

Введение в эволюционную и медицинскую геномику, часть II

Лекция 3

ФББ МГУ, весна 2008

Журнальный клуб 18 марта:

1. [Jimenez-Sanchez G, Childs B, Valle D.](#) Human disease genes. *Nature*. 2001 Feb 15;409(6822):853-5.
2. [Steward RE, MacArthur MW, Laskowski RA, Thornton JM.](#) Molecular basis of inherited diseases: a structural perspective. *Trends Genet*. 2003 Sep;19(9):505-13. Review.
3. [Di Rienzo A.](#) Population genetics models of common diseases. *Curr Opin Genet Dev*. 2006 Dec;16(6):630-6.
4. [Kryukov GV, Pennacchio LA, Sunyaev SR.](#) Most rare missense alleles are deleterious in humans: implications for complex disease and association studies. *Am J Hum Genet*. 2007 Apr;80(4):727-39.
5. [Levy S, et al.](#) The diploid genome sequence of an individual human. *PLoS Biol*. 2007 Sep 4;5(10):e254.

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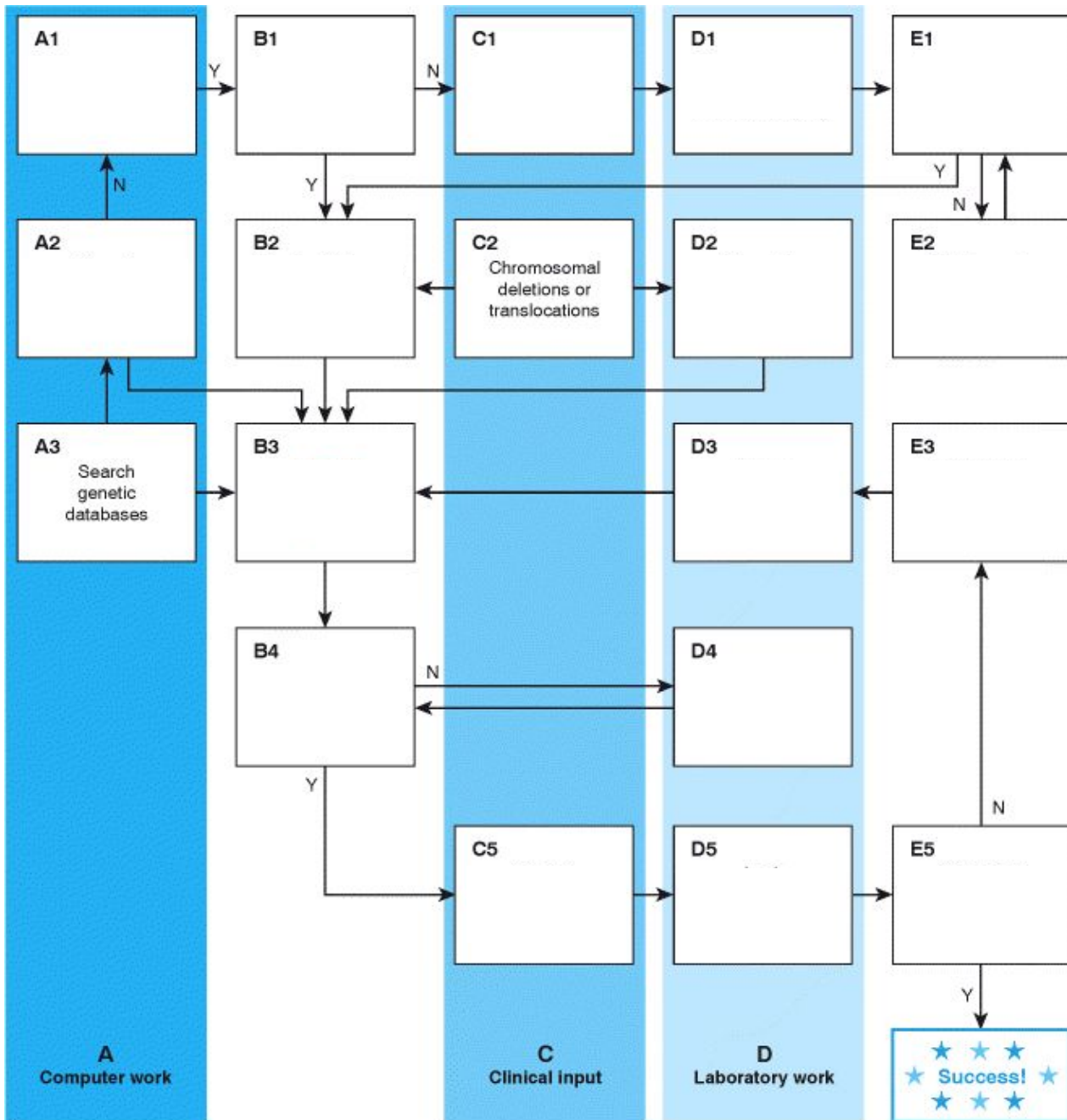


Figure 15.1. How to identify a human disease gene. There is no single pathway to success, but the key step is to arrive at a plausible candidate gene, which can then be tested for mutations in affected people.

“Human Molecular Genetics 2”,
Strachan and Read
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Стратегии поиска генов заболеваний

J. Pasternak. *An Introduction To Human Molecular Genetics - Mechanisms Of Inherited Diseases* (Wiley, 2005)

Functional/Candidate Gene Cloning: “Either a known protein that is responsible for an inherited disorder or a protein that is considered a likely candidate based on the symptoms and biochemistry of the disease” (Protein -> Gene seq -> Location, PCR, mutations, etc.)

Positional-Candidate Gene Cloning: “The disease gene is mapped to a chromosome location with polymorphic markers. Once the chromosome location is narrowed down, the human genome database is consulted for the genes within this region. From this list of genes, a likely candidate(s) is selected and a mutation detection assay is run with PCR probes based on the gene sequence derived from the database”

Сложные заболевания: примеры

- Аутизм
- Астма
- Диабет
- Ожирение
- Алкоголизм
- Гипертония
- Шизофрения

Особенности сложных заболеваний // Где тут легкий обман?

1. **Неполная пенетрантность** (Не всякая генетическая предрасположенность проявляется как болезнь)
2. **Различный возраст развития заболевания**
3. **Действие факторов окружающей среды** (Образ жизни, диета)
4. **Полигенное наследование, в т.ч. эпистатические взаимодействия**

Легкий обман: пп. 1-4. характерны также для моногенных заболеваний, так что остается

Сухой остаток: С.з. имеют наследственную компоненту, но наследуются более сложно, чем «по Менделю»

Human Molecular Genetics

Mechanisms of Inherited Diseases

Second Edition

Jack J. Pasternak
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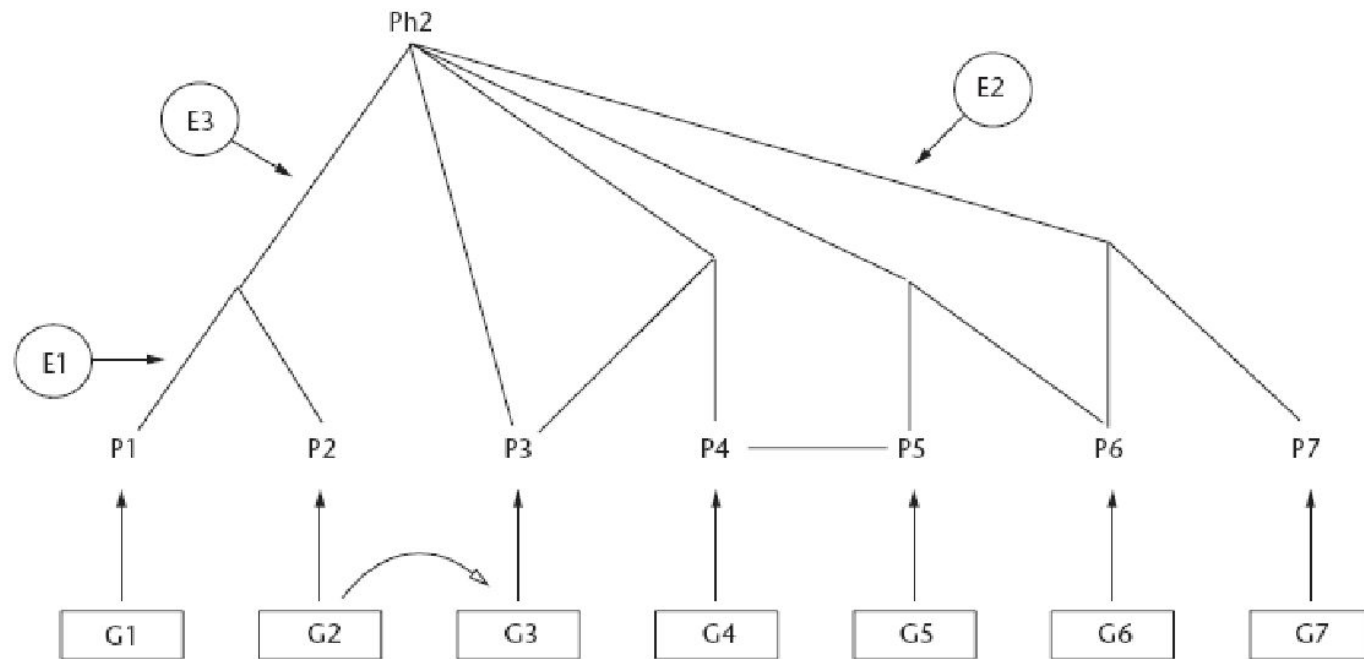


Figure 10.1 Schematic representation showing how phenotypes of a multifactorial trait are determined. (A) The primary products (P1–P7) of seven normal genes (G1–G7) form various protein–protein assemblies and with environmental factors (E1, E2) produce the Ph5 phenotype. The open arrow indicates interaction (epistasis) between genes 2 and 3. (B) The phenotypic outcome (Ph2) is due to the

Evolution revisited: упрощенный взгляд на болезнетворные аллели

Моногенные заболевания

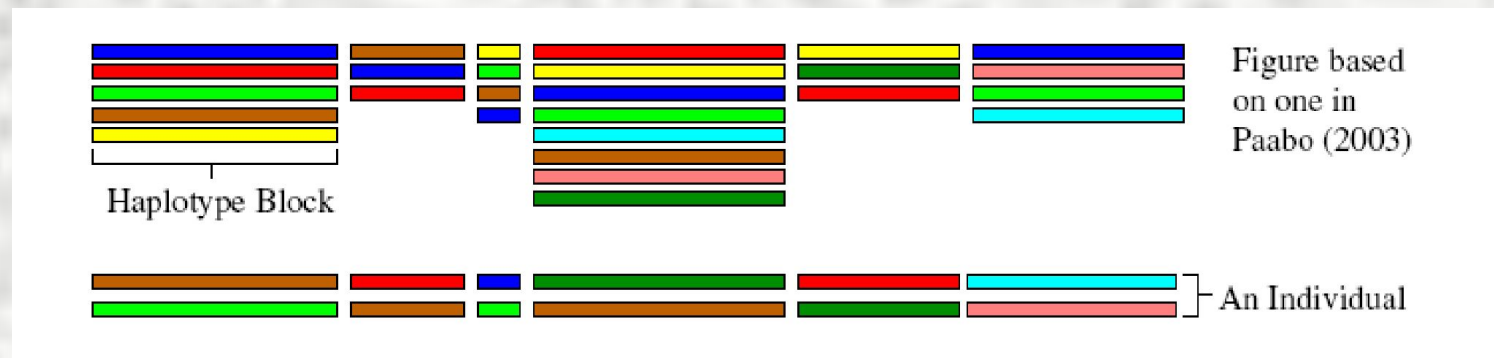
- Сильный эффект аллелей
- Мутационно-отборное равновесие

Сложные заболевания

- Слабый эффект аллелей => слабый отбор
- Давление отбора в некоторых случаях изменялось в ходе эволюции образа жизни и среды обитания

“Common disease / common variant”

CD/CV Hypothesis: Частые аллели (>1%) составляют значительную долю среди аллелей предрасположенности, и для их поиска применимы ассоциативные исследования, например, с помощью *НарМар**:



* Громкий проект 2002 г.

“Common disease / rare variant”

CD/RV Hypothesis: Редкие аллели (<1%) составляют основную долю среди аллелей предрасположенности, и нужно *ресеквенирование**

1000 Genomes - Mozilla Firefox
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http://www.1000genomes.org/index.html

1000 Genomes

A Deep Catalog of Human Genetic Variation

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INTERNATIONAL CONSORTIUM ANNOUNCES THE 1000 GENOMES PROJECT

Major Sequencing Effort Will Produce Most Detailed Map Of Human Genetic Variation to Support Disease Studies

An international research consortium has been formed to create the most detailed and medically useful picture to date of human genetic variation. The 1000 Genomes Project will involve sequencing the genomes of at least a thousand people from around the world. The project will receive major support from the [Wellcome Trust Sanger Institute](#) in Hinxton, England, the [Beijing Genomics Institute Shenzhen](#) in China and the [National Human Genome Research Institute](#) (NHGRI), part of the [National Institutes of Health](#) (NIH).

Drawing on the expertise of multidisciplinary research teams, the 1000 Genomes Project will develop a new map of the human genome that will provide a view of biomedically relevant DNA variations at a resolution unmatched by current resources. As with other major human genome reference projects, data from the 1000

LINKS

- Download the meeting report
- View the participants

* Громкий проект 2008 г.

Подтверждения гипотезы CD/CV ?

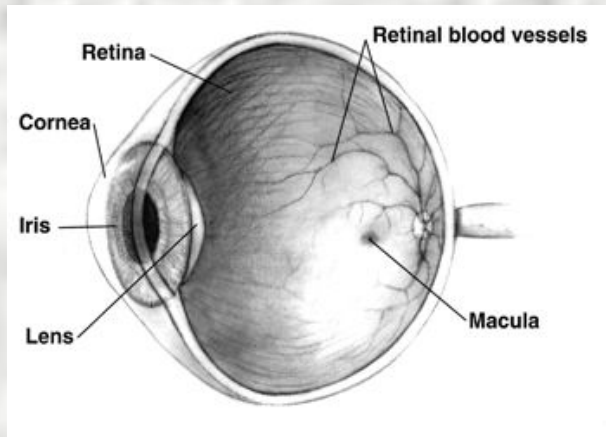
Table 3 • Some polymorphic, moderate to low risk variants

| Disease* | Locus | Change | Frequency | Relative risk |
|---------------------|----------------------|-----------------------------------|-----------------|---|
| Alzheimer's disease | <i>APOE-APOE4</i> | C112R | 0.09–0.22 | 4.0–15.0 |
| | <i>APOE-APOE2</i> | C158R | 0.04–0.08 | 0.5 |
| Thrombosis | factor V Leiden | R506Q | 0.00–0.08 (Eur) | 5.0–10.0 |
| Hemochromatosis | <i>Hfe</i> | H63D | 0.02–0.22 | 4.0 |
| NIDDM | <i>PPARγ</i> | P12A | 0.85 (Eur) | 1.25 |
| IDDM | <i>INS</i> | <i>VNTR</i> (promoter) | 0.85 (Eur) | 1.5–2.5 |
| HIV | <i>CCR5</i> | Δ32 | 0.01–0.14 (Eur) | high (resistance), moderate (nonprogression) |
| Crohn's disease | <i>NOD2/CARD15</i> | 1007fs | 0.02 (Eur) | 6.0 |
| | | G908R | 0.01 (Eur) | 6.0 |
| | | R702W | 0.04 (Eur) | 3.0 |
| Breast cancer | <i>BRCA2</i> | N372H | 0.25 (Eur) | 1.3 |
| Colon cancer | <i>APC</i> | I1307K | 0.03 (AJ) | 2.0 |
| Neural tube defects | <i>MTHFR</i> | C677T (A→V) | 0.30 (Eur) | 2.0 |
| | | A1298C (E→A) | 0.30 (Eur) | 2.0 |
| FMF | <i>MEFV</i> | P369S | 0.02 (AJ) | 7.0 |
| | | E148Q | 0.06 (AJ) | 3.0 |
| Graves disease | <i>CTLA4</i> | T17A | 0.35 (Eur) | 1.5–2.0 |
| Creutzfeld-Jakob | <i>PRNP</i> | M129V | 0.65 (Eur) | 3.0 |
| Autoimmune diseases | <i>HLA B, DR, DQ</i> | numerous amino acid substitutions | polymorphic | low to moderate |

*AJ, Ashkenazi Jews; Eur, European; FMF, familial Mediterranean fever; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus.

Age-related macular degeneration

Характерные образования в центральной части (macula) сетчатки, вызывающие нарушения зрения



Macular degeneration gene: The genes for the [complement system](#) The genes for the complement system proteins [factor H](#) (CFH) and factor B (CFB) have been determined to be strongly associated with a person's risk for developing macular degeneration. The mutation in CFH(**Tyr402His**) reduces the affinity of CFH for CRP and probably also alters the ability of factor H to recognise specific glycosaminoglycans.

Recurrence ratios for siblings of an affected individual are three- to sixfold higher than in the general population, but

Family-based analysis has resulted in only modestly significant evidence for linkage.

Age-related macular degeneration

J.Maller et al., Nat Genet (2006) 38(9):1055-9:

Генотипирование 1,536 tagSNPs у 1,238 пациентов и 934 контролей

Table 1 Association between *CFH* variants and age-related macular degeneration

| SNP | Allele | Control freq. | Affected freq | χ^2 | P value |
|------------------------|-----------------|---------------|---------------|-----------|------------------------|
| rs1061170 ^a | Риск: 7.6/2.7 C | 0.359 | 0.615 | 264.5 | 1.79×10^{-59} |
| rs1410996 | C | 0.571 | 0.808 | 272.9 | 2.65×10^{-61} |
| rs1061170, rs1410996 | | | | | 3.7×10^{-64} |
| | CC | 0.356 | 0.609 | Риск: ~15 | |
| | TC | 0.213 | 0.193 | | |
| | TT | 0.428 | 0.194 | | |

Two-letter allele symbols are used to indicate associated haplotypes of multiple SNPs.

^ars1061170 is the variant of *CFH* encoding the Y402H protein variant.

Table 2 Association between *LOC387715* variants and age-related macular degeneration

| SNP | Allele | Control freq. | Affected freq. | χ^2 | Nominal P value |
|------------|--------|---------------|----------------|----------|------------------------|
| rs10490924 | T | 0.194 | 0.455 | 315.075 | 1.71×10^{-70} |

Age-related macular degeneration

J.Maller et al., Nat Genet (2006) 38(9):1055-9:

Полиморфизм в локусе, содержащем гены системы комплемента *C2* и *CFB*, также ассоциируется с AMD:

- BF:R32Q + rs547154 (*C2*:intron10)
- BF:L9H + *C2*:E318D

Эти два редких протективных варианта принадлежат разным гаплотипам

Table 3 Association between *CFB* and *C2* variants and age-related macular degeneration

| SNP | Allele | Control freq. | Affected freq. | χ^2 | Nominal <i>P</i> value | <i>P</i> value conditional on rs9332739 | <i>P</i> value con |
|------------------------|--------|---------------|----------------|----------|------------------------|---|--------------------|
| rs9332739 ^a | C | 0.054 | 0.026 | 23.6 | 1.10×10^{-6} | X | 9 |
| rs547154 | A | 0.097 | 0.054 | 27.8 | 1.30×10^{-7} | 6.09×10^{-8} | |
| rs4151667 | A | 0.050 | 0.029 | 13.8 | 0.0002 | 0.0397 | 6 |
| rs641153 ^b | T | 0.102 | 0.045 | 51.9 | 5.5×10^{-13} | 2.99×10^{-13} | |

^ars9332739 is the variant of *C2* encoding the E318D protein variant, highly correlated to rs4151667 (BF:L9H). ^brs641153 is the variant of *BF* encoding the R32Q protein variant, highly correlated to rs547154.

Age-related macular degeneration

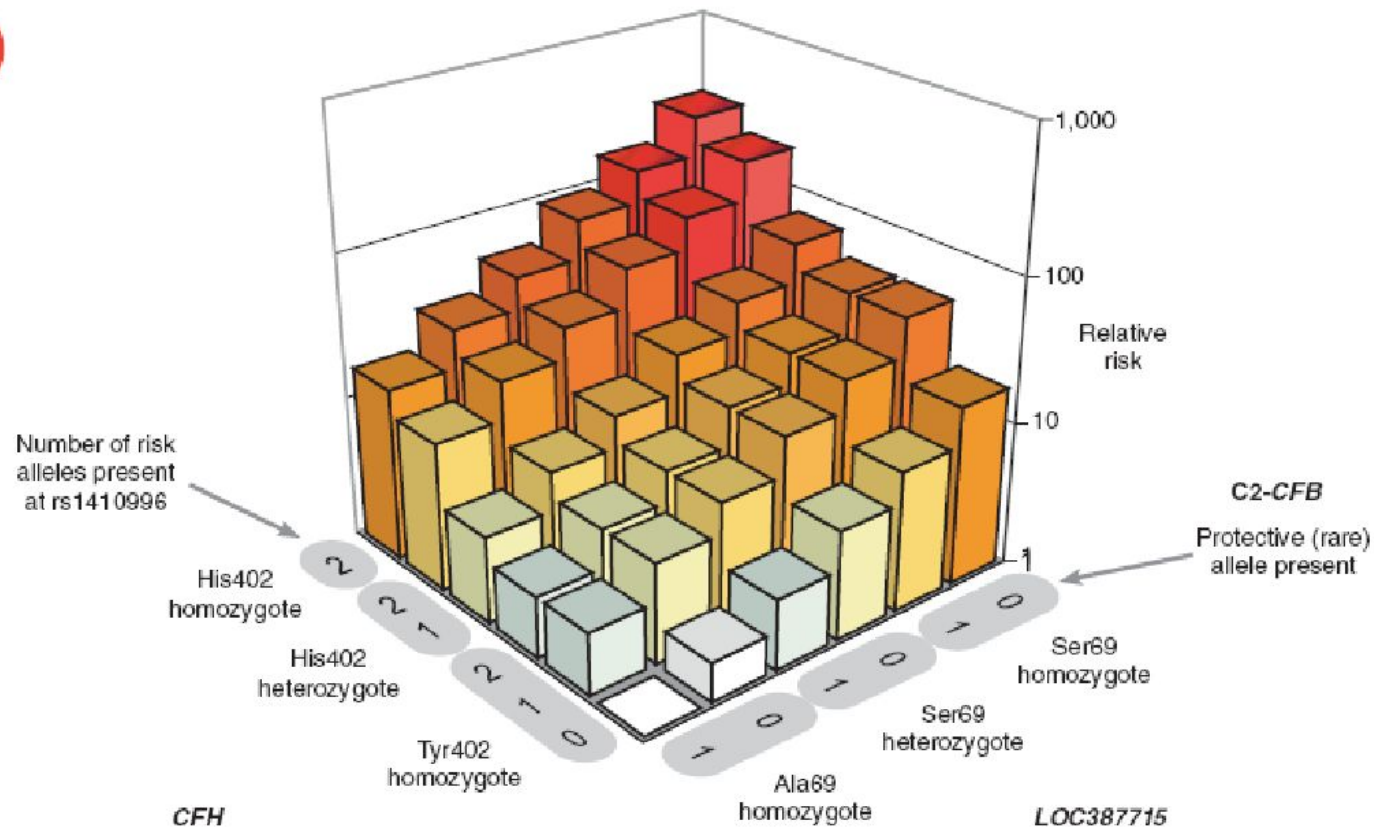


Figure 1 Relative risk plotted as a function of the genetic load of the five variants that influence risk of AMD. Two variants are in the *CFH* gene on chromosome 1: Y402H and rs1410996. Another common variant (A69S) is in hypothetical gene *LOC387715* on chromosome 10. Two relatively rare variants are observed in the *C2* and *BF* genes on chromosome 6. We find no evidence for interaction between any of these variants, suggesting an independent mode of action. (See also **Supplementary Table 4.**)

Age-related macular degeneration

Выводы:

- (i) there can **exist common alleles of substantial effect** on common disease (in AMD, these explain at least half of all risk to siblings);
- (ii) these can be found outside of 'candidate genes' and **outside of coding regions**;
- (iii) for such alleles, **association offers much greater power** and reproducibility than linkage (association results much stronger than similarly sized linkage studies);
- (iv) even for late-onset diseases with partial heritability, **common genotypes can strongly influence individual risk**;
- (v) there **need not be epistasis** among, or phenotypic sub-stratification by, genes of substantial population effect.

Влияние редких аллелей на уровень холестерина высокой плотности

Низкой уровень холестерина высокой плотности (HDL-C, «хорошего» холестерина): основной фактор риска сердечно-сосудистых заболеваний

Гомозиготы по некоторым мутациям в этих белках не имеют HDL-C вообще:

1. Apolipoprotein AI (APOA1), the major protein component of HDL;
2. The adenosine triphosphate binding cassette transporter A1 (ABCA1): efflux of cholesterol from cells to HDL particles;
3. Lecithin cholesterol acyltransferase (LCAT): catalysis of the formation of cholesteryl esters in HDL

Влияние редких аллелей на уровень холестерина высоко й плотности

The hypothesis: “rare sequence variations contribute significantly to low plasma levels of high density lipoprotein cholesterol (HDL-C)”

Results: “Of the 128 individuals with low plasma levels of HDL-C, 21 (16%) had sequence variants not present in the high HDL-C group. In contrast, only 3 (2%) of the individuals in the high HDL-C group had sequence variants not found in the low HDL-C group.

Thus, one of six individuals with HDL-C levels below the fifth percentile in the Dallas Heart Study had a rare mutation in *ABCA1* or *APOA1*”

Влияние редких аллелей на уровень холестерина высоко й плотности

Table 1. Sequence variations in the coding regions of *ABCA1*, *APOA1*, and *LCAT*. Values represent the numbers of sequence variants identified in 256 individuals from the Dallas Heart Study (DHS) (128 with low HDL-C and 128 with high HDL-C) and 263 Canadians (155 with low HDL-C and 108 with high HDL-C) (17). NS, nonsynonymous (nucleotide substitutions resulting in an amino acid change); S, synonymous (coding sequence substitutions that do not result in an amino acid change). GenBank accession numbers for DHS *ABCA1*, *APOA1*, and *LCAT* sequences are NM_005502, NM_000039, and NM_000229, respectively.

| | Sequence variants unique to one group | | | | Sequence variants common to both groups | |
|--------------|---------------------------------------|---|------------|---|---|----|
| | Low HDL-C | | High HDL-C | | NS | S |
| | NS | S | NS | S | | |
| | DHS | | | | | |
| <i>ABCA1</i> | 14 | 6 | 2 | 5 | 10 | 19 |
| <i>APOA1</i> | 1 | 0 | 0 | 1 | 0 | 1 |
| <i>LCAT</i> | 0 | 1 | 1 | 0 | 1 | 1 |
| | Canadians | | | | | |
| <i>ABCA1</i> | 14 | 2 | 2 | 3 | 7 | 5 |
| <i>APOA1</i> | 0 | 1 | 0 | 0 | 2 | 0 |
| <i>LCAT</i> | 6 | 1 | 0 | 0 | 0 | 0 |