



Human genome effort bears fruit for AMD

Newly identified variant gene may explain nearly half of cases of AMD

Researchers involved in three separate studies have reported a major breakthrough in the search to locate genes involved in age-related macular degeneration.

The results of the studies, published in the journal *Science* on April 15 [Volume 308, Number 5720], provide conclusive evidence that a gene known as “complement factor H” (CFH), may be involved in AMD.

The results provide not only a highly promising drug target for the pharmaceutical industry, but also provide the basis for a new DNA assay for clinicians to identify asymptomatic patients who may be at-risk of developing AMD.

Although several studies had implicated the role of a number of genes in AMD, those genes were linked to only a small minority of AMD cases. In contrast, the complement factor H gene could “likely explain” about 43% of cases of AMD in older adults, according to Dr Jonathan Haines, one of the researchers in the landmark studies.

Locating the complement factor H gene was achieved through a technique known as “linkage mapping” in combination with the examination of known genetic variations known as SNPs or “single nucleotide polymorphisms.”

SNPs are the most common type of sequence variation between individuals in which a single base pair is different between one individual and another. For the most part, such single base pair differences have little or no biological effect; however, on occasion, such a difference can produce serious consequences.

Variant CFH gene may disrupt complement cascade

In the case of the CFH gene, the variant of CFH detected in individuals with AMD carried a nucleotide alteration that changed the structure of the CFH protein. Although the exact molecular pathology has yet to be defined, it appears that this slight alteration leads to the development of AMD by interfering in a part of the immune system known as the “complement cascade.”

The complement cascade is an innate part of the immune defence system, consisting of over 30 serum proteins. In general, substances on the surface of microbes can trigger the complement cascade, which activates a series of biochemical steps leading to the lysis (or bursting) of invading cells. However, certain complement proteins may also help trigger inflammation.

Presumably, the CFH gene variant found in this series of studies in some manner abrogates the complement cascade in the eye, possibly in conjunction with other factors, leading to the progression of AMD.

Human Genome Project promises revolutionary changes

The Human Genome Project (HGP) will revolutionise 21st century healthcare with ground-breaking advances in diagnostics, molecular surgery, and customised drugs.

The HGP, which was completed in April of 2003, deciphered the estimated 3.2-billion letter code that constitutes the DNA of our genes. The actual attainment of the estimated 3.2-billion letter chemical code of the human species may turn out to be the “easy” part.

Understanding what it all means is most likely where the real challenge lies.

The prospect of using genes as a therapeutic tool is known as gene therapy and a major objective of genetic medicine will be the

design of therapeutic regimens directed at the molecular level of the gene itself.

Gene therapy is an attempt to discern the genetic root of a disease and treat the condition by correcting the genes involved. Such technology, although in its infancy, is currently developing at a rapid pace and possesses a very real potential for treating both genetic and acquired disorders.

An intimate knowledge of the human genome sequence is one of the foundations upon which this technology will expand in the development of molecular based gene directed medicines.

Traditional pharmaceutical companies are similarly poised to exploit the tidal wave of

genomic information. There are few drug companies in the world that do not have a major investment in the information set to emerge from the genome project. A whole science – known as genomics – has become established to characterise and analyse the relationship between gene activity and cell function.

Identifying the steps and the biochemistry between the genotype and phenotype will provide the raw material for novel drug discovery.

With the knowledge of the As, Gs, Cs, and Ts associated with particular disorders, researchers may one day be able to delicately manipulate the course of disease at its most fundamental molecular origin.

Data from Human Genome Project

SNP databases arose from the large volumes of data provided by the Human Genome Project (HGP), the multi-billion dollar research effort that identified the more than three billion nucleotides that constitute the DNA sequence of human beings.

Dr Stephen Daiger, at the Human Genetics Centre of the University of Texas Health Sciences Centre, noted that the HGP contributed significantly to the recent breakthrough.

“The human genome project is much more than the genetic map of our species. It is also a powerful set of integrated tools for solving problems in medicine and biology,” Dr Daiger pointed out in a commentary on the AMD research papers in *Science*.

“The complement cascade is an innate part of the immune defence system, consisting of over 30 serum proteins.”

“Application of these tools to a common cause of human blindness, age-related macular degeneration, implicates a common variant in the gene encoding complement factor H as a major contributor to the risk of developing the disease,” Dr Daiger added. “The new findings raise hopes for better diagnosis and treatment of macular degeneration and further validate the importance of the Human Genome Project.”

Hap Map Project

Dr Daiger commented that the data presented in the papers also benefited greatly from an allied genomic project called the International Haplotype Mapping Project, or “Hap Map” Project for short. Dr Daiger explained that “a recent goal of the Human Genome Project has been to determine the haplotype structure of human chromosomes in various ethnic groups, a goal embodied in the International Hap Map Project.”

The word “haplotype,” derives from the terms “haploid” and “genotype.” As each of the sex cells of humans (sperm and eggs) contains a haploid genotype the process of fertilisation creates a new entity with a diploid genotype. The term haplotype refers to a specific combination of two or more sequences of DNA situated close together along the length of a chromosome.

If two pieces of DNA are physically close to each other then there is less of a chance of them being separated during cell division and genetic shuffling than if they were far apart. Consequently, examination of blocks of haplotype sequence can provide useful information regarding both genetic history and the order of genes and gene variants along the length of chromosomes.

Dr Daiger explained that “haplotypes are sets of specific DNA variants that are so close together on the chromosome that they undergo recombination only very rarely. The pattern of non-recombining variants, known as haplotype blocks, reflects the evolutionary history of humans over the past 50,000 to 100,000 years. Haplotype blocks typically span tens of thousands of base pairs. One immediate benefit of the Hap Map project is that it provides a bridge between linkage mapping at millions of base pairs and SNPs mapped to single base-pairs.”

The three papers published in *Science* were authored, respectively, by:

- Dr Robert Klein at the Laboratory of Statistical Genetics of the Rockefeller University in New York;
- Dr Albert Edwards at the Department of Ophthalmology and Mc Dermott Centre for Human Growth and Development of the University of Texas Southwestern Medical Centre in Dallas (now at the Institute for Retina Research in Dallas);
- Dr Jonathan Haines at the Centre for Human Genetics Research of the Vanderbilt University Medical Centre, in Nashville, Tennessee.

The first report, published by Dr Robert Klein, found that individuals with a CFH variant that substitutes a tyrosine amino acid for a histidine at position 402 increased the likelihood of developing AMD 4.6-fold if present on one allele and 7.4-fold if present on both alleles.

The second paper, published by Dr Edwards, reported that “possession of at least one histidine at amino acid 402 (of the CFH gene) increased the risk of AMD 2.7 fold and accounts for 50% of the attributable risk of AMD.”

The final study, led by Dr Jonathan Haines, found that the CFH haplotype “significantly increases the risk for AMD with odds ratios between 2.45 and 5.57 and (that such a) common variant likely explains approximately 43% of AMD in older adults.”

Link between dysfunctional inflammation and AMD

The physical link between CFH that operates within the complement system and AMD can be found in the drusen or extra-cellular

deposits found in patients with AMD. Several components of the complement cascade have

been found in drusen deposits which have led to the hypothesis that AMD may result from

dysfunctional inflammation which incorporates inappropriate complement activation.

Dr Haines, in his publication, points out that “the biological role of complement factor H as a component of the innate immune system that modulates inflammation through regulation of complement enhances its attractiveness as a candidate AMD susceptibility gene. Inflammation has been repeatedly implicated in AMD pathology.”

Dr Edwards further elaborated upon the potential role of complement factor H stating that “complement activation has been implicated in the pathogenesis of a number of complex traits including AMD and can arise through the classical, lectin or alternative pathways. All three pathways lead to the generation of a C3 convertase enzyme and subsequent activation of the immune response, the terminal pathway pore-like membrane attack complex, and cell lysis. The alternative complement pathway is spontaneously activated and CFH is an essential inhibitor preventing uncontrolled complement activation.

Components of the terminal complement pathway and other markers of inflammation are deposited in drusen and the choroid of eyes with AMD. Abnormal regulation of the alternative pathway of complement activation by CFH is consistent with these observations.”

Role of CFH deficiency in other pathologies

Dr Klein added that further findings indicated that “drusen of similar composition to that found in AMD are found in the eyes of patients with membranoproliferative glomerulonephritis type II”. He also noted that CFH deficiency can cause the kidney disorder. Such a finding suggests a clear link between disruption of the CFH gene and the deposition of drusen.

Finally nutritional supplementation of AMD patients with zinc has been shown to slow down the progression of AMD pathology. As biochemical studies have shown CFH function to be sensitive to zinc concentrations the

observation implies a link between AMD and correct functioning of the CFH gene.

In a commentary on the combined research findings reported in Science, Dr Stephen Daiger comments that “one of the promises of the human Genome Project was that it would provide tools for identifying genetic factors that contribute to common complex diseases such as cancer and diabetes. Finding these factors would in turn, suggest possible targets for drug therapy and other forms of treatment. Taken together the three new studies and pre-existing information on complement factor H suggest a direct causal connection between the polymorphic histidine allele and life time risk for AMD. As promised, the Human Genome Project provides powerful new insights into human diseases and raises many challenging questions.”

The Human Genome Project was an enormous international effort costing in excess of \$3 billion to complete. It is now hoped that this investment will begin to pay off and the beneficiaries of such investment will undoubtedly be future generations which may have the opportunity to avail of improved diagnostic tools and eventually gene therapeutic solutions that deal with the root cause of disease.

Glossary

Allele: alternative version of the same gene that can produce distinguishable biological differences.

Amino acid: the building blocks of proteins the order of which is directed by the sequence of nucleotides in a stretch of DNA.

As, Gs, Cs, and Ts: the abbreviation of (A) adenine, (G) guanosine, (C) cytosine and (T) thymine, the four constituent building blocks of DNA.

C3 convertase enzyme: a protein involved in the complement cascade.

Complement cascade: is a term referring to a cascade of biochemical reactions involving up to 30 proteins that function in immunity to cause the lysis of antibody coated target cells.

Complement factor H: a protein involved in the complement cascade.

Complement system: refers to the system of interacting proteins that carry out the immune function of destroying targeted cells.

Histidine: one of the 20 standard amino acids that form the building blocks of all proteins. The amino acid sequence of a protein is determined by the nucleotide sequence of DNA.

Human Genome Project: the multi-billion dollar international research effort that identified the more than 3 billion nucleotides that comprise the DNA sequence of human beings.

International Haplotype Mapping Project: is an international effort to identify and chart genetic differences and similarities between human beings from a broad range of ethnic backgrounds. For more details, see: <http://www.hapmap.org>.

Lectin: a protein found in both plants and animals that can contain binding sites for sugar molecules.

Linkage analysis: a general term for any one of several statistical methods of genetic analysis applied to family based samples in order to isolate a specific stretch of DNA with a specific biological trait such as a disease.

Linkage mapping: linkage refers to the proximity of two or more pieces of DNA on a chromosome, the closer together the pieces are the lower the probability that they will be separated upon cell division; a linkage map charts the distances between specific pieces of DNA on a chromosome as well as the order of those pieces; linkage mapping is the process through which the map is constructed.

Nucleotide: a subunit of DNA consisting of a nitrogenous base (adenine, guanosine, cytosine, or thymine), a phosphate group, and a sugar.

Polymorphic histidine allele: refers to the amino acid (histidine) which is polymorphic (has more than one allelic form) at the histidine DNA sequence.

Serum proteins: proteins found in the clear liquid that separates from blood on clotting.

Single base pair: refers to one set of two nucleotides on complementary DNA strands held together by hydrogen bonds.

Single nucleotide polymorphisms: a piece of DNA in which a single nucleotide pair differs in the DNA sequence of homologous chromosomes and in which each of the alternative sequences occurs relatively frequently.

Terminal complement pathway: the final pathway of the complement cascade.