Genome-wide association studies: progress and potential for drug discovery and development

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Abstract

Although genetic studies have been critically important for the identification of therapeutic targets in Mendelian disorders, genetic approaches aiming to identify targets for common, complex diseases have traditionally had much more limited success. However, during the past year, a novel genetic approach — genome-wide association (GWA) — has demonstrated its potential to identify common genetic variants associated with complex diseases such as diabetes, inflammatory bowel disease and cancer. Here, we highlight some of these recent successes, and discuss the potential for GWA studies to identify novel therapeutic targets and genetic biomarkers that will be useful for drug discovery, patient selection and stratification in common diseases.

Genetic factors are known to have an important role in many common diseases, and the identification of genetic determinants for such diseases has the potential to provide insights into disease pathogenesis, revealing novel therapeutic targets or strategies. Genetic factors could also provide useful biomarkers for diagnosis, patient stratification and prognostic or therapeutic categorization. In addition, given that inherited genetic factors are present at birth, knowledge of these factors could facilitate timely preventative or ameliorative interventions.

During the past 25 years, genetic linkage-based studies have proved very effective in identifying causal genetic factors in Mendelian (single gene) disorders; causal genes for more than 1,300 dominant and recessive Mendelian diseases have been identified[1]. Most common diseases and endophenotypes, however, do not exhibit Mendelian inheritance, but rather feature complex, multifactorial expression and inheritance. Although linkage-based methods have been broadly applied, these studies have had little success in identifying the allelic determinants of common disorders[2]. In particular, there has been poor replication among studies, whereby an initial study identifies an allele (genotype) with large estimated genetic effects (relative risk) but subsequent studies fail to corroborate the results[3,4]. In part, this reflects the dependence of linkage-based studies on unusually informative families (with multiple affected and unaffected individuals), which induce a bias toward rare, semi-Mendelian disease subsets in subpopulations. Reports of successful identification of genetic variants in

common diseases using an approach that circumvents this limitation — genome-wide association (GWA) studies — have therefore generated considerable excitement.

Human GWA studies are based on three hypotheses: First, the common trait/common variant hypothesis proposes that the genetic architecture of complex traits consists of a limited number of common alleles, each conferring a small increase in risk to the individual[5,6]; second, the brief history of most human populations precludes sufficient generations (or meioses) to create recombination events (or mutations) between closely located, common (ancient) variants; and, third, suppression of meiotic recombination (coldspots) occurs very frequently. Thus, approximately 80% of the human genome is comprised of around 10 kb regions that exhibit reduced recombination in human populations (haplotypes)[7]. Genetic variants (alleles) within haplotypes are in linkage disequilibrium (LD). This phenomenon enables much of the recombination history in a population to be ascertained by genotyping a large set of well-spaced, common (ancient) variants throughout the genome, especially if variant selection is informed by knowledge of haplotypes. During the last 10 years, more than 10 million single nucleotide polymorphisms (SNPs) have been identified[8]. Furthermore, the International HapMap project has genotyped approximately 4 million common SNPs (occurring with a minor-allele frequency of more than 5%) in human populations and has assembled these genotypes computationally into a genome-wide map of SNP-tagged haplotypes[7]. These resources, together with array technologies for massively parallel SNP genotyping and the well-established epidemiological case-control association studies have rendered GWA feasible (BOX 1, FIG. 1).

Initial genetic association studies focused on candidate loci and exhibited a lack of replication among studies[9,10]. There were biological explanations for inconsistent results: unobserved, confounding biological sources of heterogeneity, including inconsistent or poorly defined measurements of the phenotype, heterogeneous genetic sources for the phenotype (genocopies), population stratification (ethnic ancestry), population-specific LD, heterogeneous genetic and epigenetic backgrounds or heterogeneous environmental influences (phenocopies). In addition, there were statistical reasons for irreproducibility, including failure to control the rate of false discoveries, model misspecification and heterogeneous bias in estimated effects among studies[11−14]. Also, a frequent source of non-replication was lack of power due to the limited number of individuals genotyped and phenotyped[15,16].

In order to ameliorate poor replication, GWA experiments employ multi-tiered experimental designs with discovery, replication and biological validation stages[17] (FIG. 1). Tiered designs are critical for cost-effective detection of meaningful, hypothesis-generating, genotype–phenotype associations given the large number of comparisons involved, prior probability estimates of association, sample sizes, resampling procedures and statistical significance thresholds. GWA studies also owe their statistical power to their large cohort size and high rate of SNP detection. Currently, a respected threshold for uncorrected, significant associations is $P < 5 \times 10^{-7}$ (REFS [18,19]). Alleles with moderately less significant associations, however, are often also reported, as they might indicate loci that reach the aforementioned threshold in subsequent studies.

Results of initial GWA studies

The first GWA study, published in 2002, evaluated acute myocardial infarction (AMI)[20]. The discovery, or nomination, phase comprised the examination of genotype–phenotype association signals in 65,671 coding domain SNPs (cSNPs) in 752 cases and controls (TABLE 1). Although subsequent studies have used up to 20 times this number of non-coding SNPs, gene-tagging SNPs are more informative, as the majority of true-positive associations are expected to be with genes[1]. Even more informative are screens that employ functional cSNPs,

such as nonsynonymous SNPs (nsSNPs), that are candidate, causal (risk-enhancing) gene alleles[1,21−28]. The replication, or confirmatory, phase examined associations of 26 SNPs in 2,137 individuals and confirmed association of AMI with a 50 kb region containing lymphotoxin-α (*LTA*), nuclear factor of kappa light polypeptide gene enhancer in B cells (also known as *RELA*), nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor-like 1 (*NFKBIL1*) and human leukocyte antigen (HLA)-B associated transcript 1 (*BAT1*) genes. Additional replication studies have been undertaken, some of which have confirmed an association of this region with AMI-related phenotypes and, in particular, one nsSNP in *LTA*[29−35]. The association of *LTA* with AMI was an unexpected finding, suggesting a novel therapeutic target.

A second, pioneering GWA study examined age-related macular degeneration (AMD)[36] (TABLE 1). The discovery phase sought associations of 105,980 SNPs with AMD in 96 cases and 50 control individuals. Despite the small cohort size, SNPs in the complement factor H (*CFH*) gene, including an nsSNP, showed significant association with AMD. Replication was not performed, but subsequent studies have replicated associations of *CFH* alleles with AMD[37−40]. Of all common diseases examined by GWA to date, AMD is unique in that a single haplotype explains 61% of the genetic variance, conferring a homozygous odds ratio of 7.4. To put this in perspective, this is of a similar magnitude to the classic associations of *HLA-B27* with anterior uveitis/ankylosing spondylitis and *HLA* alleles with type 1 diabetes mellitus (T1DM). Complement pathway dysregulation was a novel, unexpected association with AMD. Subsequent studies have shown an association of AMD with two additional members of the alternative complement pathway (factor B (*CFB*) and *C3*)[41,42]. These findings, together with biological validation studies, have led to the initial development of new AMD therapies, based upon complement inhibition.

In the past year, technical challenges associated with GWA were largely overcome, genotyping costs were decreased and a significant number of studies have used SNP genotyping arrays in larger population groups to produce replicated associations between individual SNP alleles and common diseases.

Inflammatory bowel disease

Five large GWA studies have examined Crohn's disease and ulcerative colitis, two histologically distinct types of inflammatory bowel disease (IBD) (TABLE 1). Four of the studies used micro-arrays featuring between 300,000 and 400,000 SNPs[18,43−45], whereas the fifth study genotyped approximately 16,000 nsSNPs[24]. Two follow-up studies sought to replicate the most significant signals from the Wellcome Trust case control consortium (WTCCC) study[18], one in a European population and another in a Japanese population[46,47]. The European study replicated significant signals of the WTCCC study, but some of the alleles failed to reach significance in the Japanese study and others were not detected. The failure to replicate signals in different studies might reflect true differences between populations, differences in phenotype ascertainment or a lack of power.

Considering the six studies of European populations, there was significant replication of specific allele associations with Crohn's disease (TABLES 1,2). Three associations were concordant in four out of five studies (representing the genes caspase recruitment domain 15 protein (*CARD15,* also known as *NOD2*), interleukin 23 receptor (*IIL23R*) and ATG16 autophagy related 16-like 1 (*ATG16L1*)). Of note, *CARD15* had previously been identified as a susceptibility gene by linkage-based approaches[48,49]. One gene, prostaglandin E receptor 4 (*PTGER4*) showed association in two out of five studies. In addition, several disease-associated intergenic segments have been replicated. IBD susceptibility genes that have been identified to date appear to coalesce into biological networks involving innate immunity, autophagy and phagocytosis[50]. In addition, alleles of two genes associated with Crohn's disease (*IL23R* and

PTPN2) have shown association with other autoimmune disorders[21,51], suggesting the existence of autoimmune susceptibility 'supergenes'. There is great interest in alleles that exhibit pleiotropic associations, as they potentially represent blockbuster targets that cross-over therapeutic categories (TABLE 2).

In common with most GWA studies to date, estimated genetic effects (relative risks) of IBD-associated loci are small[18,46]. However, as many of these variants were common, the population attributable risk — an estimate of the percentage of cases of disease that would be avoided if the allele(s) were absent — was substantial. Of several studies that looked for epistatic interactions between IBD association signals, two found suggestive evidence of epistasis involving two different pairs of genes[24,52].

Diabetes mellitus

A good example of the capabilities and limitations of GWA studies is type 2 diabetes mellitus[18,53–57] (T2DM; TABLE 1). Two studies examined association both with SNPs and haplotypes in the discovery phase[54,57]. Haplotype-based analysis can be more powerful than marker-by-marker analysis in association studies[22,58–61]. For example, haplotypes can correlate a specific phenotype with a specific gene in a small population sample even when individual SNPs cannot[62]. Case-control and family-based association studies were employed in several studies of T2DM.

The replication phases of these studies were impressive; two of them included over 9,000 replication individuals[53,54]. One study sought to replicate signals identified by the WTCCC study[18] by genotyping the most significant SNPs; 9 of 77 candidate SNPs reached a $P < 5 \times 10^{-7}$ significance level[63]. The eight genes represented by these SNPs were replicated in at least one other independent study (TABLES 1–3). The concordance of T2DM-associated genes between GWA studies is striking: of 10 novel associations, only two were unique to a single study.

Reassuringly, some of the genes identified by GWA in studies of TD2M have previously been associated with the disease in other types of genetic studies. For example, transcription factor 7-like 2 (*TCF7L2*) had previously shown linkage to T2DM in the Icelandic population, and significant association in a candidate gene association study[64]. Heterozygous and homozygous carriers of *TCF7L2* risk alleles had relative risks of 1.45 and 2.41, respectively. TCF7L2 is a transcription factor that regulates the pro-glucagon gene in entero-endocrine cells[65]. *TCF7L2* alleles have also shown associations with endophenotypes such as a lower likelihood of response to the oral hypoglycaemic drug sulphonylurea[66] and increased risk of progression to T2DM among persons with impaired glucose tolerance[67].

In common with IBD, T2DM associations exhibited small estimated-effect sizes. Some of the candidate genes from GWA studies were consistent with biological processes that have previously been implicated in the pathogenesis of T2DM, such as pancreatic islet beta-cell function and insulin biosynthesis. However, these studies also suggested new components of these processes, such as zinc transport and Wnt-signalling[56,64,68]. Validation of T2DM candidate genes as therapeutic targets will require additional studies to identify causal susceptibility alleles and to determine their precise effect on cell biology.

Three studies performed initial modelling of how loci combine to affect susceptibility to T2DM[56,63,69]. One study found evidence of epistatic interactions between two genes. Otherwise, T2DM appeared to fit a polygenic threshold model with additive/multiplicative effects of individual loci. However, until the causal alleles that underpin these association signals have been found, it is not possible to make categorical statements about the allelic architecture of T2DM.

Frequencies of T2DM associated alleles showed considerable variation between ethnic and racial groups. Despite these differences, however, T2DM-associated risk alleles were conserved between independent populations, implying an ancient origin of these polymorphisms[70].

Expansion of an initial association of an allele with a categorical trait (such as the presence of a disease) with quantitative component phenotypes (endophenotypes) is an approach pioneered with apolipoprotein E (*APOE*) alleles in Alzheimer's disease. It appears to be highly instructive in elucidating the mechanism of action of alleles in disease pathogenesis. One T2DM GWA extended its analysis to a quantitative endophenotype: T2DM-related obesity (measured by body-mass index (BMI); TABLE 1)[54,71]. Alleles associated with T2DM in the fat-mass and obesity-associated gene (*FTO*)[18,55,63] also showed an association with BMI (TABLES 2,3). Association of *FTO* with obesity has since been confirmed[72].

Two GWA studies examined T1DM. One examined 6,500 nsSNPs[28] and the other evaluated 392,575 SNPs[18]. Four T1DM, susceptibility loci had previously been identified by linkage-based methods (class II MHC alleles, *CTLA4*, *PTPN22* and insulin). GWA studies replicated the association with *PTPN22* and identified several novel loci, including *C12orf30*, *KIAA0350* (also known as *CLEC16A*) and *IFIH1* (each replicated in two studies). Twenty-one T1DM candidate genes that have previously shown linkage or association are currently undergoing replication studies[73].

T1DM, like rheumatoid arthritis and IBD, is an autoimmune disorder. Medical practitioners have long noted familial aggregation of autoimmune diseases. One study showed association of both rheumatoid arthritis and T1DM with specific polymorphisms (*IL2RA*-rs2104286 and *PTPN22*-rs6679677; TABLE 2)[18]. T1DM, rheumatoid arthritis and IBD also show association with MHC alleles[74–76]. These findings suggest common underlying aetiological pathways (and therapeutic targets) for several, common autoimmune disorders[77].

Cancer

GWA studies of cancer based on common, inherited SNPs are useful for the identification of germ-line risk alleles, but not somatic mutations. Three GWA studies sought inherited association signals in prostate cancer[53,78,79]; FIG. 2 shows details of the discovery phase of one of these studies. An association signal at chromosome 8q24 that had previously been identified by linkage analysis[80] was replicated in two GWA studies[53,79]. In addition, these studies identified a second 8q24 association, approximately 300 kb upstream from the first. As yet, the functional basis of these associations is unclear. Although individual 8q24 alleles showed modest estimated genetic effects, the cumulative effect of several loci fit a multiplicative model that conferred a population-attributable risk (PAR), that is, an expected reduction in prostate-cancer incidence if the risk alleles did not exist in the population, of up to 68%[81]. As noted above, PAR values are strongly affected by allele frequency and represent only an approximate measure of the contribution of those alleles to disease incidence.

One study of prostate cancer[78] identified a *TCF2* (also known as *HNF1B*) susceptibility allele. Intriguingly, this allele appeared to diminish the risk of T2DM (TABLE 2), possibly representing antagonistic pleiotropy. This is supported by epidemiological evidence which suggests that diabetic men have a slightly lower prostate cancer risk than non-diabetic men[82].

Another allele exhibiting association in two diseases is rs6983267 at chromosome 8q24, which has shown replicated associations with prostate and colorectal cancer[79,82–84] (TABLE 2).

Three GWA studies sought inherited associations with breast cancer[19,85,86]. Although each study identified significant novel loci, two genes and one allele were each supported in two studies.

Complex traits

In addition to common diseases, GWA studies are applicable to complex traits. One study undertook GWA with numerous quantitative and categorical memory-associated endophenotypes[87]. Despite a small discovery cohort (341 individuals), associations with the *KIBRA* (also known as *WWC1*) gene have been replicated[87−89]. A notable innovation in this study was that associations were sought with multi-scale and multi-modality endophenotypes; that is, performance in seven memory-associated tests and functional magnetic resonance image-based measures of the hippocampus during three memory-associated tests. This study provides evidence that progress can be made in the elucidation of the genetic determinants of subjective, qualitative neurologic traits by using objective, quantitative, surrogate endophenotypes.

As well as identifying novel associations, GWA studies have confirmed several susceptibility genes that were previously established by linkage analysis in large pedigrees. For example, a GWA study of late-onset Alzheimer's disease (LOAD) identified the well-established *APOE*-susceptibility allele[90]. This association was also replicated in a study that genotyped 17,343 putative functional cSNPs[23].

A remaining problem with large GWA studies is the cost of genotyping, but one study provided evidence that sample pooling strategies might help to overcome this issue. In a GWA study of bipolar disorder, investigators created 39 pools, containing DNA from 2,672 individuals[91]. These pools were used for both discovery and replication experiments. Pools were individually genotyped for 555,235 SNPs and normalized allele frequencies were inferred from intensity data. Replicates were assayed for each pool. Thirty-seven SNPs showing allele frequency differences in both cohorts were individually genotyped and one SNP retained a significant association. The aforementioned WTCCC study also studied bipolar disorder, identifying an association at 16p12 (REF. [18]). One locus, for glutamate receptor, metabotropic 7 (*GRM7*), showed association in both studies.

The rate of publication of GWA studies continues to increase. Recent studies have investigated asthma[92], nicotine dependence[93], coronary artery disease[19,26], atrial fibrillation[94], prolonged QT interval and sudden cardiac death[95,96], coeliac disease[97], lung cancer[98], psoriasis[21] and liver cirrhosis[25], among others (TABLE 1).

Initial conclusions on the utility of GWA

The utility of GWA studies for the identification of novel genomic associations with complex diseases has unambiguously been established over the past year. In general, GWA studies have employed large case–control cohorts featuring both familial and sporadic cases, categorical trait definitions and up to half a million commonly polymorphic SNPs. To date, with the exception of *CFH* in AMD, the estimated genetic effects of replicated associations have been uniformly and surprisingly small.

Encouragingly, most associated haplotype intervals identified to date are sufficiently small to feature a single gene. In large measure, this reflects the use of several, outbred populations in confirmatory, fine-mapping studies. Even when the association is within a single gene, the predisposing variant might affect an adjacent gene, as in adult lactose intolerance[99]. Although some association intervals have been found to contain a single, unequivocally functional gene variant, the causality of alleles has been established in only a minority of cases. Causal alleles

identified to date do not yet show much difference in genetic mechanism from those identified in Mendelian disorders; this could reflect ascertainment bias[1,83,84].

Many genes identified by GWA were not candidate genes previously, highlighting the hypothesis-informing value of genetic studies. Already, there are examples of potentially tractable therapeutic targets that had not previously been considered in a disease or trait. As yet, the confluence of associated genes into biological networks and pathways is at an early stage. In part, this reflects scant or incorrect annotation of many genes. There appears to be a significant conservation of associations of common alleles between human populations. Thus, to date, it appears that GWA studies are fulfilling expectations with regard to the elucidation of molecular mechanisms underpinning poorly understood, common diseases.

In the few informative studies reported to date, endophenotypes have been highly instructive in dissecting the network or pathway that is perturbed by an individual allele, which affects a complex trait. It is particularly exciting to see the application of multi-mode endophenotypes, such as combinations of psychological testing, brain imaging and gene expression[87]. This is clearly an area of potential opportunity.

The cost of enrolling the very large cohorts that are needed to discover and validate alleles with small effect sizes has hitherto precluded the collection and integration of rich, accurate clinical metadata. It is likely that future studies will use a much greater stratification of traits than the phenotypically crude studies reported so far. Recent GWA studies of breast cancer provide a good example of the added genetic complexity that can be revealed by trait stratification[19, 85,86]. In addition, following replication of associations with categorical traits, it is anticipated that targeted genotypic examination of many endophenotypes will be highly instructive in the dissection of the role of individual alleles in disease pathogenesis.

GWA studies show significant potential to redefine disease classification. In some cases, GWA studies are identifying molecular factors that enable patient stratification and might prove useful in personalized medicine. Cancers provide the clearest examples of this to date. In other cases, exemplified by IBD, GWA studies are pointing to common molecular underpinnings in diseases that were believed to be distinct. In restless leg syndrome, replicated associations have provided concrete evidence that the phenotype represents a bona fide neurological disorder[100,101]. In mental illness, there is great anticipation that GWA studies will provide an objective, molecular revision of disease categorization.

Many questions remain concerning the genetic architecture of common diseases. These include the extent of locus and allelic heterogeneity, fit with an additive-threshold model or, alternatively, the extent of epistasis (the relative contributions of rare and common, and high and low, penetrance alleles) and various types of variation, from genome rearrangements to SNPs. GWA studies are not designed to evaluate these questions. Once loci have been identified, however, methods such as deep resequencing can nominate candidate susceptibility alleles and provide data for the evaluation of genetic architecture[102]. Meaningful, individual risk determinations will require the identification of causal alleles, the development of multiplexed molecular diagnostics and significant modelling.

Future developments and implications

The trends observed in recent GWA studies are anticipated to continue. Chips with 900,000 and 1,000,000 million SNPs were recently launched and genotyping accuracies have improved. Cohort sizes are steadily increasing and biobanks of unparalleled size and phenotype definition are being established. Combinations of genotype- and haplotype-based associations are becoming more prevalent. Experimental designs and statistical methods are also becoming more uniform, enabling more meaningful meta-analysis. In particular, the emergence of

adaptive designs and the use of Bayesian inferential methods will produce a probabilistic synthesis from combined analyses[83]. Importantly, this will provide an intuitive framework for combining information from multiple studies, resulting in more effective detection and replication of weak associations[103].

As noted above, phenotypes studied to date have been crude. The use of endophenotypes is expected to increase significantly. In particular, biomarker phenotypes are anticipated to become widely used. These will probably include gene expression, proteomic, metabolomic and imaging biomarkers. As determinants of complex traits are identified, genetic stratification will become possible, potentially reducing the genetic complexity of traits and enabling the identification of additional association signals. An example of this was the recent use of periodic limb movements and serum ferritin levels in GWA studies of restless leg syndrome[100]. An area of substantial future interest for the pharmaceutical industry will be pharmacogenetic GWA studies to identify markers for patient stratification in clinical trials. Comprehensive pharmacogenetic information will, in turn, facilitate the practice of personalized medicine. Pharmacogenetic GWA studies and early adoption of personalized therapy are likely to be used in the selection of expensive or chronic medications in life threatening conditions or where the therapeutic index is narrow or adverse event concerns are high, such as cancer chemotherapy.

Despite the current excitement, GWA studies have only been able to account for a small proportion of the expected genetic variance in complex traits[24,102]. This is not surprising given current limitations. First, current GWA studies are designed to identify common risk alleles that are predicted to be important in complex disorders under the common disease/common alleles hypothesis[5,6]. Increasing evidence suggests that some complex disorders and traits, such as schizophrenia, hypercholesterolaemia and body mass, are genetically heterogeneous[104–107]. The genetic basis of such diseases is more likely to conform to the common trait/rare variant hypothesis, which proposes that many rare variants exist, with substantial allelic heterogeneity at causal loci[58,108,109]. The GWA approach is unable to detect susceptibility loci that harbour numerous, individually rare (recent), polymorphisms. Instead, a resequencing approach will be needed to identify rare alleles. Encouragingly, massively parallel sequencing methods provide a potential solution[102,107,110], suggesting disease-specific rare alleles and recent mutations that provide supplementary genotyping array content. Second, a proportion of the genome cannot effectively be examined on the basis of tag SNP genotypes. Approximately 20% of the genome is comprised of recombination hotspots that are not amenable to LD-based approaches[7]. Alternatively, at recombination coldspots, haplotype blocks might be too large for unambiguous identification of causal loci. The extent of the effect of genomic copy number variation (CNV) on association signals is not yet clear, although recent genotyping arrays do provide CNV information. Insufficient numbers of cases will be available for GWA studies of many orphan diseases, uncommon disease complications or adverse events. For some common diseases, these considerations could obfuscate a substantial proportion of the genetic variance. Supplementation of genotyping array content reflective of CNV regions should, however, circumvent some of these limitations. Use of adaptive statistical methods and resampling strategies might also circumvent the need for thousands of affected individuals in studies of orphan diseases[83].

GWA successes are creating substantial need for downstream genetics, biochemistry and cell biology efforts to confirm the biological relevance of genotype–phenotype associations and to elucidate the underlying mechanisms of disease. This is especially true of association signals in gene deserts or alleles without apparent functional consequence. Translation of the fruits of GWA studies to clinical practice will require the derivation of predictive models of the genetic architecture of complex traits that evaluate with much greater precision the contributions of factors such as epistasis, genocopies, phenocopies and penetrance.

Box 1

Useful resources and databases for genetic-based studies

- Genetic Association Database: An archive of human genetic association studies of complex diseases. http://geneticassociationdb.nih.gov/

- Schizophrenia Gene Database: An archive of genetic association studies performed on schizophrenia phenotypes. http://www.schizophreniaforum.org/res/sczgene/default.asp

- Online Mendelian Inheritance in Man: A catalogue of human genes and genetic disorders. http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM&itool=toolbar

- Human Gene Mutation Database. A catalogue of published gene lesions responsible for human inherited disease. http://www.hgmd.cf.ac.uk/ac/index.php

- Human Genome Variation Database: A catalogue of normal human gene and genome variation. http://www.hgvbase.org/

- dbSNP: A catalogue of human single nucleotide polymorphisms. http://www.ncbi.nlm.nih.gov/projects/SNP/

- GeneSNPs: A database of polymorphisms in human genes that are thought to have a role in susceptibility to environmental exposure. http://www.genome.utah.edu/genesnps/

- PharmGKB: A database of pharmacogenomics research. http://www.pharmgkb.org/index.jsp

- GeneCards: A database of human genes that includes genomic, proteomic and transcriptomic information, as well as orthologies, disease relationships, SNPs, gene expression and gene function. http://www.genecards.org/

Acknowledgments

Glossary

Genetic linkage	Co-segregation (reduced recombination) of a trait and an allele in related subjects (pedigrees) more often than explicable by chance
Dominant	An allele that confers a trait even when it is heterozygous (present as a single copy in a genome)
Recessive	An allele that confers a trait only when it is homozygous (present in two copies in a genome, one from each parent)
Endophenotype	A measurable component of a phenotype
Multifactorial	Inheritance of a trait that is attributable to two or more genes and their interaction with the environment (also known as polygenic inheritance)
Allele	The DNA code at a given locus (position) on a chromosome

Genome-wide association study	A comprehensive search of the human genome for genetic risk factors for a trait by a case-control association study involving comparisons of hundreds of thousands of alleles between unrelated subjects with and without a trait
Haplotype	A combination of alleles at linked loci (on a single chromatid) that are transmitted together more often than explicable by chance
Linkage disequilibrium	(LD). Combinations of alleles in a population that differ in frequency from that expected from random formation of haplotypes from alleles based on their frequencies
Minor-allele frequency	The allele frequency of the less frequently occurring allele of a polymorphism
Case–control association study	Comparison of the frequency of an allele between unrelated subjects with and without a trait. A difference in allele frequency between the two groups indicates that the allele might change the likelihood of the trait
Genetic association	Correlation of a trait and an allele in a population more often than explicable by chance
Genocopy	A genotype at a locus that produces a phenotype that is indistinguishable from that produced by a genotype at another locus
Phenocopy	An environmentally produced phenotype that simulates the effect of a particular genotype
Non-synonymous SNP	(nsSNP). A SNP that leads to a change in the amino-acid sequence of the gene's resulting protein and that might therefore affect its function
Odds ratio	A measure of risk that compares the probability of occurrence of a disease in a group with a risk allele with the probability in a control group
Pleiotropy	A single gene that influences multiple phenotypic traits
Epistasis	Modification of the action of a gene by another gene
Family-based association study	Evaluation of the frequency of co-transmission of an allele and a trait from parents to offspring. Co-transmission of an allele and trait to offspring more often than expected by chance indicates that the allele might change the likelihood of the trait
Antagonistic pleiotropy	A single gene that influences multiple competing phenotypes such that beneficial effects of a trait created by the gene are offset by losses in other traits

References

1. Botstein D, Risch N. Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. Nature Genet 2003;33 (Suppl):228–237. [PubMed: 12610532]

2. Freimer N, Sabatti C. The use of pedigree, sib-pair and association studies of common diseases for genetic mapping and epidemiology. Nature Genet 2004;36:1045–1051. [PubMed: 15454942]

3. Goring HH, Terwilliger JD, Blangero J. Large upward bias in estimation of locus-specific effects from genomewide scans. Am J Hum Genet 2001;69:1357–1369. [PubMed: 11593451]
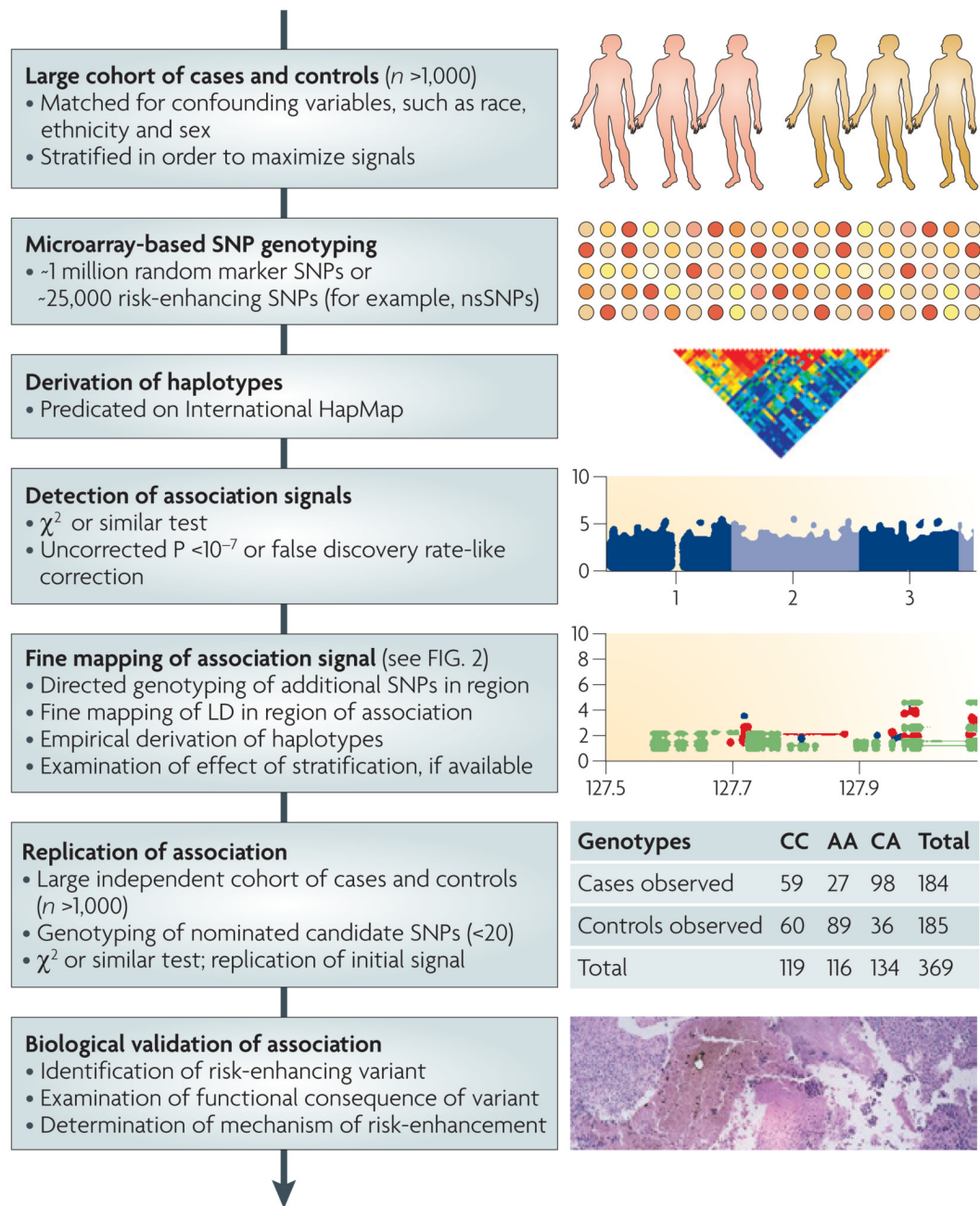
4. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nature Genet 1995;11:241–247. [PubMed: 7581446]

5. Chakravarti A. Population genetics — making sense out of sequence. Nature Genet 1999;21:56–60. [PubMed: 9915503]

6. Reich DE, Lander ES. On the allelic spectrum of human disease. Trends Genet 2001;17:502–510. [PubMed: 11525833]

7. The International HapMap Consortium. A haplotype map of the human genome. Nature 2005;437:1299–1320. [PubMed: 16255080]

8. Sherry ST, et al. dbSNP: the NCBI database of genetic variation. Nucleic Acids Res 2001;29:308–311. [PubMed: 11125122]

9. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. Genet Med 2002;4:45–61. [PubMed: 11882781]

10. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. Nature Genet 2001;29:306–309. [PubMed: 11600885]

11. Cardon LR, Bell JI. Association study designs for complex diseases. Nature Rev Genet 2001;2:91–99. [PubMed: 11253062]

12. Cardon LR, Palmer LJ. Population stratification and spurious allelic association. Lancet 2003;361:598–604. [PubMed: 12598158]

13. Redden DT, Allison DB. Nonreplication in genetic association studies of obesity and diabetes research. J Nutr 2003;133:3323–3326. [PubMed: 14608039]

14. Sillanpaa MJ, Auranen K. Replication in genetic studies of complex traits. Ann Hum Genet 2004;68:646–657. [PubMed: 15598223]

15. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nature Genet 2003;33:177–182. [PubMed: 12524541]

16. Risch NJ. Searching for genetic determinants in the new millennium. Nature 2000;405:847–856. [PubMed: 10866211]

17. Chanock SJ, et al. Replicating genotype–phenotype associations. Nature 2007;447:655–660. [PubMed: 17554299]

18. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661–678. The largest GWA study undertaken to date. [PubMed: 17554300]

19. Hunter DJ, et al. A genome-wide association study identifies alleles in *FGFR2* associated with risk of sporadic postmenopausal breast cancer. Nature Genet 2007;39:870–874. [PubMed: 17529973]

20. Ozaki K, et al. Functional SNPs in the lymphotoxin-α gene that are associated with susceptibility to myocardial infarction. Nature Genet 2002;32:650–654. The first large scale association study of a complex human disorder. [PubMed: 12426569]

21. Cargill M, et al. A large-scale genetic association study confirms *IL12B* and leads to the identification of *IL23R* as psoriasis-risk genes. Am J Hum Genet 2007;80:273–290. [PubMed: 17236132]

22. Clark AG, Li J. Conjuring SNPs to detect associations. Nature Genet 2007;39:815–816. [PubMed: 17597769]

23. Grupe A, et al. Evidence for novel susceptibility genes for late-onset Alzheimer's disease from a genome-wide association study of putative functional variants. Hum Mol Genet 2007;16:865–873. [PubMed: 17317784]

24. Hampe J, et al. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in *ATG16L1*. Nature Genet 2007;39:207–211. [PubMed: 17200669]

25. Huang H, et al. Identification of two gene variants associated with risk of advanced fibrosis in patients with chronic hepatitis C. Gastroenterology 2006;130:1679–1687. [PubMed: 16697732]

26. Luke MM, et al. A polymorphism in the protease-like domain of apolipoprotein(a) is associated with severe coronary artery disease. Arterioscler Thromb Vasc Biol 2007;27:2030–2036. [PubMed: 17569884]

27. Shiffman D, et al. Identification of four gene variants associated with myocardial infarction. Am J Hum Genet 2005;77:596–605. [PubMed: 16175505]

28. Smyth DJ, et al. A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (*IFIH1*) region. Nature Genet 2006;38:617–619. [PubMed: 16699517]

29. Clarke R, et al. Lymphotoxin-α gene and risk of myocardial infarction in 6,928 cases and 2,712 controls in the ISIS case-control study. PLoS Genet 2006;2:e107. [PubMed: 16839190]

30. Kimura A, et al. Lack of association between *LTA* and *LGALS2* polymorphisms and myocardial infarction in Japanese and Korean populations. Tissue Antigens 2007;69:265–269. [PubMed: 17493152]

31. Koch W, et al. Association of variants in the *BAT1–NFKBIL1–LTA* genomic region with protection against myocardial infarction in Europeans. Hum Mol Genet 2007;16:1821–1827. [PubMed: 17517687]

32. Laxton R, Pearce E, Kyriakou T, Ye S. Association of the lymphotoxin-α gene Thr26Asn polymorphism with severity of coronary atherosclerosis. Genes Immun 2005;6:539–541. [PubMed: 15973460]

33. Mizuno H, et al. Impact of atherosclerosis-related gene polymorphisms on mortality and recurrent events after myocardial infarction. Atherosclerosis 2006;185:400–405. [PubMed: 16054631]

34. Sedlacek K, et al. Lymphotoxin-α and galectin-2 SNPs are not associated with myocardial infarction in two different German populations. J Mol Med 2007;85:997–1004. [PubMed: 17497114]

35. Yamada A, et al. Lack of association of polymorphisms of the lymphotoxin α gene with myocardial infarction in Japanese. J Mol Med 2004;82:477–483. [PubMed: 15175864]

36. Klein RJ, et al. Complement factor H polymorphism in age-related macular degeneration. Science 2005;308:385–389. Discovery of a single variant that explains a large component of the genetic variance in a common human disease. [PubMed: 15761122]

37. Hageman GS, et al. A common haplotype in the complement regulatory gene factor H (*HF1/CFH*) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci USA 2005;102:7227–7232. [PubMed: 15870199]

38. Magnusson KP, et al. CFH Y402H confers similar risk of soft drusen and both forms of advanced AMD. PLoS Med 2006;3:e5. [PubMed: 16300415]

39. Souied EH, et al. Y402H complement factor H polymorphism associated with exudative age-related macular degeneration in the French population. Mol Vis 2005;11:1135–1140. [PubMed: 16379025]

40. Zareparsi S, et al. Strong association of the Y402H variant in complement factor H at 1q32 with susceptibility to age-related macular degeneration. Am J Hum Genet 2005;77:149–153. [PubMed: 15895326]

41. Gold B, et al. Variation in factor B (*BF*) and complement component 2 (*C2*) genes is associated with age-related macular degeneration. Nature Genet 2006;38:458–462. [PubMed: 16518403]

42. Yates JR, et al. Complement C3 variant and the risk of age-related macular degeneration. N Engl J Med 2007;357:553–561. [PubMed: 17634448]

43. Duerr RH, et al. A genome-wide association study identifies *IL23R* as an inflammatory bowel disease gene. Science 2006;314:1461–1463. [PubMed: 17068223]

44. Libioulle C, et al. Novel Crohn disease locus identified by genome-wide association maps to a gene desert on 5p13.1 and modulates expression of *PTGER4*. PLoS Genet 2007;3:e58. [PubMed: 17447842]

45. Rioux JD, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nature Genet 2007;39:596–604. [PubMed: 17435756]

46. Parkes M, et al. Sequence variants in the autophagy gene *IRGM* and multiple other replicating loci contribute to Crohn's disease susceptibility. Nature Genet 2007;39:830–832. [PubMed: 17554261]

47. Yamazaki K, et al. Association analysis of genetic variants in *IL23R*, *ATG16L1* and 5p13.1 loci with Crohn's disease in Japanese patients. J Hum Genet 2007;52:575–583. [PubMed: 17534574]

48. Hugot JP, et al. Association of *NOD2* leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001;411:599–603. [PubMed: 11385576]

49. Ogura Y, et al. A frameshift mutation in *NOD2* associated with susceptibility to Crohn's disease. Nature 2001;411:603–606. [PubMed: 11385577]

50. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448:427–434. [PubMed: 17653185]

51. Todd JA, et al. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nature Genet 2007;39:857–864. [PubMed: 17554260]

52. Raelson JV, et al. Genome-wide association study for Crohn's disease in the Quebec Founder Population identifies multiple validated disease loci. Proc Natl Acad Sci USA 2007;104:14747–14752. [PubMed: 17804789]

53. Gudmundsson J, et al. Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. Nature Genet 2007;39:631–637. [PubMed: 17401366]

54. Saxena R, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 2007;316:1331–1336. [PubMed: 17463246]

55. Scott LJ, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007;316:1341–1345. [PubMed: 17463248]

56. Sladek R, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 2007;445:881–885. [PubMed: 17293876]

57. Steinthorsdottir V, et al. A variant in *CDKAL1* influences insulin response and risk of type 2 diabetes. Nature Genet 2007;39:770–775. [PubMed: 17460697]

58. Liu PY, et al. A survey of haplotype variants at several disease candidate genes: the importance of rare variants for complex diseases. J Med Genet 2005;42:221–227. [PubMed: 15744035]

59. Morris RW, Kaplan NL. On the advantage of haplotype analysis in the presence of multiple disease susceptibility alleles. Genet Epidemiol 2002;23:221–233. [PubMed: 12384975]

60. Zhang K, Calabrese P, Nordborg M, Sun F. Haplotype block structure and its applications to association studies: power and study designs. Am J Hum Genet 2002;71:1386–1394. [PubMed: 12439824]

61. Zhang K, Sun F. Assessing the power of tag SNPs in the mapping of quantitative trait loci (QTL) with extremal and random samples. BMC Genet 2005;6:51. [PubMed: 16236175]

62. Drysdale CM, et al. Complex promoter and coding region β2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. Proc Natl Acad Sci USA 2000;97:10483–10488. [PubMed: 10984540]

63. Zeggini E, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 2007;316:1336–1341. [PubMed: 17463249]

64. Grant SF, et al. Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. Nature Genet 2006;38:320–323. [PubMed: 16415884]

65. Yi F, Brubaker PL, Jin T. TCF-4 mediates cell type-specific regulation of proglucagon gene expression by β-catenin and glycogen synthase kinase-3β. J Biol Chem 2005;280:1457–1464. [PubMed: 15525634]

66. Pearson ER, et al. Variation in *TCF7L2* influences therapeutic response to sulfonylureas: a GoDARTs study. Diabetes 2007;56:2178–2182. [PubMed: 17519421]

67. Florez JC, et al. Haplotype structure and genotype–phenotype correlations of the sulfonylurea receptor and the islet ATP-sensitive potassium channel gene region. Diabetes 2004;53:1360–1368. [PubMed: 15111507]

68. Helgason A, et al. Refining the impact of *TCF7L2* gene variants on type 2 diabetes and adaptive evolution. Nature Genet 2007;39:218–225. [PubMed: 17206141]

69. Weedon MN, et al. Combining information from common type 2 diabetes risk polymorphisms improves disease prediction. PLoS Med 2006;3:e374. [PubMed: 17020404]

70. Stephens JC, et al. Haplotype variation and linkage disequilibrium in 313 human genes. Science 2001;293:489–493. [PubMed: 11452081]

71. Frayling TM, et al. A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007;316:889–894. [PubMed: 17434869]

72. Dina C, et al. Variation in *FTO* contributes to childhood obesity and severe adult obesity. Nature Genet 2007;39:724–726. [PubMed: 17496892]

73. Rich SS, et al. The Type 1 Diabetes Genetics Consortium. Ann NY Acad Sci 2006;1079:1–8. [PubMed: 17130525]

74. Ahmad T, Marshall SE, Jewell D. Genetics of inflammatory bowel disease: the role of the HLA complex. World J Gastroenterol 2006;12:3628–3635. [PubMed: 16773677]

75. Orozco G, Rueda B, Martin J. Genetic basis of rheumatoid arthritis. Biomed Pharmacother 2006;60:656–662. [PubMed: 17055211]

76. Sia C, Weinem M. The role of HLA class I gene variation in autoimmune diabetes. Rev Diabet Stud 2005;2:97–109. [PubMed: 17491685]

77. Criswell LA, et al. Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the *PTPN22* 620W allele associates with multiple autoimmune phenotypes. Am J Hum Genet 2005;76:561–571. [PubMed: 15719322]

78. Gudmundsson J, et al. Two variants on chromosome 17 confer prostate cancer risk, and the one in *TCF2* protects against type 2 diabetes. Nature Genet 2007;39:977–983. [PubMed: 17603485]

79. Yeager M, et al. Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. Nature Genet 2007;39:645–649. [PubMed: 17401363]

80. Amundadottir LT, et al. A common variant associated with prostate cancer in European and African populations. Nature Genet 2006;38:652–658. [PubMed: 16682969]

81. Haiman CA, et al. Multiple regions within 8q24 independently affect risk for prostate cancer. Nature Genet 2007;39:638–644. [PubMed: 17401364]

82. Rodriguez C, et al. Diabetes and risk of prostate cancer in a prospective cohort of US men. Am J Epidemiol 2005;161:147–152. [PubMed: 15632264]

83. Knight JC. Regulatory polymorphisms underlying complex disease traits. J Mol Med 2005;83:97–109. [PubMed: 15592805]

84. Thomas PD, Kejariwal A. Coding single-nucleotide polymorphisms associated with complex vs. Mendelian disease: evolutionary evidence for differences in molecular effects. Proc Natl Acad Sci USA 2004;101:15398–15403. [PubMed: 15492219]

85. Easton DF, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007;447:1087–1093. [PubMed: 17529967]

86. Stacey SN, et al. Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. Nature Genet 2007;39:865–869. [PubMed: 17529974]

87. Papassotiropoulos A, et al. Common Kibra alleles are associated with human memory performance. Science 2006;314:475–478. [PubMed: 17053149]

88. Rodriguez-Rodriguez E, et al. Age-dependent association of *KIBRA* genetic variation and Alzheimer's disease risk. Neurobiol Aging. Aug 16;2007 10.1016/j.neurobiolaging.2007.07.003

89. Schaper K, Kolsch H, Popp J, Wagner M, Jessen F. *KIBRA* gene variants are associated with episodic memory in healthy elderly. Neurobiol Aging. Mar 10;2007 10.1016/j.neurobiolaging.2007.02.001

90. Coon KD, et al. A high-density whole-genome association study reveals that *APOE* is the major susceptibility gene for sporadic late-onset Alzheimer's disease. J Clin Psychiatry 2007;68:613–618. [PubMed: 17474819]

91. Baum AE, et al. A genome-wide association study implicates diacylglycerol kinase eta (*DGKH*) and several other genes in the etiology of bipolar disorder. Mol Psychiatry. May 8;2007 10.1038/sj.mp. 4002012

92. Moffatt MF, et al. Genetic variants regulating *ORMDL3* expression contribute to the risk of childhood asthma. Nature 2007;448:470–473. [PubMed: 17611496]

93. Bierut LJ, et al. Novel genes identified in a high-density genome wide association study for nicotine dependence. Hum Mol Genet 2007;16:24–35. [PubMed: 17158188]

94. Gudbjartsson DF, et al. Variants conferring risk of atrial fibrillation on chromosome 4q25. Nature 2007;448:353–357. [PubMed: 17603472]

95. Aarnoudse AJ, et al. Common *NOS1AP* variants are associated with a prolonged QTc interval in the Rotterdam Study. Circulation 2007;116:10–16. [PubMed: 17576865]

96. Arking DE, et al. A common genetic variant in the *NOS1* regulator *NOS1AP* modulates cardiac repolarization. Nature Genet 2006;38:644–651. [PubMed: 16648850]

97. van Heel DA, et al. A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21. Nature Genet 2007;39:827–829. [PubMed: 17558408]

98. Spinola M, et al. Genome-wide single nucleotide polymorphism analysis of lung cancer risk detects the *KLF6* gene. Cancer Lett 2007;251:311–316. [PubMed: 17223258]

99. Olds LC, Sibley E. Lactase persistence DNA variant enhances lactase promoter activity *in vitro*: functional role as a cis regulatory element. Hum Mol Genet 2003;12:2333–2340. [PubMed: 12915462]

100. Stefansson H, et al. A genetic risk factor for periodic limb movements in sleep. N Engl J Med 2007;357:639–647. [PubMed: 17634447]

101. Winkelmann J, et al. Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. Nature Genet 2007;39:1000–1006. [PubMed: 17637780]

102. Altshuler D, Daly M. Guilt beyond a reasonable doubt. Nature Genet 2007;39:813–815. [PubMed: 17597768]

103. Hunter DJ, Kraft P. Drinking from the fire hose — statistical issues in genomewide association studies. N Engl J Med 2007;357:436–439. [PubMed: 17634446]

104. Ahituv N, et al. Medical sequencing at the extremes of human body mass. Am J Hum Genet 2007;80:779–791. [PubMed: 17357083]

105. Cohen JC, et al. Multiple rare alleles contribute to low plasma levels of HDL cholesterol. Science 2004;305:869–872. An example of a phenotype that fits the common disorder: rare alleles hypothesis. [PubMed: 15297675]

106. Fanous AH, Kendler KS. Genetic heterogeneity, modifier genes, and quantitative phenotypes in psychiatric illness: searching for a framework. Mol Psychiatry 2005;10:6–13. [PubMed: 15618952]

107. McClellan JM, Susser E, King MC. Schizophrenia: a common disease caused by multiple rare alleles. Br J Psychiatry 2007;190:194–199. [PubMed: 17329737]

108. Pritchard JK. Are rare variants responsible for susceptibility to complex diseases? Am J Hum Genet 2001;69:124–137. [PubMed: 11404818]

109. Pritchard JK, Cox NJ. The allelic architecture of human disease genes: common disease–common variant…or not? Hum Mol Genet 2002;11:2417–2423. [PubMed: 12351577]

110. 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;80:727–739. [PubMed: 17357078]

111. Tomlinson I, et al. A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24.21. Nature Genet 2007;39:984–988. [PubMed: 17618284]

112. Zanke BW, et al. Genome-wide association scan identifies a colorectal cancer susceptibility locus on chromosome 8q24. Nature Genet 2007;39:989–994. [PubMed: 17618283]

113. Haiman CA, et al. A common genetic risk factor for colorectal and prostate cancer. Nature Genet 2007;39:954–956. [PubMed: 17618282]

Large cohort of cases and controls (*n* >1,000)
- Matched for confounding variables, such as race, ethnicity and sex
- Stratified in order to maximize signals

Microarray-based SNP genotyping
- ~1 million random marker SNPs or ~25,000 risk-enhancing SNPs (for example, nsSNPs)

Derivation of haplotypes
- Predicated on International HapMap

Detection of association signals
- χ^2 or similar test
- Uncorrected P <10^{-7} or false discovery rate-like correction

Fine mapping of association signal (see FIG. 2)
- Directed genotyping of additional SNPs in region
- Fine mapping of LD in region of association
- Empirical derivation of haplotypes
- Examination of effect of stratification, if available

Replication of association
- Large independent cohort of cases and controls (*n* >1,000)
- Genotyping of nominated candidate SNPs (<20)
- χ^2 or similar test; replication of initial signal

Genotypes	CC	AA	CA	Total
Cases observed	59	27	98	184
Controls observed	60	89	36	185
Total	119	116	134	369

Biological validation of association
- Identification of risk-enhancing variant
- Examination of functional consequence of variant
- Determination of mechanism of risk-enhancement

Figure 1. Overview of the general design and workflow of a genome-wide association (GWA) study
The discovery phase entails genotyping many case and control DNA samples and evaluation for significant associations. The replication phase involves fine mapping of association signals and independent confirmation in a second cohort. Biological validation is important for translation of GWA findings into diagnostic or therapeutic discoveries.
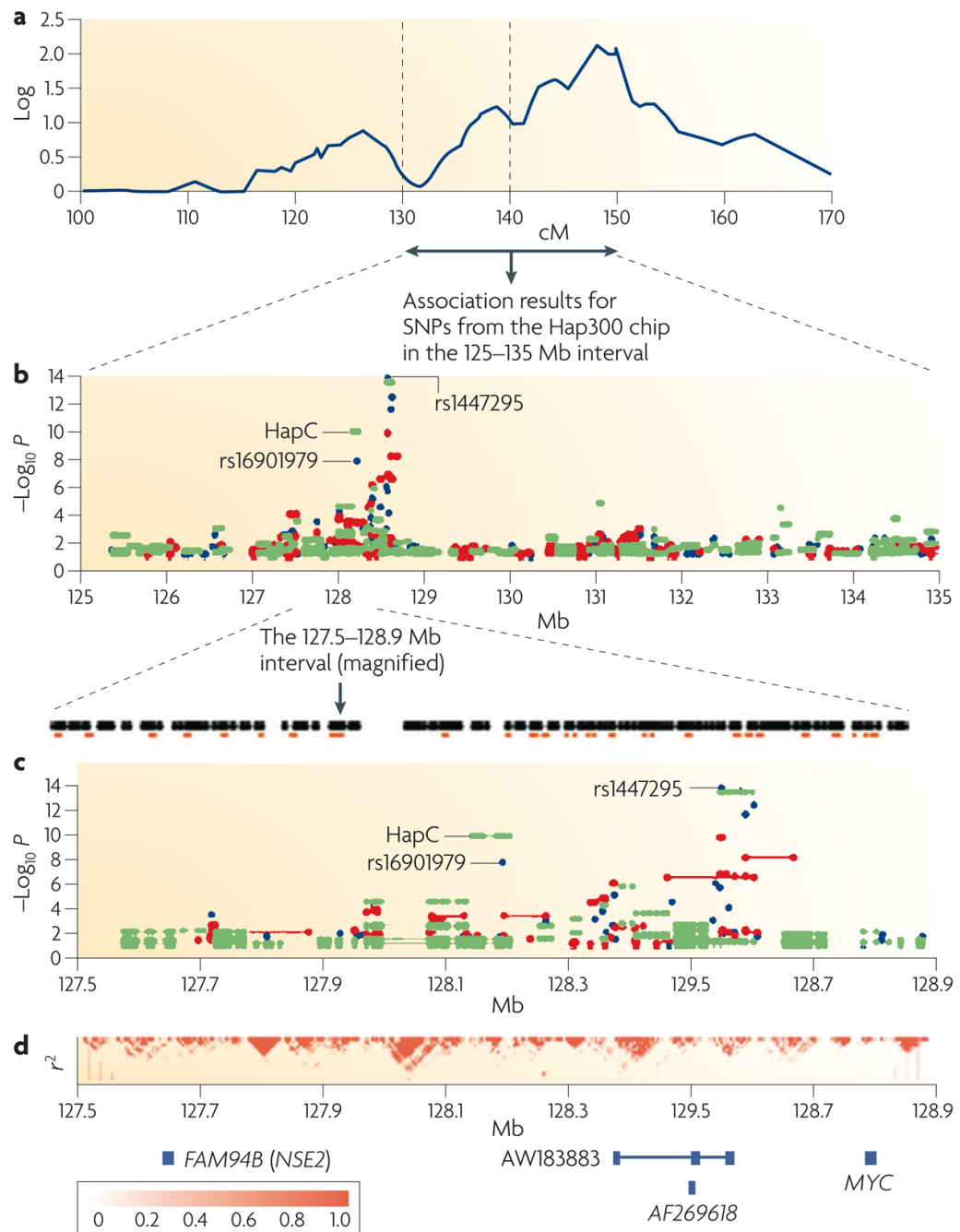
Figure 2. Schematic view of genetic linkage, GWA results, fine mapping and linkage disequilibrium structure in a region of chromosome 8q24.21 that demonstrates an association of rs1447295 and rs16901979 with prostate cancer

a | Previously reported genetic linkage scan results for chromosome 8, centiMorgans (cM) 100–170 (that is, 8q) from 871 Icelandic individuals with prostate cancer in 323 extended families. A quantitative trait locus (QTL) for prostate cancer susceptibility with log of the odds (lod) score of ~2 is shown. The interval between the two dashed horizontal lines corresponds to a previously reported admixture signal that is associated with prostate cancer. **b** | Genome-wide association (GWA) results for 1,660 single nucleotide polymorphisms (SNPs) mapping to chromosome 8 Mb 125–135 in 1,453 Icelandic individuals with prostate cancer and 3,064

controls. Association testing P values smaller than 0.1, corrected for relatedness and population stratification, are shown for single SNPs (blue circles), two SNPs (red circles) and linkage disequilibrium (LD)-block haplotypes (green circles). Four SNPs (including rs1447295) and three haplotype blocks (including Hap C, defined by 14 SNPs) show significant association signals (P $<1.58 \times 10^{-7}$). Single SNP association two-sided P values were derived using Fisher's exact test and were unadjusted for multiple comparisons. Association testing of haplotype block P values were carried out using the expectation-maximization (EM) algorithm directly for the observed data. **c** | Association results from **b**, shown in greater detail, for a 1.4 Mb interval on 8q24.21. Filled black circles represent 225 SNPs and the orange boxes represent recombination hotspots (calculated from the HapMap using the likelihood ratio test). **d** | LD between SNPs, measured by the square of the correlation coefficient calculated for each pairwise comparison of SNPs (r^2) from the Centre d'Etude du Polymorphisme Humain from Utah (CEU) HapMap population for the 225 SNPs in **c**; the blue boxes at the bottom indicate the location of the FAM84B, AF268618 and MYC genes and the AW183883 expressed sequence tag. Figure modified, with permission, from Nature Genetics REF. [53] © 2007 Macmillan Publishers Ltd.

Table 1

Discovery and replication designs of recent GWA studies

Disease	Discovery Phase			Replication Phase			Refs
	Number of individuals examined	Number of SNPs	Population	Number of individuals examined	Number of SNPs validated/tested	Population	
AMD	146	105,980	Caucasian	96	2/50	Same	36
Asthma	2,642	307,328	UK/German	2,320	0/9	German	92
Atrial fibrillation	5,026	316,515	Icelandic	17,810	2/18	Icelandic/European	94
Bipolar disorder	1,024 (pooled)	555,235	Western European	1,648	1/37	Same	91
Breast cancer	754	227,876	European	45,426	7/30	Same	85
	2,287	528,173	European	3,848	1/8	Same	19
	13,163	311,524	Icelandic	7,968	2/9	Various	86
Celiac disease	2,200	310,605	UK	2,480	5/27	Dutch/Irish	97
Colorectal cancer	1,890	547,647	Caucasian	23,121	2/18	Same	111
	2,593	99,632	Canadian	23,325	2/1,143	Same	112
Crohn's disease	1,923	304,413	European	2,150	4/37	Same	45
	1,103	16,360 nsSNP	German	2,670	3/72	Same	24
	1,475	302,451	Belgian	2,236	7/10	Same	44
IBD	1,095 + 834	308,332	European	2,885	10/27	Same	43
LOAD	1,086	502,627	Caucasian	ND	ND	ND	90
Lung cancer	673	116,204	Italian	621	0/1	Caucasian/Norwegian	98
Memory	341 (pooled)	502,627	Swiss	680	1/2	Several	87
AMI	752	65,671 cSNPs	Japanese	2,137	4/26	Same	20
Nicotine dependence	548 (pooled)	2,427,357	European	1,929	0/31,960	European	93
Obesity	4,862	490,032	British/Irish	29,596	1/1	Same	71
Prolonged QT interval	3,966	88,500	German	4,451	1/7	European	96

Disease	Discovery Phase			Replication Phase			Refs
	Number of individuals examined	Number of SNPs	Population	Number of individuals examined	Number of SNPs validated/tested	Population	
Prostate cancer	4,517	316,515 & 243,957 haplotypes	Icelandic	3,655	2/2	Several	53
	12,791	310,520	Icelandic	5,050	2/5	European	78
	2,339	550,000	European	6,266	2/2	Several	79
RLS	2,045	236,758	European	2,336	9/13	Same	101
	15,970	306,937	Icelandic	2,206	1/70	Icelandic/US	100
T2DM	2,335	315,635	Finnish	2,473	10/80	Same	55
	4,900	393,453	European	9,103	10/77	Same	63
	7,805	313,179 & 339,846 haplotypes	Icelandic/Danish	3,382	2/47	Same	57
	1,316	392,935	French	5,511	8/57	Same	56
T2DM and Triglyceride levels	2,931	386,731 & 284,968 haplotypes	Finnish/Swedish	10,850	3/107	Several	54
T1DM	3,388	6,500 nsSNPs	European	12,229	1/1	Same	28
Bipolar disorder, Crohn's disease, T2DM, T1DM, HT, RA, CAD*	4,868	392,575	UK	ND	ND	ND	18

AMI, acute myocardial infarction; AMD, age-related macular degeneration; CAD, coronary artery disease; HT, hypertension; IBD, inflammatory bowel disease; LOAD, late-onset Alzheimer's disease; ND, not determined; nsSNP, non-synonymous SNP; RLS, restless leg syndrome; RA, rheumatoid arthritis; SNP, single nucleotide polymorphism; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

*
There is overlap of the individuals genotyped in this study with REFS 58,63,80.

Table 2

Loci and variants associated with multiple diseases in GWA studies

Locus	Variant (rs)	Disease	Refs
PTPN22	6679677	RA	18
		T1DM	18
IL2RA	2104286	RA	18
		T1DM	18
PTPN2	2542151	T1DM	51
		CD	18, 46
TCF2	4430796	T2DM	53
		PC	53
FTO	9939609	T2DM	18
		Obesity	71
APOE	4420638	Triglyceride level	54
		Alzheimer's disease	90
8q24	6983267	PC	79, 81, 113
		CC	111–113
IL23R	11209026	CD	43
		Psoriasis	21

Above variants are associated $P < 5 \times 10^{-7}$. CC, colorectal cancer; CD, Crohn's disease; PC, prostate cancer; RA, rheumatoid arthritis; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

Table 3

Loci and variants exhibiting association with type 2 diabetes mellitus in GWA studies

T2DM phenotype	Locus	Variants (rs)	Refs
Susceptibility	TCF2	4430796, 7501939	78
	TCF7L2	4506565, 7903146, 7901695	18,54–57,63
	PPARG	1801282	55,63
	KCNJ11	5219, 5212	54,55,63
	SLC30A8	13266634, rs118253964	55,56,63
	HHEX	1111875	55,63
	IGF2BP2	4402960	54,55,63
	CDKAL1	9456871, 7754840, 10946398, 7756992	18,55,57,63
	CDKN2A/B	10811661, 564398	54,55,63
	Chromosome 11, intergenic	9300039	55
	FTO	9939609, 7193144, 8050136	18,55,63
Low-density lipoprotein	APOE	4420638	54
High-density lipoprotein	CETP	1800775	54
High-triglyceride level	LPL	17482753	54
	GCKR	780094	54

Above variants are associated $P < 5 \times 10^{-7}$. T2DM, type 2 diabetes mellitus.