall the potential BPD locus was found to confer a 1.4-fold increased risk (103). Finally, McQueen and collogues published a combined analysis of eleven pre-excising genome-wide scans from different populations (69-71, 78, 80-82, 85, 86, 88, 90, 92-94, 97, 102) and found a highly significant lod score of 4.2 on 6q21 further supporting the potential involvement of this chromosomal region in BPD (104).

Chromosome 8q24: In a genome-wide scan of 75 bipolar families originating from Germany, Israel or Italy significant a lod score was observed on 8q24 (Zmax = 3.6) (93). This was replicated by McInnis et al. in 65 U.S. bipolar families yielding a nonparametric lod score of 3.1 (Z_{max} = 2.1) (70). Suggestive lod scores were provided by three additional genome-wide scans: the third NIMH GI scan of 250 bipolar families (Z_{max} = 2.5) (80), Friddle et al. with 50 bipolar families from the U.S. (Z_{max} = 2.4) (100) and 13 Australian bipolar families (Z_{max} = 2.1) (87). The q-arm of chromosome 8 has remained one of the few regions that remained relatively narrow of the repeatedly replicated chromosomal regions in the bipolar genome-wide scans. In addition, in the combined genome-wide by McQueen et al. a significant lod score of 3.4 was found (104).

Chromosome 12q21-q24: In 1994, Craddock et al. reported familial aggregation of affective disorder and Darier's disease on 12q23-q24.1 (114). Subsequently, the gene for Darier's disease was identified as a sarcoplasmic/endoplasmic reticulum calcium-pumping ATPase, ATP2A2 (114). Mutational analysis of this gene identified 17 novel mutations that showed some minimal evidence for clustering in the 3' end of the gene in Darier's disease patients with neuropsychiatric phenotypes. Further evidence for linkage to 12q has been obtained in two large families from the Saguenay Lac St-Jean region of

Quebec, a settlement derived from the migration of a small number of founding families into this region in the 1830s. In this sample a nonparametric lod score of 3.9 on 12q21 and even though significant evidence of linkage was not found in the parametric two-point analysis, lod scores exceeding the threshold for replication were found for several markers (Z_{max} = 1.61) (98). A more recent genome-wide scan from the same group provided a parametric lod score of 3.4 and a nonparametric lod score of 5.1 when analyzing 18 additional families from the same region (79). In 1998 Barden et al. obtained significant evidence of linkage (Z_{max} = 4.9) in an extended family from Quebec. Unfortunately, reconstruction of haplotypes from these data did not identify a common haplotype, making it difficult to further define the critical region (115). In addition two large Danish families with multiple affected members in several generations were genotyped and a two-point lod score of 3.4 (P = 0.00002) was obtained approximately 14 cM from the ATP2A2 gene. Haplotypes were successfully constructed in each family, and a minimal overlapping region of 3.8 cM was observed in all but one bipolar patient (116). Several other genome-wide scans from different populations have all provided lod scores exceeding 2.0 (70, 73, 77).

Chromosome 13q12-q14: Two separate genome-wide scans have published significant linkage results on the same chromosomal region on 13q. Cichon et al. screened 75 German families for bipolar disorder and found a nonparametric multipoint lod score of 3.4, nonparametric two-point lod score of 3.3 and parametric lod score of 2.3 on 13q12. In 2001 Badenhop obtained a significant nonparametric multipoint lod score of 4.1 (NPL Z_{max} = 2.9, Z_{max} = 3.0) in a multiplex Australian family. By constructing haplotypes they restricted the critical linked genomic region to 6cM that included genes like the serotonin 2A receptor (89). When conducting another genome-wide scan with 13 additional families the same region still showed evidence of linkage (Z_{max} = 2.3) (87). Additional support for these findings have been obtained in two other genome-wide scans. McInnis et al. found a nonparametric lod score of 2.4 when screening 65 U.S. bipolar families (70). Maziade and colleagues combined a bipolar and schizophrenia family sample and found as a result a lod score of 2.7 on 13q14 (73). This region has been implicated in several schizophrenia linkage studies as well, suggesting a potential shared predisposition for both disorders.

Chromosome 16p13-p12: Significant linkage has been found in three recent genome-wide scans all screening bipolar families from different populations. Ekholm et al. reported a significant two-point lod score (Z_{max} = 3.4) in a Finnish bipolar study sample on 16p12 (77), which was shortly replicated by McInnis and colleagues performing a conditional analysis on 153 U.S. families (NPL Z_{max} = 3.3, Z_{max} = 2.5) (69). The most significant lod score was found in the bipolar and schizophrenia combined genome-wide scan by Maziade et al.. They found a maximum lod score of 4.1 when only analyzing the bipolar families, however when combining the entire sample the lod score dropped to 3.7. Nonetheless, this still remains a highly significant lod score, but is indicating that the lod scores are being driven by the strong linkage in the bipolar

families (73). The earlier linkage findings found by Edenberg in the first wave of the NIMH GI in 1997, they obtained a suggestive lod score of 2.5 (94). This was replicated by Dick et al. in a follow-up replication study (Wave 2 of the NIMH GI) with 56 new bipolar families yielding a maximum lod score of 2.8 (88). In genome-wide scan of two multiplex Danish families Ewald and colleagues found a two-point parametric lod score of 2.2 (74). Same region was again implicated in a genome-wide scan in Portuguese families (Zmax = 2.9) that covered the genome with a dense marker map of 11 560 SNPs (83).

Chromosome 18q21-q23: In addition to the signal on 18p, Stine et al. noted excess allele sharing at 18q21 (P = 0.0004), approximately 40 cM from the region on 18p implicated in the original report by Berrettini et al. (117). This finding was supported in a genome scan of two large Costa Rican families, where several markers that mapped to 18q22-23 yielded lod scores that were marginally positive (Z_{max} = 1.0–2.3) in each family individually as well as when combined. In addition, haplotypes were reconstructed and a large region (~ 40 cM) displayed excess allele sharing. The evidence for linkage and association was evaluated jointly in a test estimating the recombination frequency and proportion of disease chromosomes sharing a common allele to derive a linkage/association-based lod score of 3.7 and 4.1 for the best markers, respectively (118). Significant lod scores were also reported by a recent combined bipolar and schizophrenia genome-wide scan by Maziade et al.. When analyzing all the families with both BPD and SZ they found a significant parametric two-point lod score of 4.5, however this signal was not seen when only analyzing the bipolar pedigrees (73). Fallin et al. screened 41 Ashkenazi Jewish families and found a suggestive nonparametric multipoint lod score of 2.2 on 18q22 (102). The same chromosomal region was implicated by McInnis et al. when studying 65 U.S. families (NPL Z_{max} = 2.9) (70). Following these initially encouraging reports, several other groups investigated families for chromosome 18 markers. A Jewish Ashkenazi family and a Belgian family were genotyped for 14 markers, and several markers in the region of 18q21.33-q23 showed excess allele sharing (P_{min} = 0.0007) as well as a multipoint lod score of 1.34 for these markers. It is interesting to note that a small parent-of-origin effect was again detected, but it was not consistently paternal (102). Finally, Nothen et al. reported nonparametric lod scores of ·-2.0 in the region of 18q22-23 (119).

The litterature is relatively sparse on how to interpret several positive findings in the face of an equal or larger number of negative findings for the same markers. Furthermore, if impressive P values are obtained for different diagnostic models, these cannot be considered as simple replications. A single replication study meeting the P = 0.01 criterion in the face of multiple negative studies must therefore be interpreted with caution, unless it can be clearly demonstrated that the negative studies were underpowered and thus unable to detect the effect. The absence of findings in the overwhelming majority of genome scans suggests that the initial observation may have been a false positive. Of course, it is impossible to rule out a gene that confers a very low risk of bipolar disorder.

Table 3.
Genome-wide scans conducted in bipolar disorder

Study (first author, year)	Sample	Pedigrees (n)	Affecteds (n)	Number of markers (n)
Coon (1993)	Utah	8	51	328
Straub (1994)	U.S./Israeli	47		5-153/family
McInnes (1996)	Costa Rica	2	24	473
Blackwood (1996)	Brittish	12		
Polymeropoulos (1996)				
NIMH GI Wave 1	U.S.	97	320	319
Detera-Wadleigh (1997)	(Indiana University,			127
(chr.4,7,9,18,19,20,21)	John Hopkins University,			
Edenberg (1997)	Washington University,			74
(chr. 3,5,15,16,17,22)	NIMH)			
Rice (1997)				65
(chr. 1,6,8,10,12)				
Stine (1997)				53
(chr. 2,11,13,14,X)				
Admas (1998)	Australian	11	58	214
Ginns (1998)	U.S. Old Order Amish	4	50	980
Detera-Wadleigh (1999)	U.S: NIMH/Amish	22	276	607
Morissette (1999)	Quebec	2	86	332
Friddle (2000)	U.S: Hopkins	50	236	267
Foroud (2000)	U.S.	97	414	365
Murphy (2000)	Irish	45	130	65
Cichon (2001)	German/Israeli/Italian	75	275	382
Badenhop (2001)	Australian	1	6	400
Kelsoe (2001)	U.S/Canada	20	76	443
Maziade (2001)	Eastern Quebec	21	254	220
Turecki (2001)	Canadian	31	106	378
Radhakrishna (2001)	Turkish	1	34	230
NIMH GI Wave2	U.S.	56	228	296
Dick (2002)	(Indiana University,			84
(chr. 3,5,15,16,17,22)	John Hopkins University,			
Zandi (2003)	Washington University,			105
(chr. 2,11,13,14,X)	NIMH)			
Willour (2003)				107
(chr. 4,7,9,18,19,20,21)				
Ewald (2002)	Danish	2		613
Badenhop (2002)	Australian	13	69	400
Bennett (2002)	Irish/British	151	367	398
McInnis (2003)	U.S.	153	641	513
Curtis (2003)	British/Icelandic	7	68	365
NIMH GI Wave3	U.S (10 sites*)			
Dick (2003)		250	1964	391
Ekholm (2003)	Finnish	41	137	389
Liu (2003)	U.S	40	307	343
McInnis (2003)	U.S.	65	302	842
Fallin (2004)	Ashkenazi Jewish	41	97	382
Macgregor (2004)	Scottish	13	132	372
Pato (2004)	Portuguese	16	47	366
Faraone (2004)	U.S. :NIMH Wave2	97	415	319
Middleton (2004)	Portuguese	25	140	11 560 SNPs
Lambert (2005)	Irish/British	232	622	198
Maziade (2005)	Quebec	21	129	607
Ewald (2005)	Cuban	22		1 494 SNPs
Shink (2005)	Quebec	20	218	380
Venken (2005)	Swedish	9	46	380
McQueen (2005)	Combined analysis of 11 pre-existing genome-wide scns	1 067	2 423	4 510

Candiate gene studies in bipolar disorder

The selection of susceptibility candidate genes for BPD has been based on two different strategies, regional candidate genes within linked regions and genes that potentially could be functionally involved in BPD. The sequencing of the human genome and identification of numerous SNPs should substantially enhance the ability of investigators to identify disease-causing genes in these areas of the genome. General observations can be made about the difficulties in identifying disease genes for schizophrenia and bipolar disorder. It appears that studies of schizophrenia have produced more convincing evidence for linkage than those for bipolar disorder for several reasons including the use of larger sample sizes, a narrow phenotype, and more conservative and uniform data analysis.

Mapping a locus by linkage methods to a narrow region requires even more families. As a result, both positional and model candidate genes for psychiatric disease are identified with less confidence than for Mendelian disorders. Penetrance – the likelihood that a person carrying a mutation will develop the characteristics caused by that mutation – is likely to be low for most susceptibility alleles in psychiatry. This is because it is believed that many such alleles must occur in the same individual for the expression of complex psychiatric phenomena and because environmental factors probably have a large role in the expression of psychiatric disease. As a result, the concordance of susceptibility variants with disease will be incomplete. Association analysis in which variant allele frequencies are compared in cases and controls can be used to demonstrate partial association of specific variants with disease, but these will be of only moderate magnitude and require further validation. It is the reliance on statistical evidence of allelic association that most distinguishes the genetic study of complex traits from Mendelian traits in the post-genomic era. There are no accepted biological markers of psychiatric disease, and those markers that have been proposed, such as levels of hormone and neurotransmitter metabolites in serum, cerebrospinal fluid or platelets, are themselves likely to result from complex interactions between multiple gene products and epigenic factors. Most candidate genes that have been studied in psychiatry are involved in neurotransmission of the biogenic amines: 5 hydroxytryptamine (5-HT; serotonin), dopamine and noradrenaline (norepinephrine). These genes have been studied as the result of the neurotransmitter hypothesis of psychiatric disease, which has been dominant since the 1950s. This model of psychopathology was developed when the first successful antidepressant and antipsychotic medications were discovered and found to modulate the metabolism of biogenic amine neurotransmitters. As genes for components of biogenic amine neurotransmission systems were cloned and variants were identified, they were used as candidates in genetic association studies of various psychiatric disorders. These genes comprise only a small fraction of the possible candidates for psychiatric disease, and the evidence supporting their roles in psychiatric disease is not strong, particularly when compared with model candidate genes for Mendelian disorders. Thus, these genes are unlikely to represent the major determinants of psychiatric phenomena. It is possible that these genes have a role in defining mood, perception and behavior, but variants in any single one of these genes probably have only a small effect. The main weak point of the genetic approach to complex phenomena is that any single variant might have only small effects on clinically observable phenomena. The

relationship between genotype and phenotype is very stable and reliable but is also likely to be relatively weak. This is particularly important to appreciate for model candidate gene studies because the intent of these studies is to test a specific hypothesis that is built on data from studies of biological markers of CNS function. The very nature of complex psychiatric diseases ensures that there will be few major disease genes. That is, even very well designed and executed studies will identify susceptibility variants that only partially explain the risk for the disorder in individuals who possess these variants. To have convincing explanatory power, candidate gene studies in psychiatry must be very powerful statistically and supported by functional studies that (1) define a neurobiological role for the candidate gene, and (2) demonstrate an effect of associated variants on the function of the candidate gene product. There are three factors that reduce the impact of specific variants in association studies of psychiatric disease. The first is that these complex phenotypes result from the coordinated effects of variants in several genes. Besides this basic limitation of association analyses of candidate genes in complex phenotypes are the additional concerns of allelic and nonallelic heterogeneity. Allelic heterogeneity refers to different variants in the same gene that contribute to a phenotype. Allelic heterogeneity is seen commonly in Mendelian phenomena such as cystic fibrosis, familial breast cancer and familial Parkinson disease. In these occations, genotyping for specific mutations in different families would show no evidence of association. The final factor that reduces the effect of specific variants on psychiatric disease is nonallelic heterogeneity. This essentially results from phenocopies or closely related phenotypes of distinct genetic basis that are not differentiated clinically. Again there are examples of this in Mendelian genetics, as the several different genes that cause familial forms of deafness, Parkinson disease and retinitis pigmentosa. In psychiatry, this problem is likely to be exacerbated because diagnosis relies heavily on clinical asessment, which makes subtyping of psychiatric disease difficult and unreliable. In addition, pathogenomic markers do not currently exist in psychiatry. By contrast, for neurological disorders such markers can help distinguish subtypes of the condition for genetic analyses; for example, Lewy bodies in Parkinson disease. Allelic and nonallelic heterogeneity dilute the association of specific variants with disease, even when those variants are meaningfully related to the pathophysiology of disease. As a result, association studies of psychiatric phenotypes must be designed to be very powerful statistically. Few studies attempt to sample all of the common genetic variation in the gene of interest, and many use older, relatively uninformative markers. Some studies focus appropriately on genetic variation that has potential consequences on gene function, but the evidence for actual functional effects of that variation in vivo may not be clear. Several studies attempt to increase power by splitting the sample into clinically defined subgroups, but this requires statistical correction for multiple testing and it is not always clear how best to do this. Since association studies are relatively easy to carry out, many negative studies are probably never published (120). Because of these limitations, the vast majority of potentially important candidate gene markers have yet to be confidently excluded from a role in BPD. The larger problem with candidate gene association studies is that good candidate genes for BPD are hard to define. We do not yet know enough about the biochemical pathways underlying BPD symptoms - much less the large groups of genes and even larger number of genetic variants involved - to

make a truly educated guess in selecting candidate genes for study. Unless the prior probability is reasonably high (better than 1 in 1,000) that a particular gene polymorphism is in fact causally related to the disease, Bayes' theorem shows that most statistically significant association findings at the $P = 0.001$ level will actually be false positives. If we assume that there are about 30,000-60,000 human genes and that any could be a candidate for BPD, then the prior probability that a particular gene is a disease gene is no better than one in 30,000. If we only consider those genes expressed in the central nervous system (CNS), the denominator might be cut in half, but the odds are still very low. We cannot even confidently exclude the possibility that disease-relevant variants do not lie within genes at all, but rather in intergenic regions, the functional importance of which we still are far from comprehending fully. Association studies in regions of genetic linkage face more favorable odds from the start. The prior probability that a gene in a linkage region is causally involved in disease is not hard to estimate: it is approximately equal to the probability that the region is truly linked, divided by the number of genes in the region.

Animal models for bipolar disorder

The recent development of microarray technologies has made the simultaneous measurement of mRNA levels for thousands of genes possible, hence enabling gene expression profiling. The application of this approach to animal models provides a powerful tool for the discovery of novel genes involved in disorders, as many molecular genetics and histochemical experiments are not feasible in humans. Recent advances regarding the underlying neural mechanisms, etiology, genetics, and new pharmacological approaches have facilitated the development of new animal models of psychiatric disorders. The potential utility of animal models in psychiatry is quite substantial because preclinical evaluation of diverse psychotropic drugs relies on the utilization of animal tests to produce information concerning the biochemical effects of these drugs on specific targets. Nonetheless, the usefulness and acceptance of animal models in psychiatry vary widely due to obvious limitations in trying to mimic aspects of higher cognitive human functioning in other animal species. Bipolar disorder has turned out to be particularly challenging when trying to develop an adequate animal model. The alternation of mania, depression, euthymia, and mixed states that these patients often present will be extremely challenging to mimic in one and the same animal model. It is also important to remember that BPD is influenced by a variety factors, ranging from biological to genetic, as well as environmental. Today, several animal models have been proposed to reflect behavior of either one of the core features, depression or mania, however no models incorporate both components in alternating fashion and therefore fail to mimic the hallmark of BPD.

The value of animal models of BPD is evident, besides providing understanding of the pathophysiology of the illness, they also aid in developing newer pharmacologic agents for treatment. It includes models that reconstruct the etiology and pathophysiology of BPD.

3. Objectives of the study

The main aim of the current study was to elucidate the unknown genetic component of bipolar disorder. Since no predisposing gene has yet been found for this severe mental disorder, the general strategy was to localize susceptibility loci in our family sample set using a variaty of statistical analyzing strategies.

The specific aims of this study were:

1. To further investigate the previous significant linkage finding to Xq24-q27 in one family from an isolated region in Finland, and narrowing down the critical linked region. Our aim was also to validate the finding in a nationwide family sample.

2. To scan the genome for possible linked region to bipolar disorder in our nationwide family sample.

3. The aim was to confirm previous linkage findings as well as detecting new ones by re-analyzing the results from 18 different bipolar genome-wide scans using a meta-analysis strategy. By obtaining alluded results their general significance could be emphesized since they would hold true across several different populations.

4. To utilize different statistical strategies to extract linkage information for related psychiatric traits in order to increase the statistical power to detect linkage.

4. Material and methods

Please refer to the original publications (I-IV) and references for more detailed description of the studies.

4.1 Study samples

4.1.1 The ascertainment of the bipolar family sample

The bipolar study sample was collected through utilization of several nationwide registers. First, all individuals that were born between the years 1940-1969 and hospitalized for ICD-defined BPD, were identified through the National Hospital Discharge Register. This register indexes all admission and discharge dates and primary diagnoses for inpatient stays at public and private hospitals. In order to link these individuals to their first-degree relatives the National Population Register containing information on place of birth, residence, marital status and first-degree relatives for each Finnish citizen was used. With the aim of ascertaining a study sample for BPD that would be more genetically loaded, some further criterias were required from the probands; the age of onset had to be before the age of 30 and the patient had to have two or more admissions due to BPD, and in addition the probans had to have at least one sibling. Also, at least one of the parents had to be born in the Eastern part of Finland (Kuopio and North-Karelia) were BPD seemed to be enriched. Next, an extensive search was made for all available records from hospitals, clinics, general practitioners, reports from medico-legal experts and various other sources that might provide diagnostic information. Two psychiatrists, being unaware of the family relationships, made independent diagnoses of the subjects based on all available case notes according to DSM-IV diagnostic criteria for bipolar I disorder. In addition, the BPD study sample includes six families from a Finnish population based study on schizophrenia. In these families there were at least one member suffering either from bipolar disorder or schizoaffective disorder, bipolar type. Finally five hierarchical diagnostic categories were assigned to the study sample; (I) bipolar disorder type I; (II) schizoaffective disorder, bipolar type; (III) bipolar disorder type II, bipolar NOS, cyclothymia; (IV) recurrent major depressive disorder; (V) other mental disorders.

The permit for the register searches was obtained by the ethical committee of the National Public Health Institute, and the requests to participate in the study were sent to the probands fulfilling BPD diagnostic criteria through their treating psychiatrist. If permission was obtained from the proband, the family members were contacted.

4.1.2 Family P101

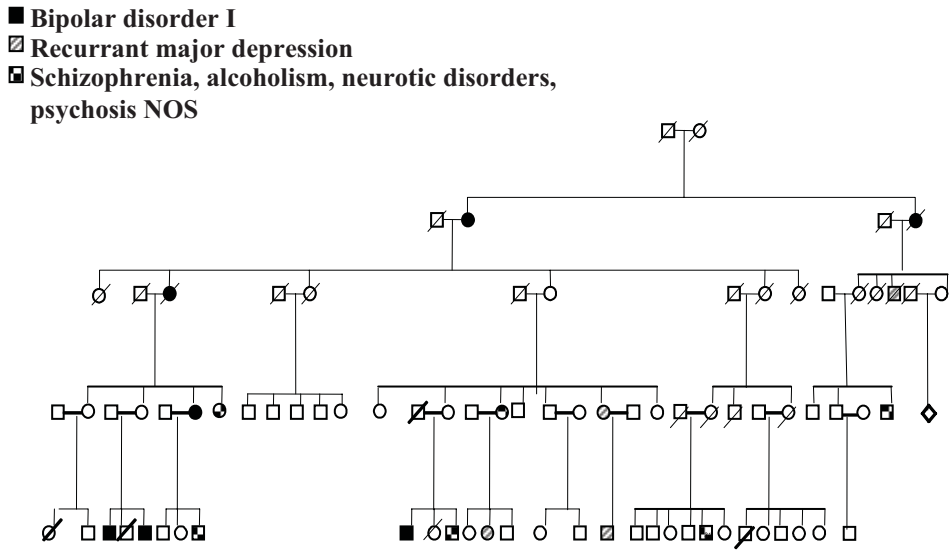
One extended BPD family, pedigree P101 was ascertained by Dr. Bredbacka. All family members were interviewed by a psychiatrist using SADS-L (121), on at least two occations. The diagnoses were made independently and blind to the family relationships

on the basis of interviews and case notes in accordance with RDC (121) and the DSM-III-R (American Psychaitric Association 1987) by two psychiatrists. The family P101 consisted of 64 individuals, representing bipolar disorder in four generations (Table 4).

Table 4.
Diagnostic description of family P101

Number of affected Individuals	*StatusI*	*StatusII*	*StatusIII*	*Status IV*	*Status V*
Family P101	8	0	0	3	6

Figure 2.
Presents the familytree of pedigree P101.



4.1.3 Nationwide family sample

The nationwide study sample consisted of 40 families in addition to family P101. Three of these 40 pedigrees represented extended pedigrees, while the remainder was nuclear families. In its entirety the family sample of the 41 families consisted of 341 individuals of whom 137 were coded as affected (Table 5). Altogether the sample consisted of 81 males and 73 females, with an average age of 55.14 years.

Table 5.
Diagnostic description of the nationwide family sample

Number of affected individuals	Affection status I	Affection status II	Status I & II
40 families	93	27	120
Only family P101	8	9	17
Total sample	101	36	137

4.1.4 Meta-analysis sample

In the worldwide BPD meta-analysis altogether 18 genome-wide scan samples were analyzed jointly. Several different diagnostic clusterings had been used in each study, hence a new diagnostic categorization was applied in the meta-analysis. Two primary narrow models were chosen, the very narrow one coding BPD-I and SZ-AFF cases as affected (347 pedigrees and 948 affected), and the narrow one including BPD-I, SZ-AFF and BPD-II cases as affected (512 pedigrees and 1733 affected) (Table 6). However, since recurrent major depression is the most common disorder in BPD families, secondary analysis on broader diagnostic classification was also conducted. The broad model included BPD-I, SZ-AFF, BPD-II and recurrent major depression as affected (593 pedigrees and 2437 affected), and the very broad model included in addition also single episode major depression and cyclothymic disorder (617 pedigrees and 2589 affected).

Table 6.
Descripton of the family samples analyzed in the meta-analysis.

STUDY SITE	FIRST AUTHOR	Fam *	Aff **	M1 ***	M2 ****	BPD-I	SZ-AFF	BPD -II
Very narrow analysis:								
NIMH	Detera-Wadleigh	97	424	264	336	232	32	72
U.K/Irish	Bennett	151	367	288	325	288	12	25
Columbia	Liu	39	297	115	208	101	14	93
Finland	Ekholm	41	132	107	107	95	12	2
Sydney 1	Badenhop	13	69	40	44	33	7	4
Sydney 2	Schofield	15	63	41	46	31	10	5
Quebec	Morissette	5	56	42	42	39	3	5
Edinburgh	Blackwood	7	41	27	36	27	8	1
Costa Rica	McInnis	2	24	24	24	22	2	
Total				**948**		**868**	**80**	
Narrow analysis:								
Hopkins/ Dana	McInnis	65	301		232	129	6	97
Bonn	Cichon	75	245		128	104		24
NIMH-IM	Detera-Wadleigh	22	160		118	64	18	36
USCD	Kelsoe	20	76		48	33		15
UC London	Curtis	7	74		39	24		15
Total					**1733**	**1222**	**124**	**394**
Broad analysis:								
Ottawa	Turecki	31	106			33	3	25
Antwerp 1	Van Broeckhoven	10	56			14	1	15
Utah	Coon	8	51			20		12
Antwerp 2	Van Broeckhoven	9	47			22	2	6
Total						**1311**	**130**	**452**

* Fam : Number of families
** Aff: Number of affected subjects
*** M1: Model 1 (BPD-I and SZ-AFF)
**** M2: Model 2 (BPD-I, SZ-AFF and BPD-II)

4.1.5 Combined analysis sample

In the combined analysis two Finnish genome-wide scan study samples were pooled, one bipolar study sample and one schizophrenia study sample. The bipolar family study sample that was already earlier described consisted of 41 families and 137 affected individuals. The larger schizophrenia genome-wide scan study sample was ascertained using the same collection strategy, consisted of 238 schizophrenia families and 591 affected individuals. The aim of this study was to test shared phenotypic entities of both disorders in order to unravel some shared genetic etiology that could underlie the several similarities seen in phenotypes of the two conditions, such as age of onset, lifetime risk, suicide risk, gender distribution, and heritability estimates (122). Two shared phenotypic entities were selected for testing, affective and psychotic symptoms. In this combined analysis, all the previous diagnostic categories from the original scans were disregarded and the entire family material was recoded. Individuals diagnosed with affective or psychotic symptoms were coded as affected in the respective classes. The affective phenotype included major depressive disorder, dysthymic disorder, cyclothymic disorder as well as depressive disorder NOS. The psychotic phenotype included schizophrenia, schizophreniform disorder, schizoaffective disorder, bipolar I disorder with psychotic features, major depressive disorder with psychotic features, delusional disorder, shared psychotic disorder, brief psychotic disorder as well as psychotic disorder NOS. Therefore, only families that contained individuals with either affective or psychotic phenotypes were included in these analysis, resulting in a combined study sample of 352 families for affective phenotype (309 affecteds) and psychotic phenotype (841 affecteds). However, there was overlap between the families and individuals entered into the affective phenotype and psychotic phenotype groups. In the bipolar study sample all 40 families entered into both affective and psychotic groups, altogether 113 individuals displayed symptoms from both diagnostic categories. In the schizophrenia study sample all 312 families entered into both affective and psychotic groups. In this sample set 158 individuals displayed symptoms from both diagnostic categories (Table 7). In order to make the results of the combined analysis comparable with that of the original data, both BPD and SZ scans were re-analyzed including only those families that were informative for the combined analysis using broad DSM-IV diagnostic categories for BPD and SZ, respectively 6 (BPD: bipolar disorder type I, II and not otherwise specified (NOS), schizoaffective disorder of bipolar type, cyclothymic disorder or recurrent major depressive disorder, SZ: schizophrenia, schizoaffective disorder, included the DSM-IV diagnosis of bipolar disorder type I, II and NOS, schizoaffective disorder, schizophreniform disorder, delusional disorder, brief psychotic disorder or psychotic disorder NOS, as well as schizoid, schizotypal or paranoid personality disorder).

Table 7.
Description of the study sample analyzed in the combined analysis

		Affective phenotype	Psychotic phenotype
Bipolar disorder	Families	40	40
	Affected	128	123
	Controls	203	208
Schizophrenia	Families	312	312
	Affected	181	718
	Controls	1681	1144
Combined	Families	352	352
	Affected	309	841
	Controls	1884	1352

4.2 Laboratory methods

4.2.1 DNA extraction

20-30ml of venomous blood was into ETDA tubes, and DNA was extracted according to a standard protocol (123).

4.2.2 Genotyping

All microsatellite markers in the studies were amplified using PCR. The fluorescently labeled PCR products were electrophoretically separated, either with an automated laser fluorescence DNA sequencer ABI 377 (Perkin-Elmer), using GENESCAN (version 2.1) fragment-analysis software, or with an LI-COR DNA 4200 Genetic Analyzer (LI-COR Biosciences). The alleles were identified using the GENOTYPER program (version 2.0; Perkin-Elmer) or genotyping software SAGA version 5.1 (LI-COR Biosciences). Two researchers checked independently the interpretation of alleles. The microsatellite markers were all di-, tri- or tetranucleotide repeats selected from the Marshfield Medical Research Foundation (Weber sets 6 and 9). The average intermarker interval in the genome-wide scan was ~ 7.7cM and the maximum 20 cM. Map positions were derived primarily from the Marshfield integrated map.

4.2.3 Statistical methods

In all studies the Mendelian inheritance of alleles in the pedigrees was confirmed prior to statistical analysis by the PedCheck program (124), as well as with SimWalk2 (125) and Mendel (126). Also all subjects were classified either as affected or unknown. By using this affected only (AO) model problems caused by incomplete penetrance of the disease and genetic ambiguity of the unaffected phenotype was circumvented.

In the fine-mapping study of the X-chromosome (study I), the genotype data were analyzed using five hierarchical diagnostic categories: (I) bipolar disorder type I, (II) schizoaffective disorder; bipolar type, (III) bipolar disorder type II, bipolar NOS (not otherwise specified) and cyclothymia, (IV) recurrent major depressive disorder, (V) other mental disorders.
In the parametric linkage analysis, both dominant and recessive modes of inheritance were tested. The required parameters set were as follows: the disease prevalence at 1%, disease-allele frequency 0.005 (dominant model) and 0.100 (recessive model), and allowing a phenocopy rate of 0.1%. We used the ANALYZE package, an accessory program to the LINKAGE package, to perform the two-point analysis. This package includes both programs used, MLINK and the HOMOG program, which tests the genetic heterogeneity between families.

The affected sib pair (ASP) analysis was performed by the SIBPAIR program, and two different association analyses were performed by the haplotype relative risk (HRR) and transmission disequilibrium test (TDT). All the analyses, including the association analyses were performed using all the sibships in the pedigree structures.

Both nonparametric and parametric multipoint analyses were performed using only the diagnostic categories that gave the highest lod score in the two-point analyses by the GENEHUNTER program.

In order to assess the significance of the linkage results a simulation study for the diagnostic categories I-II was carried out by the SIMULATE program.

In the genome-wide scan study (study II), the data were analyzed using two different diagnostic categories enclosing the following DSM-IV diagnosis (I): BPD-I and SA-BP and (II): BPD-I, SA-BP, BPD-II, BPD-NOS, cyclothymic disorder and rMDD.

Again, both dominant and recessive modes of inheritance were tested in the parametric analysis. In these analyses the penetrance of the disease allele was set at 0.9. The disease allele frequency was set at 1% and the phenocopy rate at 0.1% in the narrow diagnostic category (I), and for the broader spectrum a disease allele frequency of 5% and a phenocopy rate of 4.5% was used. In addition, one extended pedigree (family P101) was also analyzed separately from the other 40 families, in order to assess its influence on the lod scores due to its significant large size (n = 64).
The analysis of the genome-wide scan was divided into two stages, in the first stage the preferred primary analyses methods were parametric (MLINK, HOMOG) and nonparametric (SIBPAIR) two-point linkage analyses.

In the fine-mapping stage the association analyses and three-point analyses were performed for selected linked regions. The association analyses were performed using the PSEUDOMARKER program (23), which is a linkage analysis software for joint linkage and/or linkage disequilibrium analysis. We utilized LD given linkage statistics of the program, which calculates association free of the effect of linkage. The association results were corrected for multiple testing using the Dunn-Šidák correction ($\alpha' = 1 - (1 - \alpha)^k$). The MLINK program was also used to perform three-point analysis. This analysis was not performed with flanking markers moving the disease across the map, because of the known propensity for false exclusions in that method. To avoid the known negative side effects of multipoint analysis, we performed a multipoint analysis in which the markers were placed in a fixed order and in which the disease locus was allowed to vary outside the map of markers. Using this method, we found that meioses uninformative for some markers can be scored for nearby markers, thus allowing all meioses in all families to be scored in the analyses.

A simulation study estimating type I error rates was carried out by the SLINK program for the two peak markers, D4S1629 and D16S769. The exact same conditions under which the best lod scores were obtained for these two markers were reproduced in the simulation analysis eg. same family structures, statistical models and allele frequencies were used. The markers were simulated 10000 times under null-hypothesis (theta = 0.5), subsequently it was elucidated how many times the same or exceeding maximum LOD

scores were retrieved by analyzing these 10.000 replicates using ANALYZE program. The empirical *P*-values obtained were then Dunn-Šidák corrected.

In the meta-analysis study (study III), a rank-based genome scan meta-analysis method (GSMA) was utilized (60, 127, 128). The linkage results attained from each group were maximized across the different transmission models for each marker. And based on the GSMA method the genome was divided into 120 30-cM bins that were defined by markers from the Genethon map (CEPH-Geneton Integrated map). The average bin width according to the Marshfield map was 29.1 cM. Each marker from each study was placed within one of these bins, and for each study in a given analysis, the maximum linkage score or minimum *P*-value was selected within each bin and the bins were assigned a rank (R_{study}) in ascending order. The average rank (R_{avg}) for each bin was then computed across the study. Also a weighted analysis was performed taking in consideration the sample size in each study. Here, each R study value was multiplied by its study's weight (√N[affected cases]), divided by the mean of this value over all studies. Two pointwise *P* values were determined by permutation (500 permutations per analysis): P_{AvgRnk} and $P_{ord.}$

In the combined analysis (study IV), the two-point linkage analysis was performed using the MLINK program. For the genotyping data originating from the SZ genome-wide scan study a disease-allele frequency of 0.001 was used, assuming no phenocopies. For the genotyping data originating from the BPD genome-wide scan study a disease-allele frequency of 0.005 was used, allowing a phenocopy rate of 0.1%. The results presented are the summed lod scores maximized over recombination fraction in increments of 0.02. The conservative approximation of the population allele frequency from the individual study sample was used. To refrain from introducing false positive inflation of the linkage evidence due to different genetic background of the separate samples, the population allele frequencies were estimated separately for the two samples, linkage analysis performed separately and the lod scores subsequently added at every recombination fraction for the two samples.

5. Results and discussion

5.1 Replication of the X-chromosomal linkage finding

In an earlier study we repoted significant evidence of linkage (Z_{max} = 3.54) for BPD to chromosome Xq24-q27.1 in an extended pedigree from the late settlement region of the genetically isolated population of Finland. This particular family displayed a distinct X-chromosomal haplotype covering a 19 cM wide region that co-segregated with BPD. In the present study our aim was two demensional, on one hand we wanted to replicate this linkage finding in a larger set of nuclear families. And on the other hand, we wanted to test if this particular pedigree showed evidence of linkage to other chromosomal regions in the genome. Since the extended family originated from an isolated region in Finland that overall presents a higher prevalence of BPD, we chose a nationwide replication sample in an attempt to evaluate if this X-chromosomal finding represented a more general genetic predisposion to BPD.

In the first part of the study, we analyzed eight microsatellite markers scattered over the 13cM wide critical linkage region on Xq24-q27 with the population-wide nuclear family samples (n = 40). However, since two new subjects of family P101 had been added and two diagnoses had changed in reassessment, the extended pedigree P101 was also re-analyzed in the present study. In addition, new more robust statistical parameters were utilized in the present study in comparision to the previous study (age dependent liability classes versus affected only model). When applying of the new statistical parameters to the original family-setup of P101, the maximum lod score of 3.54 dropped to 2.17. When including the new individuals and the changed diagnosis into the equation a maximum lod score of 1.89 was achieved for the original peak marker (Table 8). When excluding family P101 from the analysis and only testing the 40 nuclear pedigrees, a maximum lod score of 1.34 was obtained, hence meeting the guidelines for replication of a linkage finding ($Z \geq 1.2$) (Table 8). When testing the entire sample including all 41 families a maximum lod score of 2.78 was obtained shifting the linkage peak to the next marker telomericly located of the original linkage peak. In the present study the co-segreagting haplotype could again be seen in all individuals affected by bipolar disorder type I.

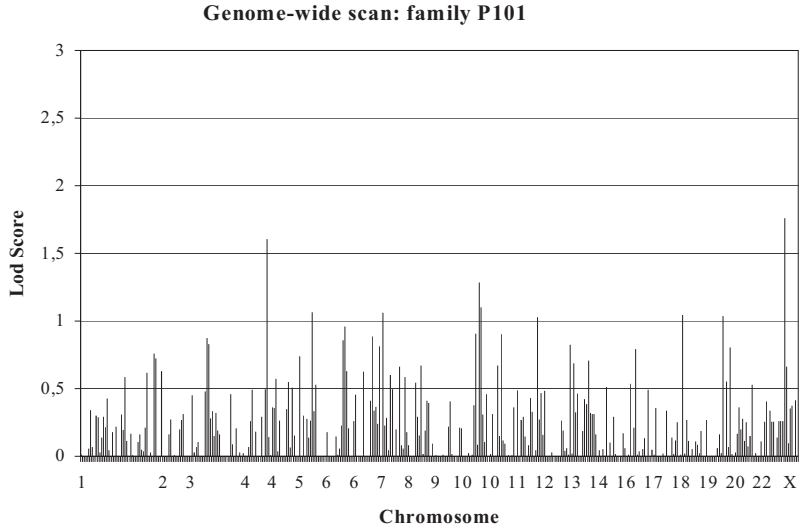
Table 8.
Here the maximum two-point lod score results under diagnostic category II for the 40 new families (and for family P101) are presented.

MARKER	θ = 0.0	θ = 0.1	θ = 0.2	θ = 0.3
DX737	- 19.52 (0.71)	- 2.87 (0.45)	- 0.60 (0.45)	0.05 (0.30)
DXS1047	- 10.53 (1.84)	1.58 (1.44)	**2.14** (1.02)	1.44 (0.61)
DXS994	- 11.89 (**1.89**)	- 0.25 (1.54)	0.90 (1.16)	0.84 (0.76)
HPRT**	- 16.26 (1.77)	- 1.86 (1.41)	0.07 (1.04)	0.44 (0.65)
DXS1062	- 16.93 (0.11)	- 2.88 (0.08)	- 0.80 (0.04)	- 0.14 (0.02)
DXS6854	- 13.73 (1.72)	- 0.98 (1.41)	0.44 (1.06)	0.59 (0.69)
DXS102	- 14.54 (0.07)	- 3.05 (0.05)	- 1.02 (0.03)	- 0.29 (0.01)
DXS984	- 15.24 (-1.97)	- 3.01 (-1.17)	- 0.90 (-0.60)	- 0.20 (-0.27)

**Hypoxanthine Phosposyltransferase
Theta (θ) indicates recombination fraction

In the second phase of the study, we scanned the genome for any other potential linked regions for family P101. Although, these analyses showed that a gene predisposing this family to bipolar disorder is very likely located in this X-chromosomal region also 4q32 showed signals of modest linkage.

Figure 3.
Shows the maximum two-point lod score results for family P101 for all the markers in the BPD genome-wide scan.



In conclusion, only a fraction of the families provided any evidence of linkage to this region suggesting that a relatively rare gene predisposing to BPD is enriched in the extended family P101. The genome-wide scan for BPD predisposing loci in this large pedigree indicated that this particular X-chromosomal region provides the best evidence of linkage genome-wide, suggesting a X-chromosomal gene with a major role for the genetic predisposition of BPD in this family (Figure 3).

5.2 Genome-wide scan in a nationwide BPD family sample

We conducted a genome-wide scan with aim to identify loci predisposing to bipolar disorder by utilizing the benefits of an isolated population and a nationwide Finnish family-set. To our knowledge, this is one of the largest genome-wide scans targeted on narrowly diagnosed bipolar disorder. Furthermore, we have followed a strict family ascertainment strategy, e.g. by ascertaining the probands nationwide using criteria of an early age-of-onset (first bipolar episode under 31 years of age) and requiring two or more hospitalizations due to BPD-I or SA-BPD. We have thus made an effort to include more genetically loaded BPD cases in this study sample.

A total of 27 markers showed a maximum two-point LOD score of 1.0, three of which produced a LOD score > 2.0 (4q32, 12q23 and Xq25), and one > 3.0 (16p12) (Table 9). Regions on 4q32, 12q23 and 16p12 were fine-mapped by including additional unaffected family members. One extended pedigree (family P101) was systematically analyzed separately from the remaining 40 families due to its significantly larger impact in statistical analyses. This family alone could exert a major influence on the linkage results, possible outweighing the contribution of all other families. Nonetheless, it did not seem to have a major effect on autosomal locus findings. This particular family P101 was ascertained already decades ago and has ever since been the primary family included in our genetic studies. Through these previous studies significant evidence of linkage to Xq24-q27 was found. Family P101 was here included into the statistical analyses of the genome-wide scan, since multiple affected individuals provided significant linkage and phase information for putative additional loci.

Table 9.
Show the markers in the genome-wide scan resulting in Z_{max} > 1.0 for the 40 families and family P101 separetly.

CHR	cM	40 fam.		P101	
		Diagnostic category	Z_{max}	Diagnostic category	Z_{max}
Chr.1	175.62	Broad	1.0	Broad	1.0
Chr.1	212.44	Broad	1.8	Broad	0.3
Chr.1	215.17	Broad	1.8	Narrow	0.2
Chr.3	102.64	Broad	0.8	Broad	0.8
Chr.3	124.16	Broad	1.1	Broad	1.1
Chr.3	134.64	Narrow	0.8	Narrow	1.0
Chr.3	138.0	Broad	1.1	Broad	1.1
Chr.4	157.99	Broad	2.4	Broad	2.6
Chr.5	52.55	Broad	1.1	Narrow	1.4
Chr.5	59.3	Broad	0.2	Narrow	1.2
Chr.7	50.29	Narrow	1.4	Narrow	1.0
Chr.8	26.43	Narrow	1.5	Narrow	1.5
Chr.8	77.89	Broad	1.4	Broad	1.4
Chr.8	164.47	Broad	1.6	Broad	1.6
Chr.9	44.28	Narrow	1.3	Broad	1.2
Chr.11	33.02	Broad	1.1	Broad	1.1
Chr.11	131.26	Broad	1.0	Broad	1.0
Chr.12	109.47	Broad	2.0	Broad	2.0
Chr.14	40.68	Broad	1.3	Broad	1.1
Chr.14	66.81	Broad	0.7	Broad	1.1
Chr.14	105.53	Broad	1.6	Broad	1.1
Chr.14	125.88	Narrow	1.0	Broad	1.1
Chr.16	50.6	Narrow	3.4	Broad	2.5
Chr.17	10.72	Broad	1.2	Broad	1.2
Chr.X	82.07	Narrow	1.3	Narrow	1.3
Chr.X	97.89	Broad	1.2	Broad	1.2

Narrow diagnostic category = BPD-I and SA-BPD,
Broad diagnostic category = BPD-I, SA-BPD, BPD-II, BPD-NOS, Cyclothymic Disorder, rMDD.

All three chromosomal regions that were fine-mapped gained further, eventhough modest support from the additional marker and subjects. The most significant two-point lod score of 3.4 was observed on 16p12 and the same linkage region obtaining a maximum three-point lod score of 2.7. Also modest support was found in the association analysis (P = 0.0680) (Table 10). Chromosome 4q32 obtained a lod score of 2.6, which was strengthened in the three-point analysis to a three-point lod score of 3.6. This region was also significant in the association analysis (P = 0.0319) (Table 10). Despite the large amount of linkage evidence between bipolar disorder and the chromosomal region 12q24, we found no further support in the finemapping stage.

Table 10.
Maximum two-point linkage results from the fine-mapping stage and well as Dunn-Šidák corrected association results.

Chr	Marker	cM	MOI*	Diag.**	Family material	Two-point lod score: $Z_{max}(\alpha)$ ***	Association test: *P*-value
4	D4S3049	155	ASP	Narrow	40 fam.	0.9	0.031
4	D4S1629	158	Dom	Broad	41 fam.	2.6	0.983
4	D4S1528	162	Rec	Broad	40 fam.	1.9	0.968
12	D12S1607	108	Dom	Narrow	41 fam.	0.5 ($\theta = 0.24$)	0.965
12	PAH	109	ASP	Broad	40/41 fam.	2.0	1.000
12	D12S78	112	Dom	Narrow	40 fam.	0.4 ($\theta = 0.22$)	0.642
16	D16S403	47	Dom	Broad	40 fam.	0.4	0.068
16	D16S769	51	Dom	Narrow	40 fam.	3.4 ($\theta = 0.22$)	0.999
16	D16S3093	52	Dom	Broad	40 fam.	0.8	0.759

* Mode of Inheritance ** Diagnostic category *** Alpha (α) if maximum LOD score is obtained from analysis allowing for heterogeneity
Family material included in the two point analyses; 41fam = all families, 40 = excluding family P101 from the analyses
Mode of inheritance; ASP = Affected Sib-Pair, Dom = dominant mode of inheritance, Rec = recessive mode of inheritance.

In conclusion, two promising chromosomal regions on 4q32 and 16p12 emerged from our genome-wide scan study, both obtaining maximum lod scores exceeding 3.0. And since both regions have been implicated in several other independent studies from other populations, these findings meet the guideline given by Kruglyak et al. as statistically significant. It is encouraging to find similar genetic findings in the Finnish study sample, even though it represents an extreme population due to the isolation. Thus, this should stress the general significance of these results.

5.3 Meta-analysis of worldwide genome-wide scans in BPD

The inconsistency in the linkage results in BPD has long been evident. In large this is due to the small sample sizes utilized, thus in order to increase the power of linkage to produce a more generalized picture of genetic presidposition to BPD, meta-analysis are conducted. In these analyses, the results from different genome-wide scans are combined and analyzed in a combined fashion. Here, in this study 18 genome-wide scans conductede world-wide were analyzed together. The selected studies were consistent in that they achieved reasonably even marker coverage of the genome, used well-established linkage statistical methods, diagnosed mood disorders on the basis of modern diagnostic instruments and criteria, and included predominantly European-ancestry subjects. However, the studies did vary in sample size, evenness of marker spacing, the number of linkage analyses that were applied, community-based versus more ethnically homogeneous samples, and the typical size of pedigrees, hence also a weighted analyses were performed.

Using the rank-based genome scan meta-analysis (GSMA) method, three different models were analyzed; a phenotypically very narrow model (Model 1), a narrow (Model 2), and a braod model (Model 3). The overall results are summarized in Table 11.

Table 11.
P_{AvrRnk} * values for Models 1-3 weighted and unweighted

Bin	Marshfield location (cM)	Cytogenic posistion	WEIGHTED			UNWEIGHTED		
			Model 1 P_{AvrRnk}	Model 2 P_{AvrRnk}	Model 3 P_{AvrRnk}	Model 1 P_{AvrRnk}	Model 2 P_{AvrRnk}	Model 3 P_{AvrRnk}
1.4	83-113	1p32.1-q31.1	.013			.016		
1.8	201-231	1q31-q32				.070	.041	.057
2.6	128-154	2q22.1-q23.3		.036	.082		.014	
3.6	146-173	3q22.1-q25.31					.047	
5.1	0-31	5pter-pter		.065	.097		.038	
7.6	122-148	7q34-qter		.079	.043			.059
8.1	148-190	8pter-p22		.044			.049	
8.6	137-167	8q24.21-qter		.045	.037		.069	.064
9.2	27-53	9p22.3-p21.1	.006			.009	.087	
9.3	53-84	9p22.1-q21.32	.026	.080	.085	.073		
10.3	62-91	10q11.21-q22.1	.008			.043		
11.3	47-72	11p13-q13.3			.054		.085	.025
14.2	40-74	14q13.1-q24.1		.100			.016	.011
14.3	74-105	14q24.1-q32.12	.043	.003	.006		.017	.040
17.2	25-63	17p12-q21.33	.043			.070		
18.1	0-24	18pter-p11	.033			.038		
18.2	24-62	18p11-q12.3	.033	.013	.015	.038	.027	.018
18.3	62-96	18q12.3-q22.1	.055	.061	.048	.059	.023	.019
19.4	75-105	19q13.33-p12.3	.017			.021		
20.1	0-21	20pter-p12.3			.058			.045
21.2	25-57	21q21.3-qter			.071		.042	.052

* P_{AvrRnk} presents the pointwise probability of observing a given R_{Avg} for a bin in a GSMA of n studies, determined by theoretical distribution (unweighted analysis only) or by permutation test.

Several clear patterns were seen in this study. First, and a bit surprisingly, a genomewide level of significance (P_{AvgRnk}) was not reached in any of the analysis. Levision and colleagues established criteria for linkage in the simulation studies they conducted that nevertheless were not met here. Reasons behind this could be several, for one the differential sample ascertainment strategies, diagnostic batteries, and linkage methods used might result in this outcome. Alternatively, linkage might be detected only in certain populations or subsets of pedigrees that will be masked by the background noice.

Secondly, overlapping results with a previously meta-analysis conducted in BPD by Bardner and Gershon were hardly seen. Nonetheless, same study samples were not analysed, in addition different statistical methods as well as phenotypical models were utilized in conducting the meta-analysis.

Third, the meta-analysis showed very little support for overlapping genetic susceptibility loci in BPD and schizophrenia. However this might change with the use of a larger BPD sample set, and a more selective phenotype focusing on subjects with overt psychotic symptoms as hallucinations or delusions, as well as with combined analyses of schizophrenia and psychotic BPD data.

Overall, it seems like disease loci that have a high enough relative risk to be detected even this kind of large survey are rare in BPD. Instead, it seems more plausible that these results are the outcome of loci of modest effect producing inconsistent and weak evidence for linkage with variable peak locations. The loci with higher relative risks appear to only be detected in specific population- or even family sets, where the risk locus is enriched. In summary, the present study provided some support for the following chromosomal regions 14q, 9p-q, 10q, 18p-q, and 8q that could contain common loci that are weakly linked to BPD in the various populations.

5.4 Combined analysis of BPD and SZ; investigating psychotic and affective symptoms

In this study our aim was to evaluate the existence of a common genetic predisposition for shared phenotypic entities in schizophrenia and bipolar disorder, which both represent prevalent psychiatric disorders with genetically complex backgrounds. The vast amount of overlap between the clinical features, familial aggregation and even observed genetic linkage peaks have led to speculation of common predisposing genetic factors among the two disorders. By combining two Finnish genome-wide scans in schizophrenia and bipolar disorder respectively, we were able to increase the sample size significantly and hence also the statistical power. This strategy enabled the detection of two loci showing significant evidence of linkage to affective symptoms on chromosome 6p21 and psychotic symptoms on chromosome 5q14 respectively, that both went unnoticed in the original genome-wide scans.

By testing for linkage of shared phenotypes of clinical relevance, we found evidence for linkage of affective and psychotic symptoms exceeding that obtained in either one of the original separate genome-wide scans for bipolar disorder or schizophrenia on a total of 11 chromosomal regions. Many of these regions are supported by linkage studies in psychiatric disorders and should thus be considered good targets for further genetic studies. The strongest linkage finding, a maximum LOD score of 4.7 for affective symptoms was obtained on 6p21 in the immediate vicinity of Dysbindin, a gene previously found to be associated to schizophrenia. Another interesting finding was the linkage of psychotic symptoms to 5q14 ($Z_{max} = 3.8$). The results demonstrate the value of the analyses of shared trait components in study samples ascertained for related but separate diagnostic entities. This strategy enables analyses of larger samples; hence increasing the power to detect linkage that otherwise might be overlooked.

In summary, 21 marker loci exceeded lod scores of 1.5 in the combined analysis for either affective or psychotic disorder. The linkage results are shown in Table 12, where also the lod scores obtained in the original genome-wide scan analysis for schizophrenia and bipolar disorder is presented for comparison. The results suggest that these genomic regions are likely to contain genes affecting the risk for broadly defined affective or psychotic disorder rather than for SZ and BPD (Table 13). Thus, this demonstrates the feasibility of combining genotypic data from studies originally ascertained for separate disorders that potentially have overlapping genetic backgrounds, and increasing sample size and, consequently, statistical power.

Table 12.
Shows the markers in the combined analysis that exceeded a maximum lod score of 1.5 for either affective or psychotic symptoms in comparison to the linkage results obtained from the original BPD and SZ genome-wide scans.

Marker (SZ)	Marker (BPD)	cM	Chr.	Aff. phen. (Z_{max})	Psych. phen. (Z_{max})	BPD-scan (Z_{max})	SZ-scan (Z_{max})
D6S1017	D6S1017	63	6p21	**4.7**	0.1	0.0	0.5
D5S428	D5S1725	98	5q14	0.2	**3.8**	0.1	0.0
D4S1629	D4S1629	158	4q32	**2.6**	0.3	2.5	0.0
D1S1660	D1S1660	212	1q31	**2.6**	0.4	1.7	0.5
DXS1193	DXS1193	98	Xq28	0.1	**2.5**	0.6	0.0
DXS1192	DXS8013	86	Xq27	1.5	**2.5**	0.4	1.0
D8S263	D8S1128	140	8q24	0.7	**2.2**	0.3	0.4
D5S647	D5S2500	69	5q11-12	**2.1**	2.0	0.2	3.5
D4S2397	D4S2397	43	4p15	0.1	**2.0**	0.0	0.4
D1S1723	D1S1723	215	1q32	**1.9**	0.3	0.9	1.0
D20S103	D20S103	2	20p13	0.7	**1.9**	0.5	0.1
D16S415	D16S753	58	16p11-12	0.2	**1.9**	0.1	0.6
D16S539	D16S621	125	16q24	**1.9**	0.0	0.2	0.0
D5S1501	D5S1501	85	5q14	0.3	**1.8**	0.2	0.4
D6S1721	D6S1006	27	6p24	**1.8**	0.1	0.0	0.2
D16S769	D16S769	51	16p12	1.3	**1.8**	2.9	0.7
D9S930	D9S302	123	9q32	0.0	**1.7**	0.0	0.1
D16S746	D16S3093	52	16p11-12	0.5	**1.6**	0.4	0.0
D3S2465	D3S2465	112	3p12	**1.6**	0.1	0.0	0.0
D3S2406	D3S2406	103	3p13	**1.5**	0.0	1.5	0.3
D2S1391	D2S1391	186	2q32	0.0	**1.5**	0.0	0.3

Table 13.
Summarizes the susceptibility loci for affective and psychotic phenotypes obtained in our combined analysis, along with the maximum lod scores ($Z_{max} \geq 2.0$) achieved on that cytogenic band.

Affective phenotype loci	Psychotic phenotype loci
6p24-21 (Z_{max} = 1.8 and 4.7)	5q14 (Z_{max} = 3.8)
4q32 (Z_{max} = 2.6)	Xq27-q28 (Z_{max} = 2.5)
1q31-q32 (Z_{max} = 2.6)	8q24 (Z_{max} = 2.2)
5q11-q12 (Z_{max} = 2.1)	4p15 (Z_{max} = 2.0)

6. Concluding Remarks and Future Prospects

General observations can be made about the difficulties in identifying disease genes for psychiatric disorders like schizophrenia and bipolar disorder. Yet it appears that the unraveling of the genetic predispositions to schizophrenia is a step ahead of bipolar research. It seems like several factors contribute to this trend including the use of larger samples, a narrower phenotype, and more conservative and uniform data analysis in the schizophrenia studies. However, the most significant advantage in schizophrenia research is a more clear-cut and reliable phenotype presentation due to the non-cycling nature of the disorder, and in addition excellent endophenotypes exist for schizophrenia. The development of similar phenotypic traits or markers is critical in BPD since these may represent a more direct expression of underlying genes and thus be far more informative of the aggregate psychiatric phenotypes. Some of the endophenotypes that may underlie mood disorders include circadian rhythm, stress reactivity, and mood, sleep, and appetite regulation, however, more research needs to be conducted before the validity of these endophenotypes can be confirmed. However, endophenotypes like these could potentially be extended to animal models giving rise to powerful research tools by enabling biological validation tests in *in vitro* or transgenic animal model systems.

For the foreseeable future, linkage studies will remain the preliminary effort for systematic molecular genetic research in BPD. Even though the results from linkage studies have been far less thriving than originally anticipated, rapid developments of the genotyping and gene-expression technologies are now enabling cheaper, faster and more precise massive high-throughput research. Nevertheless, the sample sizes to date are still too trivial to achieve the relevant statistical power needed to detect the underlying predisposing genotypes. Thus, it is of great importance that more future efforts to combine samples through methods such as meta-analysis or combined analysis are taken, especially since individual susceptibility genes are likely to confer only modest increases in risk for bipolar disorder and therefore making their detection even more challenging. Encouragingly, several chromosomal regions have repeatedly been implicated by independent groups from different populations suggesting that true linkage signals may have been uncovered. The identification of the actual disease-predisposing variant in the usually large regions is a challenge that requires a combination of genetic and biological strategies and hypothesis. For example the involvement of epigegenetic factors such as DNA methylation, histone deacetylation, chromatin modification, RNA interference, RNA editing and DNA rearrangement have long been proposed in BPD. And since epigenetic processes increase the complexity of genomic responses by allowing short-term fine-tuning of the genome, and provide a mechanism for preserving information on environmental exposures, it is set to again emerge as an important avenue of research.
Yet even the most successful genetic mapping experiments will never tell the whole pathophysiologic story, thus it must be further complemented with gene expression studies, studies in model organisms, and, ultimately, basic neurobiology.

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