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THE OUTLOOK FOR LINKAGE RESEARCH IN PSYCHIATRIC DISORDERS

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Summary—Analysis of the distribution of diagnoses in families (segregation analysis) has not, so far, advanced us toward the goal of resolving the inheritance of the major psychiatric disorders. Recent advances in genetics, particularly the development of new molecular genetic linkage methods, have made it, at least theoretically, feasible to scan the entire human genome, so that in a properly designed and executed set of studies it will be possible to demonstrate whether there exists single locus transmission for a particular disorder, and to map the locus.

We present here an analysis of feasibility of moderate-sized pedigrees (approximately 15 persons, two generations) and affected-sib-pair sets in such studies. Eight to ten moderate-sized pedigrees are sufficient to perform definitive mapping under the tested conditions, of recessive or additive inheritance, low penetrance of the heterozygote, and a genetically homogeneous disorder. The affected-sib-pair method required 25 pairs of sibs to detect linkage under such conditions in a recessive model, and 45 in a dominant model. When there is genetic heterogeneity, moderate-sized pedigrees are much less useful, but the affected-sib-pair method retains considerable power.

1. INTRODUCTION

IF WE CONTRAST the traditional major psychiatric disorders, schizophrenia and manic-depressive illness, with other inherited disorders having significant behavioral symptoms, such as the profound mental retardations, Huntington's, and Alzheimer's diseases, we have in the latter group of disorders a number of well-defined biologic–genetic events, including metabolic and degenerative disorders transmitted at a single genetic locus, cytogenetic abnormalities, and inherited pathophysiological abnormalities in the brain whose precise mode of inheritance is undetermined. This evidence includes the aminoacidurias and lysozomal storage disease, which are single locus causes of mental retardation, trisomy 21 and the fragile X chromosome which are cytogenetic abnormalities associated with retardation, an arbitrary DNA sequence on chromosome 4 which is a linkage marker for the Huntington's disease gene, and the findings of specific degenerative events in the brain associated with Alzheimer's disease (LEROY, 1983; GUSELLA *et al.*, 1983; WHITEHOUSE *et al.*, 1983). For the major psychiatric disorders, on the other hand, although there is abundant evidence from clinical twin, family, and adoption studies implying that they are genetically transmitted, up to this time there has been no firmly established linkage marker or inherited pathophysiologic event associated with any of them (GOLDIN and GERSHON, 1983; NURNBERGER *et al.*, 1986). It is self-evident that demonstration of such a marker or pathophysiologic event would greatly enhance our understanding of the psychiatric disorders. This paper is a consideration of some of the theoretical and technological issues

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that need to be dealt with, in order to find valid and broadly applicable single locus markers, or to demonstrate convincingly that none exist.

Recent advances in genetics, particularly the development of new molecular genetic linkage methods, have made it at least theoretically feasible to scan the entire human genome, so that in a properly designed and executed set of studies it will be possible to demonstrate whether there exists a marker for a particular disorder (Botstein *et al.*, 1980). However, one must raise the issue of whether these techniques are applicable to the psychiatric disorders, in view of the complex inheritance of these disorders. By complex inheritance, we refer to the variable age of onset, the variable penetrance that must be invoked if single locus inheritance is assumed (because of the observed non-Mendelian ratios of ill and well relatives of patients), and the apparent biological heterogeneity and non-genetically caused cases within each of the major psychiatric disorders.

2. CONSTRAINTS ON LINKAGE ANALYSES IN BEHAVIORAL DISORDERS

It is reasonable to consider whether single locus inheritance actually exists in psychiatric disorders, even under conditions of complex inheritance. In the affective disorders as well as in schizophrenia, segregation analyses (of distribution of diagnoses in pedigrees of patients) are generally inconsistent with single locus inheritance as the sole genetic mechanisms in large data sets (GOLDIN and GERSHON, 1983; NURNBERGER *et al.*, 1986; GOLDIN *et al.*, in press). The presence of single locus inheritance on a multifactorial background is consistent with some data from schizophrenia (RISCH and BARON, 1985), and there has been reported one large pedigree of bipolar affective illness, from the Amish, consistent with single-locus dominant inheritance detected by linkage to a marker (EGELAND *et al.*, 1987).

However, studies of the transmission of psychiatric disorders are complicated by several factors such as uncertainties in the definition of clinical illness, variable age of onset of illness, incomplete penetrance of susceptible genotypes and biases due to decreased reproduction by ill individuals. This last factor has a large effect in schizophrenia where the prevalence of illness in parents of probands is about half of that in siblings and offspring; this can be taken into account in specialized analyses, as performed by RISCH and BARON (1985). In addition, psychiatric disorders may be heterogeneous in etiology with the possibility of multiple genetic subtypes and environmentally caused subtypes. Some of these factors can be taken into account when analyzing familial diagnostic data, but it is not possible to exclude the possibility that even when major locus transmission is rejected for the sample as a whole, a substantial subgroup of families have an illness caused by a single major locus.

Simulation studies have provided us with an understanding of some of the limits of major locus detection from family diagnostic information (GOLDIN *et al.*, 1981, 1984). We simulated single locus transmission of a dichotomous trait in samples of moderate sized pedigrees and varied the mode of inheritance. The models were chosen to be realistic for complex, "common" disorders, a category in which several major psychiatric disorders are included. We found that if the model was nearly recessive or intermediate (i.e. "low" heterozygote penetrance), a major locus was not consistently detectable by segregation analysis (GOLDIN *et al.*, 1981, 1984). If the transmission was dominant or additive, then

the major locus was detectable. This was true even when a substantial proportion of cases were phenocopies (i.e. genetically normal, or sporadic).

For models with low heterozygote penetrance, we also simulated a marker locus linked to the trait locus, and found that there were parameters of inheritance where segregation analysis could not detect a major locus, but it could be detected by linkage analysis (using the lod score method) as long as linkage was close and there were not a large proportion of phenocopies in the population. These included low heterozygote penetrance (10–15%) in a common disorder (2% population prevalence). These studies only examined a limited number of possible parameters of interest. For example, we did not consider models with multiple genetic subtypes. Nonetheless, the results give us a general indication of the limitations of segregation analysis and the increased power of linkage analysis to detect the presence of single gene transmission of an illness.

If we accept the exclusion of single locus inheritance from the analyses of actual data sets, and the limits on these exclusions from the simulation studies, we are left with two classes of conditions under which single locus inheritance might still be present and detectable in these disorders. The first condition is within the parameter sets of complex inheritance where linkage markers allow detection but segregation analysis does not. This condition can be classed as a unitary hypothesis about any given psychiatric disorder (that there is one generally present inherited form in the population). The second condition is one not considered in the segregation analyses or in the simulations: that there are genetically heterogenous subgroups of inherited illness. We present here preliminary results on the power of linkage detection under each of these conditions.

Linkage generally present: complex inheritance

Since our investigative group is currently engaged in a study of bipolar manic-depressive pedigrees selected from a clinical population, we first consider the expected lod score under different models of inheritance for structures of two actual pedigrees with affective disorder that we have ascertained. In our ascertainment procedure, we selected pedigrees for the presence of a sibship of 4 or more adult individuals, with at least two affected and one unaffected members, and an additional segregating sibship in the pedigree.

The two pedigrees we used for this simulation are shown in Fig. 1. Fourteen individuals had been examined in one, and 15 in the other, from two generations, with information on the grandparents restricted to psychiatric history. We simulated the genotypes at the disease locus based on specific genetic models for the illness and on the phenotypes in the pedigree structure. Two models were used, a recessive model ($q = 0.2, f = 0.9, 0.005, 0.005$, where q is gene frequency and f refers to the penetrances), and an additive model ($q = 0.04, f = 0.9, 0.45, 0.005$). Under each model, the population lifetime prevalence is 4%. As can be seen from the penetrances, some phenocopies are allowed for since the penetrances of the "normal genotypes" are not zero. A linked marker locus was also simulated, at different levels of recombination (0.01, 0.05, 0.1, 0.5). It was assumed that the marker locus is highly polymorphic. A full description of the simulation process and precise outcomes of the simulations will be presented elsewhere. Here, we give a summary of results to give a sense of the required sample size for linkage detection in a series of pedigrees such as these.

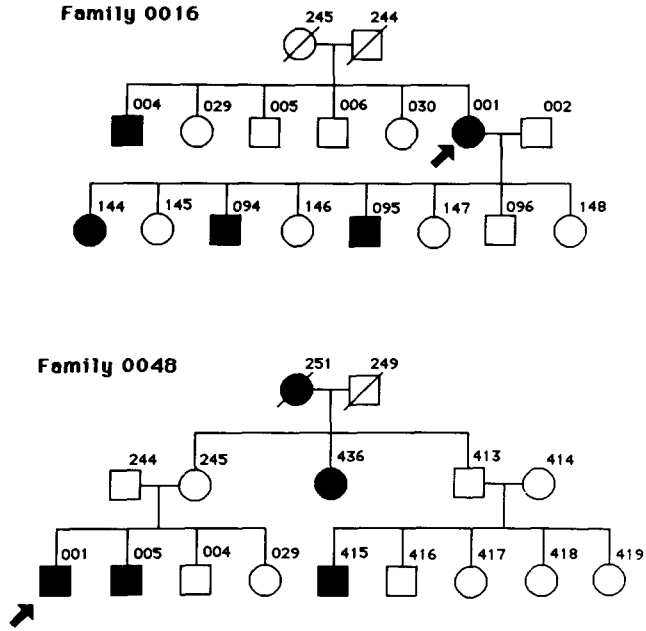

FIG. 1. Actual pedigrees and diagnoses used in simulations.

We computed the lod scores for each pedigree in 100 simulations per model, and then averaged the scores (over the two pedigrees) to calculate the average lod score per pedigree at each value of recombination fraction (theta) for each model. At theta = 0.1, the maximum lod scores were between 0.2 and 0.3 (Fig. 2). This value of theta has been proposed as a practical maximum distance between a disease locus and a marker locus for screening the genome (BOTSTEIN *et al.*, 1980). Therefore, 10–15 pedigrees like these would be needed firmly to establish linkage (lod score > 3). Eight familes would be needed to reject linkage (lod score < -2). We note that 8 families would be sufficient for scanning the genome, since exclusion is firm, and since the lod score for the linked locus would be very suggestive and would undoubtedly prompt further study.

An alternative method for linkage analysis is to sample affected-sib pairs (ASP). This method was developed to bypass some of the assumptions required by the lod score method applied to pedigrees (THOMSON and BODMER, 1977; SUAREZ *et al.*, 1978). For example, if only ill sibs are sampled, one need not be concerned with estimating penetrance, or with the problem of whether well individuals with a marker allele are genetically vulnerable or recombinants.

Using the method of BLACKWELDER and ELSTON (1985), we computed the number of affected sib pairs needed to detect linkage with 80% power under several models of inheritance for each of two disease prevalences. A complete description of the computations is presented in GOLDIN *et al.* (in preparation). The models were chosen to reflect possible modes of inheritance for bipolar I or schizophrenia (1% population prevalence) and major affective disorder (7% population prevalence). The two genetic models considered were

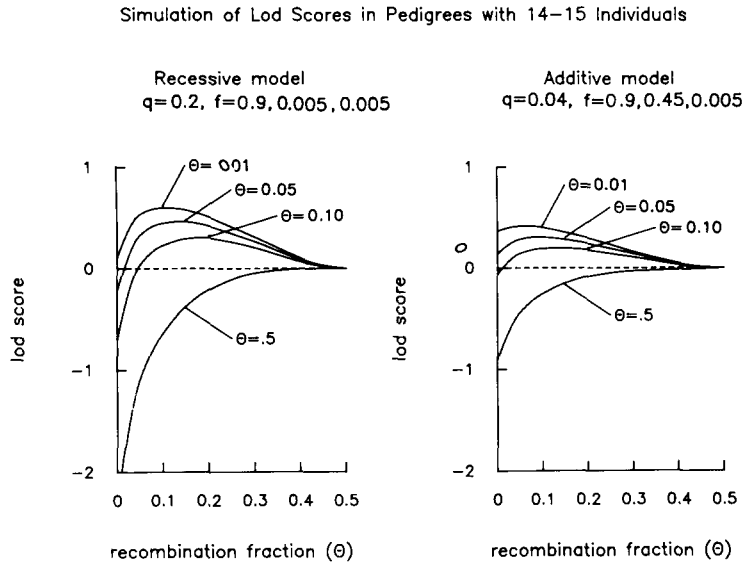

FIG. 2. Simulation of lod scores in pedigrees with 14–15 individuals.

recessive and dominant. For the 1% prevalence disease, the parameters for recessive inheritance were $q = 0.25, f = 0.16, 0, 0$; for dominant inheritance $q = 0.01, f = 0.5, 0.5, 0$; for the 7% prevalence disease, recessive inheritance $q = 0.27, f = 0.9, 0.005, 0.005$; and dominant inheritance $q = 0.068, f = 0.5, 0.5, 0.005$. For the rarer disorder, a sample size of 25 pairs is sufficient to detect linkage up to 10% recombination under either model (Fig. 3). For the more common disorder, a sample size of approximatley 45 is needed (Fig. 4).

Genetic heterogeneity

There are several conceivable investigative strategies to detect linkage under this condition. These include study of population isolates, and use of the affected sib-pair method in large populations.

We have computed the number of affected sib pairs required to detect linkage under the same models as above, but assuming only 50% of pairs are linked. For the rare disorder at theta = 0.1, approximately 60 pairs are needed under the recessive model, and 120 pairs are needed under the dominant model (Fig. 3). For the common disorder, approximately 70 pairs are needed under the recessive model. For the dominant model approximately 250 pairs are needed for theta = 0.1, and 99 for theta = 0.05 (Fig. 4).

We also considered the power of this method with more extensive heterogeneity, with only 25% of families linked. The sample size needed for the recessive model of a "rarer" disorder (1% prevalence) under 10% recombination was approximately 200 affected sib pairs, which is about 3 times the number required when 50% of families are linked. Thus, it appears that the affected sib pair method is powerful for detecting linkage under conditions of moderate heterogeneity. As expected, it is more powerful for "rarer" disorders.

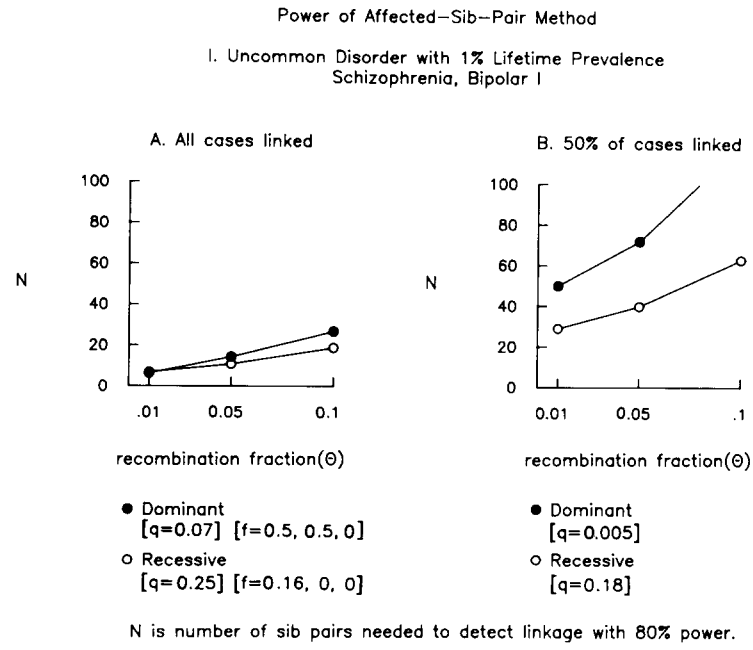

FIG. 3. Power of affected-sib-pair method. Uncommon disorder with 1% lifetime prevalence (Schizophrenia, bipolar I). *N* is the number of sib pairs needed to detect linkage with 80% power.

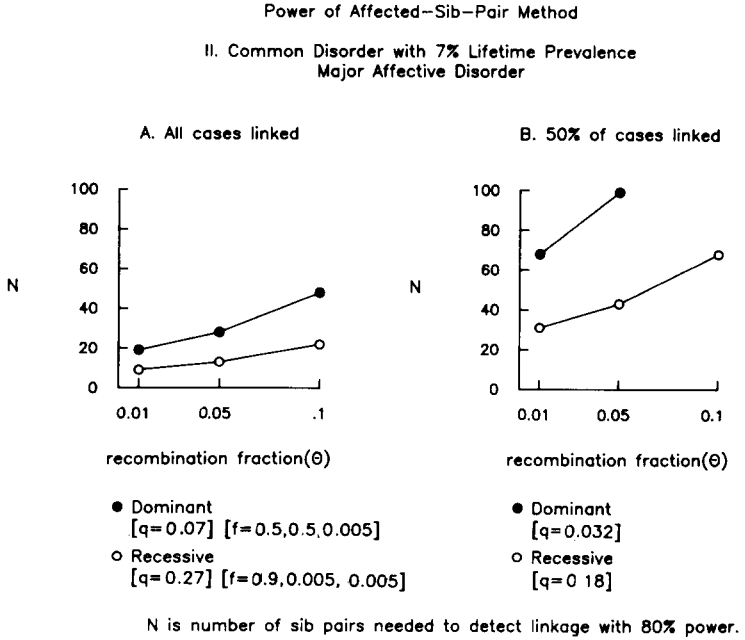

FIG. 4. Power of affected-sib-pair method. Common disorder with 7% lifetime prevalence (Major Affective Disorder). *N* is the number of sib pairs needed to detect linkage with 80% power.

The power of the lod score method under these same conditions (complex inheritance and heterogeneity) is not well defined, although methods for considering heterogeneity are available (MORTON, 1956; SMITH, 1963). RISCH and BARON (1982) have applied Morton's method to analyze heterogeneity of X-linkage of bipolar illness under these conditions, but the general power of the lod score pedigree method has not been fully explored.

Large pedigrees from population isolates may also serve to resolve heterogeneity. The advantages of dense pedigrees from population isolates include the presumption of genetic homogeneity of illness, increased frequency of "helper" alleles, and ease of identifying distant branches of large pedigrees. The disadvantage is that disorders found in an isolate may be rare and of no epidemiological significance in the larger population, despite some clinical similarities to a more common disease. However, this possibility can be readily tested once a linkage is found.

The intellectual appeal for scanning the genome is that it allows the presence of a gene of illness to be excluded under essentially all reasonable conditions of single-locus inheritance. The amount of scanning needed is clearly a function of what one considers reasonable conditions, including the anticipated amount of heterogeneity, and the complexity of the mode of inheritance. But within those limits, it is of some importance that one be confident that if linkage has not been detected, it is not there. For this reason, despite the size of the investigative effort needed to scan the genome for markers linked to the major psychiatric disorders, we consider this effort worth undertaking.

3. METHODS OF MARKER IDENTIFICATION

Historically, the current excitement in linkage mapping of medical disorders stems from two developments. First, is the realization that disorders of considerable medical importance, such as juvenile diabetes and ankylosing spondylitis, which were long thought to be polygenic/multifactorial in their inheritance, in fact had single locus inheritance which could be demonstrated by linkage or association to the HLA system (MCMICHAEL and MCDEVITT, 1977; THOMSON and BODMER, 1977). The second source of excitement over linkage markers is the use of Southern blots to demonstrate restriction fragment length polymorphisms (RFLPs) (KAN and DOZY, 1978). It is the RFLPs that have so greatly increased the number of known human polymorphisms (WILLARD *et al.*, 1985), and provided the striking successes in mapping disorders which had long been unmappable, such as the mapping of Huntington's disease to an arbitrary single-copy DNA sequence on chromosome 4 (GUSELLA *et al.*, 1983). But it is, we think, worth putting single-copy RFLPs into perspective as one of several new methods for polymorphism detection. As important as this method now is, it is arduous, and may ultimately prove not to be the most efficient and rapid method possible for genomic scanning.

Several other methods have promise for scanning the genome more rapidly, by allowing examination of multiple loci with each laboratory procedure. These include use of repetitious DNA sequences on Southern blots, 2-dimensional high resolution electrophoresis of proteins, and chromosome banding methods based on molecular genetic techniques.

In the classical use of the Southern blot in linkage, genomic DNA from informative individuals is digested into fixed length fragments (by use of enzymes known as restriction endonucleases), electrophoresed on a gel, and bonded to a membrane (the preparation at

this point is a Southern blot). A radioactive cloned gene is then hybridized to its homologous fragment(s) on the blot, which is then placed against a photographic film. If genetic differences between the individuals have produced differences in the lengths of the garment(s) containing the cloned gene, the gene will have hybridized to different positions on the blot. Thus, the fragment length for one indivual for that gene can be distinguished from that of another individual; this is restriction fragment length polymorphism.

It is easiest to conceive of a polymorphism of a unique, single-copy DNA fragment. But a substantial proportion of the human genome consists of repetitious DNA segments, which have been replicated and re-inserted into numerous points of the genome, over the course of evolution. There are two major difficulties in using repetitious DNA on Southern blots: the difficulty in establishing the chromosomal localization of each of the numerous bands produced by a probe for a repeated sequence, and the difficulty in "reading" of the complex patterns that the repetitious DNAs produce on Southern blots (BOTSTEIN *et al.*, 1980). Instead of the one, two, or three band patterns that the single copy RFLPs produce on a blot, the repetitious DNAs may produce from 13 or so bands to 300,000 bands (in the case of the 'Alu' sequences). Recently, JEFFREYS *et al.* (1985a, b) and GILL *et al.* (1985) identified a "mid-repetitious" DNA sequence (that is, one with an intermediate number of repetitions). The variability of this repeat sequence has permitted its use as a DNA "fingerprint" to identify individuals on the basis of the numerous polymorphisms observable on the tens of bands of the blot, although these polymorphic differences are not yet resolved into all their component loci.

Another approach to visualizing multiple polymorphisms on a single preparation is to view them *in situ,* on their chromosomal sites. With traditional banding methods using dyes, or recent methods using restriction enzymes on chromosomes *in situ* (BIANCHI *et al.*, 1985) or other *in situ* methods (BULLERDIEK *et al.*, 1985), polymorphisms may in the future be directly identified at numerous points on each chromosome. This may allow scanning of a considerable portion of the genome by computerized image analysis of chromosomal preparations from each member of a pedigree, with considerably fewer laboratory procedures needed than we now anticipate for RFLPs.

High-resolution 2-D electrophoresis of proteins offers another method of screening for multiple linkage markers with a single laboratory procedure. In a survey of 186 human lymphocyte proteins, 19 independent polymorphic protein systems were observed (GOLDMAN *et al.*, 1985). Each of these polymorphic systems consisted of 2–3 allelic charge variants that displayed Mendelian inheritance with codominant expression in heterozygotes and strict genotypic concordance in monozygotic twins (GOLDMAN *et al.*, 1982; GOLDMAN and MERRIL 1983). With improvements in automated methods of analyzing the complex two-dimensional protein electrophoretic patterns it may prove possible to detect even more polymorphic loci, since it is not uncommon to be able to visualize as many as 1000 separate proteins in a single gel.

4. CLINICAL RESOURCES IN PSYCHIATRIC DISORDERS

Although progress in the technology of linkage marker evaluation may be anticipated, the magnitude of clinical effort and commitment needed in properly ascertaining, engaging cooperation, and systematically diagnosing the individuals in families of patients does not

appear reducible. From the computations described above, it is evident that a sizeable number of individuals, either in pedigrees of sib pairs must be investigated in order to detect or exclude single gene components of the genetic transmission of psychiatric disorders. These numbers are larger than have been assembled, to our knowledge, at any one location. It therefore appears imperative that a massive effort be mounted to assemble sufficient clinical material, and that these resources be shared through making cells and clinical information freely available among linkage investigators.

We propose to create a repository of cells and pedigree information from families with multiple cases of schizophrenia, manic-depressive illness, alcoholism, or Alzheimer's disease. Large sets of affected sib pairs, and of moderate sized pedigrees from the general population, as well as unique large pedigrees, would be included. These would serve as an international resource for molecular geneticists to perform gene mapping studies on these disorders. The advantage of the repository is that it enables investigators in molecular genetics to study these disorders without needing to develop their own clinical collaboration. Furthermore, the size of the collection that could be built by the repository is larger than any reasonable sized investigative group could develop, but would be sufficient for linkage scanning according to our computations. The alternative is to have fewer linkage studies applied to fewer pedigrees, thus delaying progress in answering the key questions of whether there is single gene transmission in these psychatric disorders, and if there is this form of transmission, to map the illness gene(s).

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