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GENOME-WIDE CASE/CONTROL STUDIES IN HYPERTENSION: ONLY THE "TIP OF THE ICEBERG"

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Abstract

Recent advances in genome technology have enabled genome-wide searching for disease predisposition loci, using dense SNP and haplotype maps. Over the past year, such approaches have yielded positive results in human hypertension. Here we outline factors underlying the rationale for the approach, and consider reasons for false positive and negative results. While the approach has positive results, typically the trait-associated loci explain only a small fraction of the heritable fraction of trait variance. Finally, we consider alternative approaches and emerging strategies to probe the role of heredity in control of blood pressure.

Keywords

Hypertension; genomics; association

COMPLEX TRAITS: EMERGING GENOME-WIDE APPROACHES

Hypertension

Essential/idiopathic hypertension (HT), the most common cardiovascular disease, with a prevalence of >30% of adults[1], is a major risk factor for stroke, heart and renal disease. Family history data of affected individuals coupled with disease concordance rate in twins has established that both genetic and environmental factors determine susceptibility to HT. The heritability (h^2) of HT (and blood pressure [BP]) is typically estimated from twin and family studies in the range of up to ~50%, [2] likely with multiple contributory genes and even gene-by-gene interactions. Human population and animal studies have implicated several important physiological pathways contributing to the clinical presentation of essential HT that enable functional candidate gene association studies in addition to more comprehensive genome wide linkage or association studies.

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Genetic linkage

In families or sibling pairs, linkage mapping has been used to identify chromosomal regions (genetic loci) that co-segregate during meiosis with a phenotype. This approach is indirect and in most genome-wide studies has been used to identify only relatively large genomic regions that potentially harbor genes of interest; further efforts, including fine mapping and sequencing, are necessary to identify causal genes. Further shortcomings or challenges include the susceptibility of the linkage approach to locus heterogeneity (more than one causal gene), the possibility that the final phenotype is the net effect of genes that both raise and lower the BP, phenotypic heterogeneity arising both from phenocopies (multiple forms of the same disease with different etiologies), and uncertainty as to what constitutes an appropriate phenotype (blood pressure vs. hypertension). In late-onset diseases, additional ambiguity can arise when individuals with putative variant alleles develop the disease later in life or in a much milder form (incomplete penetrance). Finally, hypertension can result from gene-by-environment (GxE) interactions, and it may be more difficult to characterizing the heterogeneous environmental exposures, particularly lifetime exposures, than to assay genotypes. There is no way to easily identify or correct for such heterogeneities in complex disease such as hypertension, and therefore, linkage analysis is best suited for single-gene (Mendelian) disorders in which genes are more highly deterministic and the correlation between genotype and phenotype is robust[3]; indeed, linkage remains the “go-to” technique for investigation of traits that segregate in families in such as way as to suggest a major gene process with minimal environmental contribution, e.g., the monogenic hypertensions[4].

Genetic association

By contrast, genetic association correlates particular alleles, or diploid genotypes, with a trait. Association may be statistically more powerful than linkage for complex traits, especially in the setting of locus heterogeneity; however, the number of markers required to probe for association on a genome-wide (i.e., hypothesis-free) basis may be quite large (see below) [5, 6]. In the case of essential hypertension, while there have been numerous reports of associated genes, only a few of these findings have been replicated. Explanations for non-replicability might include: i) statistical significance criteria may have been improperly applied, e.g., no correction for multiple comparisons, with use of a nominal $p<0.05$ level leading to false positives; ii) differences between the populations studied arising from differences in environmental exposures and genetic background; iii) phenotypic misclassification; and/or iv) population stratification leading to false positive associations.

Genetic architecture of complex traits: Relative role of common (high frequency) versus rare (low frequency) susceptibility variants

A priori, there is substantial uncertainty about what kind of genetic variation (or “architecture”) might underlie complex human traits, including quantitative traits such as HT or BP[7, 8, 9]. While HT and BP have substantial heritability, ranging up to ~50% based on twin or family studies [10], whether the bulk of the contributing allelic variation to common genetically complex diseases consists of common variants with weak effects (CD/CV) or rare variants with individually stronger effects (CD/RV), is generally unknown. Emerging results from the HapMap/CD/CV approach suggest that, while common genetic variants can be identified, they may account for only a small proportion of heritable trait variance[11]. Testing the CD/RV hypothesis is challenging unless rare variants produce distinctive phenotypes, e.g., PHA-II (pseudohypoaldosteronism type2) [12], because even the latest technologies are probably yet insufficiently powerful for the task of uncovering novel rare allelic variants. The question of the contribution of rare variants to the population variation of BP remains open [9].

Genome-wide association (GWA) studies—The availability increasingly dense panels of variant alleles that can be typed with automated techniques in increasingly large numbers of people have led to the development of GWA approaches to complex traits. There are a number of advantages of GWA compared to linkage disequilibrium approaches, including the hypothesis-free nature of GWA (unconstrained by prior assumptions about biological pathways contributing to the trait) and the fact that the genomic regions harboring "hypertension" genes identified by GWA are smaller and more easily dissected by direct sequencing. Indeed, it will eventually be possible to perform GWA analyses of all genomic variants and directly assess the effect of each in genomically-defined subpopulations, an approach that may help to circumvent some of the problems noted above that arise from the "phenotype-based" approaches currently employed.

Current GWA approaches are exemplified by the International HapMap project[13–15] <www.HapMap.org>, which takes advantage of the effect of the historical meiotic recombination events that have fragmented the genome into stretches of DNA sequence now shared between related individuals. These "blocks" can be identified by the extent of linkage disequilibrium (or "LD"), i.e., the tendency of sufficiently nearby sequences to segregate together even during repeated meiotic recombination. The HapMap project[13] first quantified genome-wide LD relationships in several populations chosen for their geographic diversity, in order to facilitate the process of selecting a minimal set of markers that could (indirectly) capture the bulk of the signals from the un-typed (functional) markers during GWA. Depending on the degree of LD in a block, the genetic information contained in the genomic segment can be captured by a few (sometimes a single) genetic markers, which markedly increases the efficiency of interrogating the genome compared to genetically tagging every gene. It is the assumption of the HapMap approach that case-control studies can identify disease-predisposing alleles of sufficiently high (minor allele) frequency can be detected by a combination of LD within blocks and appropriately spaced common marker variants that span the many LD blocks within the genome. Since the typical extent of LD in unrelated humans varies by biogeographic ancestry group, ranging from ~3–10 kbp in blacks up to ~30–50 kbp in whites[16], a much denser set of variants (typically 500,000 SNPs) than that commonly used for linkage disequilibrium mapping (typically with an average spacing of ~3.3 $\times 10^9/400 = \sim 10^7$ bp (or ~10 Mbp) is necessary. Such dense SNP mapping array sets are now available in platforms developed by Affymetrix <http://www.affymetrix.com>, Illumina <http://www.illumina.com>, or Perlegen <http://www.perlegen.com>. Because of the very large number of relatively independent LD "blocks" within the genome that can now be interrogated with SNP assays, the threshold for statistical significance (in the face of multiple potential comparisons) must be adjusted downward, typically to the level of $p<5\times10^{-8}$, or even lower [13–15]. To augment confidence in such findings, replication of the result in an independent population sample is useful, taking advantage of the joint (or multiplicative) probability.

GWA RESULTS IN HYPERTENSION: GENOME WIDE SUCCESSES AND LIMITATIONS OF THE HAPMAP-BASED COMMON DISEASE/COMMON VARIANT (CD/CV) HYPOTHESIS

Prior to 2009

Wellcome Trust Case Control Consortium (WTCCC) [17]—In a large 2007 study of several common diseases, ~2000 HT cases and ~3000 unphenotyped (BP uncertain) shared population controls in the UK were subjected to GWA with Affymetrix 500K SNP chips, which yielded ~470K usable SNPs. The WTCCC study failed to yield significant ($p<5\times10^{-7}$) associations with HT. However, while unphenotyped population controls should not impair a case/control study for a rare disease, in a common trait such as HT, failure to phenotype the

population "controls" is likely to misclassify up to ~25% of such "controls. We elaborate on the effect of such misclassifications on the statistical power below. Even so, when the 6 variants with the highest significance ($p<10^{-5}$) in the WTCCC were typed in an independent sample of 11,433 phenotyped subjects from the NHLBI Family Blood Pressure Program), one variant (rs1937506, located in a ~500 kb gene "desert" on chromosome 13q21) achieved association (p as low as 0.004) in subjects of European and Hispanic ancestry[18]. Two of these 6 variants (rs6997709 and rs7961152) also displayed nominal associations with SBP or DBP in a Korean population sample of 7551 subjects[19]. Later in (2009) the WTCCC hypertension cases were included in a larger GWA study of hypertension (see below) that did identify potential causal variants[20].

Framingham Heart Study—In a 2007 report from the US NHLBI Framingham Heart Study, an initial SNP genome scan of >1000 individuals with a 100K marker Affymetrix SNP chip (yielding ~70K usable autosomal markers) was negative for hypertension [21], but as noted above this average SNP density (averaging ~30 kbp inter-marker distance) is probably insufficient to "tag" the majority of LD blocks across the human genome. Once again, at higher SNP density (~500K), the Framingham subjects contributed to a successful 2009 GWA for hypertension (see below)[22].

GWA studies in hypertension have yielded positive results only since January of 2009 [20, 22, 23]

Amish STK39—In early 2009, a hypertension GWA in an Amish population sample [23] discovered an association with STK39, a Ser/Thr protein kinase likely involved in control of ion transport in the distal nephron; replication in >7000 individuals lends credence to the result.

CHARGE and GlobalBPgen—In mid-2009, 2 reports involving >60K people of European ancestry genotyped for ~500K SNPs (on Affymetrix or Illumina platforms) appeared in Nature Genetics: the CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) and GlobalBPgen (Global Blood Pressure Genetics) consortia studies [20, 22]. The CHARGE consortium included 29.136 subjects, while GlobalBPgen included 34,433 subjects: both studies were primarily performed in subjects of European ancestry. These two exceptionally well-powered reports documented many novel SNP loci with significant and reproducible/replicated effects on BP and hypertension (CHARGE: 13 for SBP, 20 for DBP and 10 for hypertension; GlobalBPgen: 8 for SBP or DBP) [20, 22], although the odds ratio of each such locus on trait was very modest (typically <1.1:1), and the cumulative effects of the novel loci together explained perhaps ~1% of population BP variance [20, 22], while twin and family studies (such as our own) indicate that up to ~50% of BP variance is heritable [10].

Korea KARE—In the Korea Association Resource (KARE) project, 8842 subjects were analyzed for associations at ~350K SNP loci on the Affymetrix platform, with replication in 7641 independent samples[24]. Of note for the CHARGE and GlobalBPgen consortia (see above), KARE found an SBP association on chromosome 12q21 in the region of the Ca^{2+}-translocating ATPase *ATP2B1* (rs17249754, $p=1.3\times10^{-7}$), which had also been identified as a putative candidate in the CHARGE study.

African Americans—In a sample of 1017 African Americans genotyped at >800K SNP loci (Affymetrix 6.0 platform), genome-wide significance for SBP (though not DBP or the binary trait) was detected in/near 4 loci: PMS1, SLC24A4, YWHA7, IPO7, and CACANA1H[25]. Of note for the pathophysiology of hypertension, SLC24A3 encodes a sodium/potassium/calcium exchanger, while CACANA1H encodes the $alpha_1$ (pore-forming) subunit of the T-type voltage-gated Ca^{2+} channel.

Germany/Estonia/UK—This study began in southern Germany, with 1644 subjects genotyped at ~400K SNPs on the Affymetrix 500K platform, and progressed to replication in Estonia and the UK for a total of 8142 subjects. Investigators discovered an association with HT ($p=5.3\times10^{-8}$) for a region (tagged by rs11646213) on chromosome 16q23.3 upstream of the *CDH13* (T-cadherin) gene [26].

Caveats and limitations of the HapMap GWA approach: Fraction of trait variance explained by common haplotype-tagging genetic variants (estimated by the coefficient of determination, R^2)

The assumptions underlying the HapMap GWA approach have been subjected to extensive critiques[27], but success over the past 2 years in a variety of complex traits attest to the undeniable power of the method. Despite such successes [14, 15], it is becoming apparent that the HapMap/CD/CV approach is able to capture or explain only a very small % of common trait variability (as estimated by the coefficient of determination, or R^2) when applied to even highly heritable traits such as height and BMI. The general term of a "missing heritability" problem has been applied in this setting [28].

Height—A compelling example of this gap in our knowledge arises from the trait of height, for which twin and family studies have long established heritability (or the % of trait variability attributed to genetic variance) at up to ~90% [29]. However, while dense (~500K) SNP mapping identified contributions of up to 20 novel loci to height, the cumulative effect of these 20 loci explains only ~3% of population height variability[11].

BMI—A recent meta-analysis of genetic effects on BMI in the GIANT (Genetic Investigation of ANthropometric Traits) consortium[30], involving ~32,000 individuals genotyped at ~500K SNP loci, with replication in ~59,000 subjects, revealed association of 8 loci with BMI: 2 had been previously reported (FTO and MC4R) and 6 were novel (TMEM18, KCTD15, SH2B1, MTCH2, GNPDA2 and NEGR1). However, the cumulative effect of all 8 loci explained only ~0.84% of trait (BMI) variance, despite the fact that twin and family studies consistently reveal BMI to be a highly heritable trait, with heritability estimated at >80%[31].

BP/Hypertension—Given the poor performance of GWA studies in highly heritable traits, it is perhaps not surprising that even for GWA studies that have detected variants associated with SBP, DBP or hypertension, R^2 is low. As noted above, the CHARGE[22] and GlobalBPgen[20] consortia established and replicated the effects of up to 8 novel loci on BP. However, the cumulative effect of these 8 loci explained only ~1% of trait (BP) variance[22].

The relatively small aggregate R^2 values for genetic variants contributing to such traits (typically in the range of only <1–3%) are difficult to understand, given the substantially greater heritabilities (h^2) of such traits, where h^2 is the proportion of phenotypic variance accounted for by additive genetic variance, as estimated by family studies[32]. The lack of explanatory power for common genetic variants on such traits might suggest that initial CD/CV genetic discoveries might not be primarily useful for "personalized medicine", or predictions within an individual, but instead for discovery of new pathways for physiological or pharmacological exploration. For example, initial GWA results for hypertension[20, 22] suggest components of several previously unsuspected pathways to be operative in BP elevation. Thus additional approaches will be required to identify much if not most of the source of complex trait genetic determination (read below).

Why do GWAs "underperform" in hypertension and other "complex traits"?

Trait misclassification—McCarthy et al[14] have systematically considered the effect of trait misclassification genetic association studies. The study of such a common trait as HT may

render case/control approaches especially susceptible to diluting effects of misclassification, thereby basing the approach towards the null. Misclassification is a potentially critical problem in the WTCCC where 3000 *un*-phenotyped (no BP measurement) controls were drawn from a population with a high (>25%) prevalence of hypertension. While the use of unselected/un-phenotyped population controls is certainly a valid procedure for many diseases (especially those that are relatively rare), for a very common malady such as hypertension, such an uncharacterized control group may result in a substantial reduction in statistical power. In Figure 1, we re-estimated power for the WTCCC hypertension case/control study (Figure 1), using variance components tools[33] <http://pngu.mgh.harvard.edu/~purcell/gpc/>, and applied a spectrum of reasonable assumptions (disease allele relative risk; marker and disease allele frequencies; D' [index of linkage disequilibrium]) to the unselected population controls. Our analysis suggests that the WTCCC GWA study does not have adequate power to discover hypertension predisposition loci: even at an overly optimistic D'=1.0 (i.e., the marker allele *IS* the disease allele), statistical power (1-beta) declines to only <10%.

Common (relatively high minor allele frequency) variants as contributors to hypertension: Caveats—Biallelic SNP variants for HapMap GWA arrays are deliberately selected for relatively high minor allele frequencies, optimally >10–15%. Secondly, required sample size to detect the effect of a rare allele on a trait is necessarily greater than that for a common allele; the corollary is that any given sample size will demonstrate decreased power for detection of the rarer allele effect; for example, given an odds ratio (effect size) of 2.0 for a genetic variant, the statistical power to detect such a variant with a 30% allele frequency with 90% confidence would require a sample size (cases and controls) of ~1000, while a variant of similar effect but a 1% frequency would require a much larger sample size of ~20,000 ([34], Fig 2). As we discuss above for common variants, even when such approaches are successful in identifying trait-susceptibility loci, the fraction of trait variance explained is typically far less than the heritability. Assuming that the marker SNPs selected perform as expected, i.e., capture most of the important common variants contributing to hypertension, a likely conclusion is that less common (rare, at <1–5% minor allele frequency) alleles dominate complex trait determination. The previously cited examples of height[11], in which, despite trait heritability approaching ~90% that only ~3% of trait variation can be explained by CD/CV approach, as well as the corresponding examples of BMI (heritability of >80%, yet <1% of trait variance explained)[30] and BP (heritability approaching ~50% yet only ~1% of trait variance explained) [20, 22] strongly suggest that additional, perhaps less common genetic variation shapes trait determination. The role of rare variants has recently been confirmed for several common diseases (see below).

Local SNP-by-SNP: Haplotypes—Haplotypes are ordered sequences of genetic variants along a chromosome, such as SNPs that may capture information beyond that of each single SNP. A recent re-analysis of HT in the WTCCC revealed that the C-A-A haplotype at rs11632637–rs7182413–rs11037474 (on chromosome 15q26.2) is associated with hypertension at genome-wide significance ($p=2.8\times10^{-8}$) [35]. This compares to an association signal of lower significance (at $p=5.7–7.9\times10^{-6}$) that was previously detected in this chromosomal region in the WTCCC, using a single-SNP-at-a-time approach.

Genetic *interactions* (epistasis)—For complex and to date poorly understood traits such as HT, the genetic architecture of disease predisposition may not necessarily be monotonously limited to unifactorial marker-on-trait influences, but instead depend on gene-by-gene (or epistatic) interactions to affect phenotypic traits such as blood pressure. While such interactions are likely to exist, the introduction of one extra degree of freedom during marker-on-trait analyses may reduce statistical power to detect them.

One example of how to detect epistasis is the pathway analysis approach to the likely polygenic basis of common disease in the WTCCC GWA results, which identified several candidate pathways that were not apparent using standard single-SNP GWA statistical approaches[36]. For HT, even without genome-wide significant associations in the original WTCCC GWA, a network of pathways, many centering on dopaminergic transmission, was detected by analysis of the most strongly HT-associated SNPs. Apparent interconnection among these pathways suggests that multiple, but related, genetic mechanisms may underlie HT susceptibility. Understanding what pathways are etiologically important in hypertension may suggest intermediate phenotypes that can improve the utility of genotyping for identifying individuals at risk as well as approaches for tailoring antihypertensive therapy.

Gene-by-environment interactions—Ecological comparisons between Westernized and more traditional populations point to a necessary permissive role for environmental factors in the development of hypertension. Except for sodium, environmental factors promoting hypertension are poorly characterized in individuals studied to date in GWA studies. Our inability to account for the impact of environment on final phenotype introduces an element of variation which compounds that introduced by trait variation. In addition, the effects of the history of an individual's environmental exposure may affect the final phenotype even if the environmental factor is not present at the time the individual is studied. The impact of *in utero* nutritional state on adult blood pressure is one such example of the chronic effect of an early environmental factor[37].

PERSPECTIVES AND FUTURE DIRECTIONS

Some have declared that efforts to discover genetic variation underlying BP variation should now cease[38], and instead focus upon the existing GWA results of the CHARGE[22] and GlobalBPgen[20] consortia; however, other prominent voices argue strongly that, for complex traits, GWA has typically discovered only a small % of the genetic variance underlying the trait, in what has been termed the "missing heritability" (h^2), a problem extending to such diverse traits as height, BMI, dyslipidemia, type 2 diabetes, and early onset myocardial infarction[28]. Hence, new strategies may be required to move forward discovery in this setting.

Rare (relatively low minor allele frequency) genetic variants

As noted above, recent WGA studies have not identified common genetic variants with major phenotypic effects[20, 22]. Leading to the question of how much multiple *rare* alleles might contribute to variation in common traits such as BP. Even though "uncommon" variants are individually unusual, the relative risk (as estimated by the odds ratio) conferred by each such variant may be substantially higher than that for more common variants [9]. In an illustration of this strategy for hypertension, we re-sequenced a locus critical for catecholamine storage, chromogranin A (*CHGA*), and discovered a low (~3%) frequency functional variant, Gly364Ser (Figure 2), that had a profound effect on autonomic activity as well as risk for HT [46]. Similarly, Lifton et al [47] re-sequenced three genes subserving renal tubular salt reabsorption – *SLC12A3* (NCCT), *SLC12A1* (NKCC2) and *KCNJ1* (ROMK) – in subjects from the most extreme BPs in the population, and thereby discovered a series of rare, non-synonymous loss-of-function variants associated with lower BP [47]. Resequencing at candidate genes or pathways in individuals with extreme phenotypes has also revealed evidence for rare alleles that contribute to risk for obesity[48] and dyslipidemia[48–52].

This approach can only be currently implemented by re-sequencing across candidate loci in phenotyped individuals [9], or ultimately by "exome"-wide[39] resequencing enabled by the development of "exon capture" microarrays[54], although restriction to exons may miss numerous variants conferring quantitative changes in the amount of a gene product (rather than qualitative changes in sequence). For example, we have described roles for transcriptional-

regulatory variants in several genes contributing to the human sympathochromaffin phenotype and ultimately BP, including *CHGA*[40], *CHGB*[41], *SCG2*[42], *TH*[43], *DBH*[44], and *NPY1R*[45]. Eventually, genome-wide resequencing may be enabled by higher-throughput "next generation" sequencing platforms[53], and pilot projects utilizing these technologies are now being undertaken by the "1000 Genomes Project" international consortium <http://www.1000genomes.org>.

Phenotypes: Trait extreme values

Better phenotyped cases and controls should improve the yield of future GWA studies of HT. One strategy to maximize the efficiency of quantitative trait studies is to derive the sample(s) from the extremes of the trait distribution[55]. For example, we have conducted candidate genotyping in a community-based sample from the upper and lower 5th percentiles of BP among >50,000 people in a health maintenance program. This approach has >90% statistical power to detect genes contributing as little as 3% to trait (blood pressure)variation[56]. The trait-extreme approach to BP genetics has already yielded progress on the CD/RV front, providing evidence that multiple rare variants in renal salt transporters influence BP in the population[47]. Analysis of the trait as a continuous rather than dichotomous variable also enhances statistical power, and may also enable multivariate approaches to correlated traits[57].

Phenotypes: Intermediate traits

GWA analysis of "intermediate" (or "risk") phenotypes for HT, in addition to BP itself, should assist in defining the genetic roots of HT, for several reasons. Ideal properties of such traits include pathogenic potential, substantial heritability (h^2; the % of trait variance accounted for by genetic variance), and expression very early in the course of development of a complex disease trait such as HT [58]. Advantages of such traits may include: enhanced statistical power for marker-on-trait associations as a result of earlier trait penetrance with greater h^2; empirical partitioning of the heterogeneity within a complex trait into "endophenotypes"; and understanding the longitudinal course of disease trait development. Such approaches can best be implemented in twin or family studies, wherein relative pair correlations yield h^2 estimates[58].

Gene-by-environment (GxE) interactions

Studies in both humans [59] and rodents [60] indicate that organisms at genetic risk of developing hypertension display exaggerated cardiovascular responses to environmental stressors, thereby documenting the importance of GxE interaction in the development of hypertension. Indeed, GxE effects are likely to contribute to many (if not most) complex traits [61]. GWA approaches to hypertension thus far have not accounted for or incorporated such interactions, which would necessitate more extensive information on such environmental triggers as diet and stress. With introduction of the necessary one additional degree of freedom in analyzing GxE effects, statistical power to detect associations will fall, unless sample size is increased substantially[62]. Nonetheless, understanding such GxE effects may allow more rational introduction of environmental interventions in an attempt to prevent future occurrence of disease in at-risk individuals[63]. For example, in studies on twin pairs we have recently documented that the BP response to environmental stress is influenced by genetic variation at several points within the adrenergic pathway, including catecholamine biosynthesis (at tyrosine hydroxylase [*TH*][43] and DBH[44]), storage (at chromogranin A [*CHGA*][40, 64] or B [*CHGB*][41], and *SCG2*[42], or post-receptor signal transduction (at rho kinase [*ROCK2*] [65]. In addition, widespread genetic variation within the adrenergic pathway seems to influence the vascular response to environmentally-triggered increments in catecholamine release[66].

Additional approaches

Manolio et al[28] have summarized several additional potential areas for genomic exploration in complex traits, including exploiting the value of family studies, structural variants such as copy number variation (copy number polymorphism) and inversions, “epigenetics” or change in phenotype as a consequence of events other than DNA sequence variation (such as DNA CpG methylation, or chromatin remodeling), extension of GWAs to probes for causative variants with lower allele frequency, and systematic polymorphism discovery at loci already associated with the complex trait by GWAs. Finally, twin pairs[67] (including MZ twins) may present unique opportunities for uncovering GxE interactions[68].

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ABBREVIATIONS

BP	Blood Pressure
CD/CV	Common Disease/Common Variant
CD/RV	Common Disease/Rare Variant
GWAs	Genome Wide Association study
h^2	Heritability (% of trait variance accounted for by genetic variance)
HapMap	Haplotype Map Consortium
HT	Hypertension
LD	Linkage Disequilibrium
SNP	Single Nucleotide Polymorphism
WTCCC	Wellcome Trust Case Control Consortium

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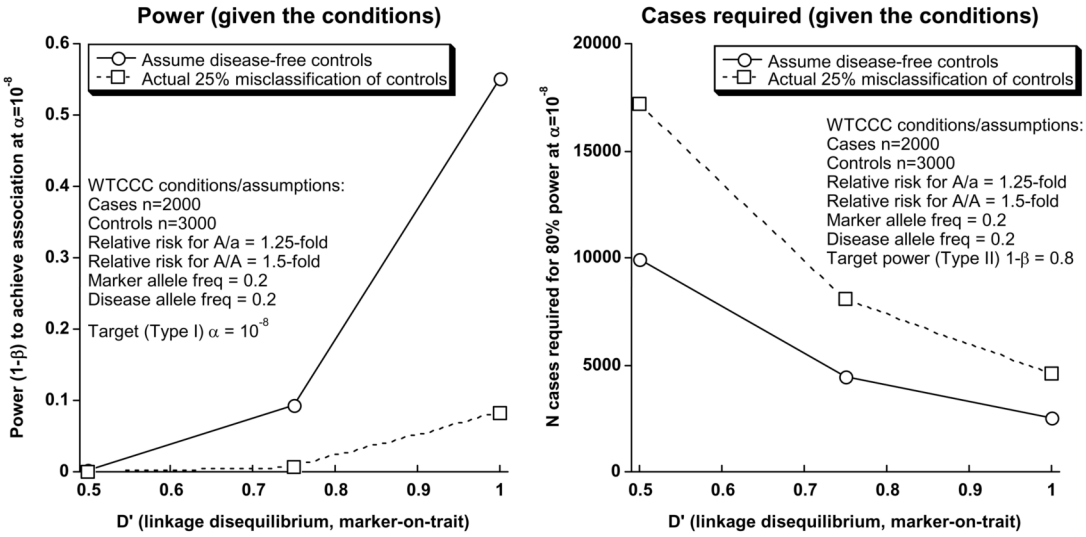

Figure 1.

Re-estimation of statistical power for hypertension in the Wellcome Trust Case Control Consortium (WTCCC). Computation of power used variance components tools, implemented at <http://pngu.mgh.harvard.edu/~purcell/gpc>, considering a spectrum of reasonable assumptions (disease allele relative risk; marker and disease allele frequencies; D' [linkage disequilibrium]), as well as the effects of the likely ~25% trait misclassification in the "control" group.

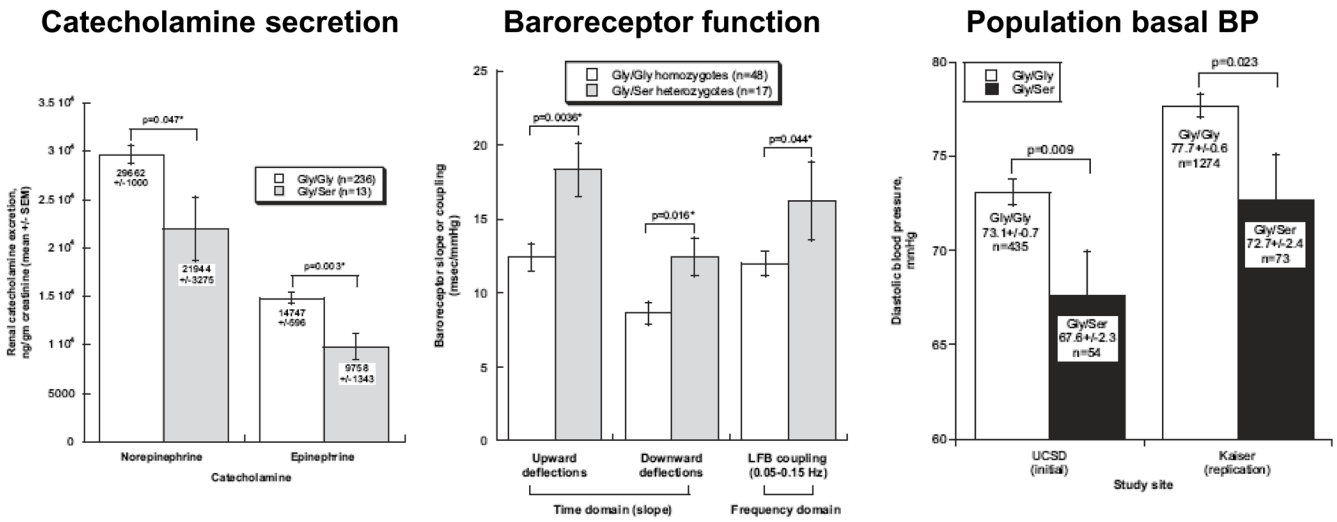

Figure 2. Common Disease/Rare Variant (CD/RV) hypothesis. Human *CHGA*/Catestatin Gly364Ser (~3% minor allele frequency)

Profound effects on autonomic function and BP *in vivo*. Left: Effects of Gly/Ser heterozygosity on catecholamine secretion. Center: Effects of Gly/Ser heterozygosity on autonomic function (baroreceptor sensitivity as measured in either the time or frequency domains). Right: Effect of Gly/Ser heterozygosity on resting DBP in the population, with replication in an independent sample. *CHGA* Gly364Ser is rs9658667. Reproduced from F Rao et al[46].