A disease approach using linkage analysis: Still in geneticists' quiver.

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ABSTRACT: Linkage analysis, based on Mendel's law of independent assortment, has contributed the most to the late advance of human genetics. In this article we present a way of approaching a disease by the use of this technique. The first step is to use a set of families where the disease segregates. Large pedigrees are preferable. Then all the members of these families are analyzed using an initial set of markers to detect an area of high interest. A common approach is to re-examine the same families with markers spaced closer to the chromosome in order to narrow the relevant area. Later, taking into account the existing knowledge, the hypothesis of the candidate gene is made and we try to prove it. The final study, as well as the final result are both influenced by the kind of families that are chosen, the suitability of the markers and the criteria used for proving a candidate gene hypothesis. Mendelian diseases were the first to be studied, but the real challenge for geneticists is detecting loci influencing complex diseases and linkage analysis remains still an effective approach.

Key Words: Linkage analysis, Mendelian disease, Heterogeneity, Candidate gene.

INTRODUCTION

During the last few decades, huge advance has been made in human genetics in point of detecting the loci of human traits and diseases and linkage analysis is one of the techniques that contributed the most to this advance.

Linkage analysis is based on Mendel's second law, the law of independent random assortment which deals with the joint behavior of alleles at two loci. When two genetic loci, the marker locus and the trait locus, lie proximally on the same chromosome, they tend to cosegregate within families. The loci are linked. The first to realize this was Morgan TH[1] with his experiments on Drosophila melanogaster while the first genetic linkage in man was made by Bell J and Haldane JB when they noted the linkage between haemophilia and colour-blindness[2]. Meanwhile, Morton NE proposed as a statistic to assess possible linkage the logarithm of an odds ratio score (LOD score)[3]. The odds ratio is the odds of occurrence of observed genotypes in the framework of the proposed model under the assumption of linkage to the odds computed on independent assortment. Thus, high LOD scores favor the linkage hypothesis and score close to 1 favor independent assortment. In particular, a LOD score of 3 is considered to be a statistical significant evidence for linkage, while a score of -2 is evidence against it[4,5].

Linkage studies were primarily used for diseases showing Mendelian inheritance which were caused by highly penetrant alleles and there are numerous successful examples of it. On the other hand, the first results after using the same technique for complex traits and diseases were not statistically significant with linkage being hard to find[6]. This article presents the basic principles and steps that could be followed to approach a disease with the use of linkage analysis. Many examples are described, but in the same time some problems that are found frequently are also presented.

The starting point

Linkage analysis studies begin with a set of families in which the disease segregates. The diagnosis of the phenotype should be unambiguous. The pedigrees are drawn and a pattern of inheritance is estimated. Large

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families with multiple cases have a greater power on revealing a clear pattern of inheritance and detecting the disease locus than multiple small families, and thus they should be preferred[7].

Simple Mendelian diseases such as cystic fibrosis[8,9], Huntington disease[10] and polycystic kidney disease[11], with a least ambiguous diagnosis and a one-to-one correspondence between phenotype and genotype were the first to be successfully studied. When the diagnosis is not certain and there is disease heterogeneity or complex and unclear inheritance we must be far more precautious or else linkage studies will fail[12].

Approach by the use of linkage analysis

The selected families, including those of their members who do have the phenotype and those who do not, are analyzed for linkage using a group of markers. The first markers that were used were the various blood group loci. Anderson DE linked the naveoid basal cell carcinoma syndrome (NBCCS) with Rhesus locus[13] and Bird TD et al linked Charcot-Marie-Tooth with the Duffy locus[14], both on chromosome 1, while Harper PS et al linked myotonic dystrophy with the Lutheran blood group locus[15]. But the real revolution came with the proposal of genome-wide linkage analysis using anonymous DNA polymorphisms[16]. Restriction fragment length polymorphisms (RFLPs), simple sequence repeats and today simple nucleotide polymorphisms (SNPs) and microsatellite DNA are all used as markers, providing us with some useful results[17,18,19].

An initial set of markers spaced about 5-10 cM apart is used in order to detect an area of high linkage interest[12]. Naylor SL et al examined 14 families with cystic fibrosis for linkage with three markers. His results revealed significance linkage and indeed his analysis indicated a specific order; COL-pJ3.11-CF-MET[20]. Moreover, Lebo RV et al using a small set of markers detected the gene of CMT disease in an area of 18 cM between the genes of Duffy blood group and antithrombine III (AT3)[21].

Linkage studies have been reinforced by the construction of genetic maps of the human chromosomes, an idea which was proposed by Botstein D et al[16]. Soon after Botstein's idea, maps of the different chromosome were presented[22,23,24], maps which were continuously enriched by newer and more in number polymorphisms. This kind of map used Lebo RV et al in his research[21,23]. A genetic map of the whole human genome was constructed by Green P et al using RFLPs[25]. Modern maps using as polymorphisms the huge number of SNPs and microsatellite DNA are the first part of the genetic approach of both complex and mendelian diseases.

After detecting an initial linkage within a region, the standard procedure is to re-examine the same pedigrees with markers placed more closely in that area of interest[12]. This is what Michon L et al did in their work over congenital microphthalmia[26]. Morle L et al had detected the responsible gene in a 13.8 cM region on chromosome 15q12-q15[27]. Michon L et al re-examined this region with new microsatellite DNA markers and managed to restrict it into two smaller regions with size 1.9 and 2.5 Mb respectively, regions which are very closely spaced on chromosome 15[26]. In more favorable cases this procedure results in a small number of genes in the region that might be relevant with the disease, otherwise there can be more than a hundred of them as candidates.

Heterogeneity

A common problem that linkage studies had to cope with from the early beginning was locus and disease heterogeneity[7]. Locus heterogeneity refers to a situation where the same disease can be caused by multiple loci independently. This particularly means that when the sample of the study is consisted of a large set of families, it is likely that all families are not linked to the same locus. Disease heterogeneity exists in diseases that have subtypes, grades or stages. A standard approach for dealing with heterogeneity is by splitting the large sample of families into multiple smaller ones[7]. Families in which the disease has common pattern of inheritance or common clinical symptoms and laboratory findings, and as a result the disease in them may be of the same subtype, can be presented as a new sample and can be analyzed separately.

A very useful example for understanding heterogeneity is that of Charcot-Marie-Tooth disease (CMT). CMT is an extremely heterogeneous disease, as it appears with a great variety of clinical symptoms and inheritance patterns[28]. Therefore it has heterogeneity - clinical, genetic and hereditary. Initial studies showed linkage with the Duffy blood group locus

on chromosome 1[14]. On the other hand, other studies failed to confirm such linkage. Finally two research groups, Dyck PJ et al and Bird TD et al, proposed the hypothesis of heterogeneity. They named the families which showed linkage to Duffy locus as type B and those which did not show linkage as type A and studied them separately[29,30]. This was the first step that led to the detection of the responsible genes and their proteins, myelin protein zero (MPZ) and peripheral myelin protein 22 (PMP22) respectively[31,32].

Candidate genes

Candidate gene is a gene which is localized in the relevant region that linkage studies have already recognized and its protein product seems to be related to the disease[12]. As mentioned above, this region may include a number of genes that may vary from less than ten to more than some hundreds. Linkage analysis is able to identify the region, but in order to consider a gene as a probable candidate more information is required. Physiological and pathophysiological knowledge, previous genetic studies and animal models, they all should be taken into account[33]. Genome Database and Online Mendelian Inheritance in Man (OMIM) are useful tools for today's researchers as they can provide them easily and rapidly, with a list including all the genes in the relevant region[12,34].

At this point, linkage analysis becomes insufficient by itself and genetic studies need help from other techniques. Sequencing is the technique used next[35,36]. Sequencing the candidate gene can accentuate the responsible for the disease mutation. However this is not the end of the research. The most important thing is to reassure that what has been found is not another new polymorphism. Cosegregation of the mutant allele with the disease, absence in controls and low frequency in general population are the criteria which must be fulfilled[37]. Finally, functional, tissue expression and animal model studies offer the crucial additional support for the definite connection between a single gene or a single mutation and a specific disease or phenotype in general[12]. Following all these steps and based on previous work, Miki Y et al identified BRCA1 in the centre of the suspected 600-kb region of 17q21 between markers D17S1321 and D17S1325 showing that the essential criteria are fulfilled[37]. Before that, but using similar methodology, Hayasaka K et al identified MPZ as the responsible gene for CMT1B[38] and afterwards Boerkoel CF et al linked periaxin mutations with Dejerine-Sottas neuropathy[39].

However all the candidate genes studies do not reach the desirable success. In fact, many unsuccessful studies have anteceded a single one successful. The first candidate gene for CMT1B was the gene of Fc-gamma receptors which in the end was not affirmed[21]. In the same way Michon L et al excluded three candidate genes for congenital microphthalmia (CKTSF1B1, CX36 and KLF13) as they found no mutations in these genes after PCR-amplification and sequencing in affected subjects and healthy controls[26].

DISCUSSION

As Botstein D and Risch N wrote, "connecting phenotype with genotype is the fundamental aim of genetics"[12]. During the last decades, many steps have been made towards this direction and linkage analysis, since the experiments of Morgan TH[1] and Bell J[2], has been a pioneer. Despite the fact that Risch N and Merikangas K declared linkage studies' death in 1996[40], they continue to be used till today to prioritize other genetic techniques.

The availability of computer software to process the huge amount of data from linkage studies as well as the nomination of new DNA polymorphisms which made linkage studies cheaper and faster were the cause of the scientific explosion at the 1980s and afterwards that lead to the connection of hundreds of genes with diseases. The main polymorphisms used today are microsatellite DNA and SNPs. They are both highly polymorphic and equally dispread through the genome. There is a strong argument concerning the suitability of the markers used for each study[41,42,43,44]. Microsattelite markers are considered to be more informative than SNPs, although SNPs are more abundant. Furthermore, a larger number of SNPs is needed to achieve information content similar to that of microsatellite DNA, but on the other hand, SNPs result in higher LOD scores and narrower linkage region. In conclusion, the currency of new software for the statistical process of the linkage studies' data and industrial arrays with a large number of SNPs will boost their use.

Firstly, the main interest concerned the mendelian diseases. They show a simple pattern of inheritance

and in most cases they are caused by rare, highly penetrant alleles. Nowadays, genetic studies have turned their interest on complex diseases and complex traits. This interest is pleaded because of their great prevalence and great effect on total human morbidity and mortality. Detecting complex traits is a real challenge for geneticists as complex diseases are the result of many genes acting together, each one of which has a small effect on the final phenotype and moreover, they receive major environmental impact which is not completely understood yet[45]. Genetic studies on complex diseases aim to clear the darkness that exists on the way environmental and genomic factors affect each other and result to the final phenotype. This can increase our knowledge over complex diseases and can reveal new therapeutic targets. Criswell LA et al with their analysis over rheumatoid arthritis showed the potential importance of sex and other quantitative components of the disease on genetic factors that may finally influence the risk of specific manifestations,[46] while Zhang Y et al found linkage between variants on chromosome 13q14 and atopy and severe clinical asthma[47] and Johnson L et al's meta-analysis showed linkage evidence between BMI and chromosome 8p[48].

From the above, it is easily comprehensible that linkage analysis, despite being old, is not superannuated at all. It still plays a key role in modern genetic panorama. The late advances in Bioinformatics which have made genome-wide scans a reality and the results of the combination with techniques such as mapping studies, association studies (GWAS) and new generation sequencing, show that linkage analysis is a powerful tool in the geneticists' quiver.

Μια προσέγγιση νόσου χρησιμοποιώντας την ανάλυση σύνδεσης: Παραμένει ακόμα στη φαρέτρα των γενετιστών.

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ΠΕΡΙΛΗΨΗ: Η ανάλυση σύνδεσης, η οποία βασίζεται στον νόμο της ανεξάρτητης κληρονόμησης του Μέντελ, έχει συνεισφέρει τα μέγιστα στην πρόσφατη πρόοδο της γενετικής. Στο παρόν άρθρο παρουσιάζεται ο τρόπος προσέγγισης μιας ασθένειας με την χρήση αυτής της μεθόδου. Το πρώτο βήμα συνίσταται στη σωστή επιλογή μιας ομάδας από οικογένειες στις οποίες η ασθένεια κληρονομείται. Προτιμούνται τα μεγάλα γενεαλογικά δένδρα. Στη συνέχεια όλα τα μέλη αυτών των οικογενειών αναλύονται με τη χρήση μιας αρχικής ομάδας από δείκτες με σκοπό την ανίχνευση περιοχής υψηλού ενδιαφέροντος. Μια κοινή προσέγγιση αποτελεί η επανεξέταση των ίδιων οικογενειών με δείκτες που βρίσκονται πιο κοντά μεταξύ τους στο χρωμόσωμα έτσι ώστε να περιοριστεί η σχετική περιοχή. Έπειτα, λαμβάνοντας υπόψη την υπάρχουσα γνώση, καταστρώνεται η υπόθεση του υποψήφιου γονιδίου και γίνεται προσπάθεια απόδειξής της. Η τελική μελέτη, όπως επίσης και το τελικό αποτέλεσμα, επηρεάζονται και τα δύο από το είδος των οικογενειών που θα επιλεχθούν, την καταλληλότητα των δεικτών και τα κριτήρια που θα χρησιμοποιηθούν για την απόδειξη της υπόθεσης του υποψήφιου γονιδίου. Οι μεντελικές ασθένειες ήταν οι πρώτες που μελετήθηκαν, ωστόσο η πραγματική πρόκληση για τους γενετιστές είναι η ανακάλυψη γενετικών θέσεων που επηρεάζουν τις σύνθετες ασθένειες και η ανάλυση σύνδεσης παραμένει ακόμα μια αποτελεσματική προσέγγιση.

Λέξεις Κλειδιά: Ανάλυση σύνδεσης, Μενδελικές ασθένειες, Ετερογένεια, Υποψήφιο γονίδιο.

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