Predicting genotype-specific gene regulatory networks

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Abstract

Understanding how each person's unique genotype influences their individual patterns of gene regulation has the potential to improve our understanding of human health and development and to refine genotype-specific disease risk assessments and treatments. However, the effects of genetic variants are not typically considered when constructing gene regulatory networks, despite the fact that many disease-associated genetic variants are thought to have regulatory effects, including the disruption of transcription factor (TF) binding. We developed EGRET (Estimating the Genetic Regulatory Effect on TFs), which infers a genotype-specific gene regulatory network (GRN) for each individual in a study population. EGRET begins by constructing a genotype-informed TF-gene prior network derived using TF motif predictions, eQTL data, individual genotypes, and the predicted effects of genetic variants on TF binding. It then uses message passing to integrate this prior network with gene expression and TF protein-protein interaction data to produce a refined, genotype-specific regulatory network. We used EGRET to infer GRNs for two blood-derived cell lines and identified genotype-associated, cell-line specific regulatory differences that we subsequently validated using allele-specific expression, chromatin accessibility QTLs, and differential ChIP-seq TF binding. We also inferred EGRET GRNs for three cell types from each of 119 individuals and identified cell type-specific regulatory differences associated with diseases related to those cell types. EGRET is, to our knowledge, the first method that infers networks that reflect individual genetic variation in a way that provides insight into genetic regulatory associations that drive complex phenotypes.

EGRET is available through the Network Zoo R package (netZooR v0.9; [netzoo.github.io](https://github.com/netzoo/netzoo)).

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1 Introduction

The mechanisms by which disease-associated genetic variants exert their effects on phenotype remains an unanswered question. Genome-wide association studies (GWAS) have found that phenotype-associated genetic variants typically have modest effect sizes, generally lie outside of coding regions, and likely influence gene regulation [1, 2, 3, 4]. These findings are consistent with the observation that genetic variation in transcription factor (TF) binding sites and their flanking regions explains a substantial amount of the heritability of many diseases and complex traits [5]. What is lacking are methods that can effectively predict genotype-specific TF regulatory network structure, helping to explain the genotype-phenotype link at the level of the individual.

EGRET (Estimating the Genetic Regulatory Effect on TFs) is a method built on a simple premise: genetic variants that affect both transcription factor binding in a gene’s regulatory region and the expression of that gene should produce an alteration in an individual’s gene regulatory network (GRN). More specifically, such a variant should alter the edge weight connecting a TF and its target gene in a specific individual’s regulatory network if that person carries the variant. EGRET begins with an initial guess of TF-to-gene edges using TF binding motifs identified within the human reference genome. For variants lying within TF binding motifs in a gene’s promoter, EGRET determines whether the variants are likely to influence the binding of the TF and the expression of the gene. If a variant meets these criteria, then the initial estimate of the TF-to-gene edge is modified. EGRET then uses message passing [6] to integrate these modified edges with TF-TF interaction data and population-level gene expression information to construct individual-specific GRNs where the weight of an edge connecting a TF to a gene reflects the confidence that the TF regulates that gene.

We validated EGRET in two ways. First, we inferred and compared networks for two genotyped cell lines and found that predicted genotype-specific, differentially regulated genomic regions were enriched for genotype-affected chromatin accessibility, allele-specific expression, and differential TF binding as determined by ChIP-seq; each provided independent evidence that EGRET networks capture biologically relevant genetic disruptions in gene regulation. Second, we used EGRET to infer 357 individual and cell-type specific GRNs (three cell types, 119 individuals) and showed that EGRET networks captured cell-type specific, genetically influenced regulatory disruptions in relevant disease processes. Notably, these disease-related regulatory disruptions affected network modularity in different cell types, indicating that the effects of genetic variants extended beyond differential regulation of genes to the alteration of higher-order regulatory processes through changes in network structure.

These results show that EGRET can identify genetic changes that alter cellular functions and the causal role played by disease-associated variants. Because the only individual-specific data EGRET requires is genotype data, it can be used to understand genetic effects in many cohorts with SNP chip or whole genome sequencing data, such as TOPMED [7] and the UK biobank [8].

2 EGRET - Estimating the Genetic Regulatory Effects on TFs

2.1 The EGRET algorithm

EGRET uses several sources of information to capture the impact of genetic variants on TF-to-gene regulatory relationships and construct individual-specific GRNs (Tables 1 and S1, Figures 1A and S1). The first is a TF-to-gene reference motif prior network M derived, for example, from motif scans of a reference genome ([9], Supplementary Note S1) that estimates which TFs bind to promoter regions to regulate target gene expression. Second, EGRET requires eQTL data either from the study population or from a public database from the cell type of interest. Third, EGRET uses genotype information in the form of genetic variants of the individual(s) for which GRNs are being constructed, and fourth, EGRET uses predictions of the effect of these genetic variants on TF binding (Supplementary Note S2) to modify the reference motif prior M , producing a genotype-specific “EGRET prior” E .

Specifically, for each input genotype, EGRET selects SNPs (A in Figure 1B) that (1) are within motif-based TF binding sites in the promoter regions of genes, and (2) have a statistically significant eQTL association (β in Figure 1B) with the expression of the adjacent gene. EGRET then uses QBiC [10] to identify SNPs within TF motifs that significantly affect TF binding (q in Figure 1B), thus selecting genetic variants in each individual that are predicted to affect both gene expression and TF binding. The effect of a SNP s on TF i ’s regulation of gene j is then defined as the product $|q_{s_{ij}} A_{s_{ij}} \beta_{s_{ij}}|$. Modifier weights to the reference motif prior are calculated by adding these effects per TF-gene pair, allowing for the fact that a gene might have more than one variant in its promoter region affecting the binding of a particular TF. The EGRET prior network E is constructed by subtracting the modifier from the reference motif prior:

$$E_{ij} = M_{ij} - \sum_s |q_{s_{ij}} A_{s_{ij}} \beta_{s_{ij}}|$$

thus penalizing the reference motif prior when the individual in question contains a genetic variant with sufficient evidence to suggest it may alter gene regulation (Figure 1B, Supplementary Note S3). This EGRET prior, E , is then combined with two additional inputs (Supplementary Note S4). The fifth input to EGRET is a TF-TF interaction network (P) derived from protein-protein interactions, that reflects the fact that TFs can form complexes through protein-protein interactions to cooperatively regulate expression. Lastly, EGRET uses gene expression data to calculate a co-expression matrix C under the assumption that genes which are co-regulated are likely to exhibit correlated expression.

EGRET then uses a message passing network integration framework introduced previously [6] to search for regulatory consistency among the E , P , and C matrices (Supplementary Note S5). This message passing process updates all three input matrices, boosting those relationships that show agreement between associations captured in E , P , and C while down-weighting others. Upon convergence, the primary output is an individual-specific, complete, bipartite GRN (E^*) that captures genotype-specific regulatory effects. EGRET repeats this process separately using genotype information for each individual, producing a collection of individual-specific genotype-informed GRNs (Figure 1C). These networks can then be examined to identify features that are unique to specific genotypes, are associated with particular phenotypic states, or both. It is important to note that EGRET GRNs E^* are complete graphs,

meaning that an edge exists between all TFs and genes considered. However, it is the edge weights that indicate the strength of the relationship between the respective TFs and genes, with a higher weight indicating a higher likelihood of a regulatory relationship.

It is also worth noting that the data for M , P , C , as well as the eQTLs can be obtained from publicly available resources. Thus, one can construct an EGRET network for a given cell type in an individual of interest simply by providing the genotype information for that individual and relying on publicly available data (for example, databases such as the Genotype Tissue Expression Project - GTEx [11]) for the remaining model inputs.

2.2 Regulatory disruption scores

EGRET inferred edge weights can be used to quantitatively estimate the predicted regulatory effects produced by SNPs on a given gene, TF, or TF-gene relationship (Table S2). A higher edge weight between a TF i and a gene j is interpreted as a higher confidence that the TF binds the promoter of and regulates the expression of gene j . To assess the effects of SNPs on gene regulation, we define and calculate three different regulatory disruption scores for nodes and edges in a given genotype x (Figure 1D). The *edge disruption score* $d_{x_{ij}}^{(E)}$ quantifies the extent to which a TF-gene regulatory relationship is disrupted by genetic variants. The *gene disruption score* $d_{x_j}^{(G)}$ assesses the extent to which a gene has disrupted regulation due to genetic variants in its promoter region. The *TF disruption score* $d_{x_i}^{(TF)}$ is a measure of the extent to which a TF's genome-wide regulation is disrupted by genetic variants. These scores are defined per edge/node in each genotype-specific EGRET network by comparing it to a baseline network constructed using no genotype information and applying message-passing to M , P , and C (instead of E , P , and C):

$$d_{x_{ij}}^{(E)} = |E_{x_{ij}}^* - B_{ij}^*|$$

where $E_{x_{ij}}^*$ denotes the weight of edge ij in the EGRET network for individual x and B_{ij}^* is the edge weight for edge ij in the baseline network predicted without using genotype information. This score quantifies the extent to which edges are disrupted by variants in a given individual-specific network (E^*) compared to a baseline genotype-agnostic regulatory network (B^*).

Similarly, TF disruption scores $d_{x_i}^{(TF)}$ and gene disruption scores $d_{x_j}^{(G)}$ are calculated by taking the sum of edge disruption scores around the specific TF or gene in question:

$$d_{x_i}^{(TF)} = \sum_j |E_{x_{ij}}^* - B_{ij}^*|$$

$$d_{x_j}^{(G)} = \sum_i |E_{x_{ij}}^* - B_{ij}^*|$$

It is worth noting that disruption scores are all greater-than or equal to zero and that a higher edge disruption score corresponds to a larger difference between the EGRET and baseline edge weights for a particular TF-gene edge. However, because of the manner in which the EGRET prior is created - by penalizing edges involving a TF motif which contains an eQTL variant with a significant negative QBIC

effect - only regulatory disruptions are modeled, and not the creation of “new” regulatory relationships where none potentially existed before.

3 Applications of EGRET

3.1 EGRET finds regulatory differences between two genetically distinct cell lines

We tested whether EGRET could distinguish genotype-specific patterns of gene regulation by analyzing two blood-derived cell lines, GM12878 and K562. We chose these cell lines because high quality genome sequences are available for both cell lines [12, 13] providing high confidence variant calls; this was especially useful for K562 because the cell line is aneuploid [13]. In addition, both cell lines have had relatively large numbers of TFs mapped by ChIP-seq (110 TFs for GM12878 and 204 TFs for K562 in the ReMap 2018 database [14]), allowing us to use differential TF binding as a way of validating regulatory differences.

To build genotype-specific EGRET priors (E) for GM12878 and K562, we generated a reference motif prior M using FIMO [9] identifying TF motifs in the promoter regions of genes ($[-750, +250]$ relative to transcription start sites) and modified this using eQTL data for lymphoblastoid cell lines (LCLs) from GTEx [11], the cell lines’ respective genotypes, and SNP effect predictions from QBiC (Supplementary Notes S1-S3). Comparing edges in E against M predicted 1,520 genotype-altered prior edges for GM12878 and 1,182 for K562 (Figure S2) out of a total of 39,690,052 possible edges.

Next, we used the TF-TF interaction data as used by Sonawane and colleagues [15] to construct P , and LCL gene expression data from GTEx to construct C (Supplementary Note S4). Genes and TFs with low expression, defined as having non-zero values in < 50 samples, were filtered out. Performing message passing between E , P , and C produced the final genotype-specific EGRET networks E^* for GM12878 and K562 (Supplementary Note S5). For comparison, we constructed a baseline PANDA GRN using M as input to the message passing with P and C . We calculated the edge disruption score for each TF-gene pair in each cell line’s EGRET network. Because of the relatively small number of genotype-altered edges in the EGRET priors, the majority of edge disruption scores are very close to zero in both cell lines (Figure S3).

For each cell line, we compared both EGRET’s predictions of TF binding and the baseline PANDA networks to an empirical network based on ChIP-seq data [14] (Supplementary Note S6.1). At multiple cut-offs for the edge disruption scores, EGRET networks outperformed the baseline network prediction of TF binding for variant-disrupted edges (Tables S3 and S4, Supplementary Note S6.2). Based on these analyses, we considered variant-impacted scores to be those at or above 0.35 and a “high” disruption score to be anything at or above 0.5.

To capture changes in the disruption score between different genotype-specific networks, we calculated a “regulatory difference score” $R_{ij}^{(E)}$ (Table S2) for each edge between genotypes GM12878 (g) and K562 (k), defined as:

$$R_{ij}^{(E)} = \left| d_{gij}^{(E)} - d_{kij}^{(E)} \right|.$$

The magnitude of this score is the difference in edge disruption scores between GM12878 $d_{gij}^{(E)}$ and K562 $d_{kij}^{(E)}$ and reflects the assumption that genetic differences between cell lines will cause differences in predicted regulatory TF-gene interaction strength. A high value of $R_{ij}^{(E)}$ suggests a difference in the edge disruption scores for the edge $TF_i - G_j$ between the two cell lines, which is interpreted as that relationship being disrupted in one cell line but not the other. Conversely, a value of $R_{ij}^{(E)}$ close to zero indicates that the edge disruption scores for the edge $TF_i - G_j$ are similar and therefore that the regulatory relationship is either disrupted in both cell lines or remains unaltered in both cell lines.

We again used the cell line-specific ChIP-seq regulatory networks (Supplementary Note S6.1) to construct a *differential ChIP-seq regulatory network* by taking the absolute value of the difference between the GM12878 ChIP-seq network and the K562 ChIP-seq network. This allows us to assign a score of 1 to edges which show differential TF binding (a TF binds the promoter region of a gene in one cell line but not the other) and a score of 0 to edges which show the same pattern of TF binding (a TF either binds the promoter region of a gene in both cell lines, or neither). This scoring allows us to validate the framework modeled by the regulatory difference score $R_{ij}^{(E)}$. We found that edges with high differential regulation scores $R_{ij}^{(E)}$ were enriched for edges showing differential TF binding in the differential ChIP-seq regulatory network (Fisher p-value = 2.4e-226, T-test p-value = 2.296e-07).

We highlight two examples of genotype-specific promoter binding of TFs identified through the EGRET network analysis. First, the edge between the TF RELA and the gene SLC16A9 (ENSG00000165449) has a regulatory difference score of 6.099744, with $d_{gij}^{(E)} = 0.000256$ in GM12878 and $d_{kij}^{(E)} = 6.1$ in the K562. These scores suggest that the binding of RELA to the promoter region of SLC16A9 is disrupted in K562, but not in GM12878. The positions of eQTLs, genetic variants, and ChIP-seq binding regions for RELA in both genotypes (Figure 2A) indicate that an eQTL variant is present in the promoter region of SLC16A9 (purple track in Figure 2A), is associated with the expression of SLC16A9, resides within a RELA binding motif, and is predicted by QBiC to affect the binding of RELA at that location; the disrupting variant is present only in K562 (orange track in Figure 2A) and not in GM12878; this prediction is confirmed by the presence of a RELA ChIP-seq binding range in GM12878, but not in K562 (teal track in Figure 2A). As a second example, consider the edge between the TF ARID3A and the gene PMS2CL (ENSG00000187953), with a regulatory difference score of 1.0564 and $d_{gij}^{(E)} = 0.0096$ in GM12878 and $d_{kij}^{(E)} = 1.066$ in K562, suggesting that the binding of ARID3A to the promoter region of PMS2CL is disrupted in K562, but not in GM12878. This prediction is confirmed by ChIP-seq-derived TF binding data in the region (Figure 2B). Both of these examples of genotype-specific TF binding are within the top 20 edge disruption scores for K562 for edges with confirmed differential binding between K562 and GM12878 ChIP-seq experiments.

Because an edge in an EGRET network implies a *regulatory* relationship, as opposed to simply presence of binding by a TF, we wanted to further validate our network predictions against assays that captured changes in gene expression. We used data from an in-vitro allele-specific expression (ASE) assay (Biallelic Targeted Self-Transcribing Active Regulatory Region sequencing — BiT-STARR-seq) performed in LCLs [16] (Supplementary Note S6.3). We calculated regulatory difference scores per gene and found that the 101 genes having highest differential regulation scores $R_j^{(G)}$ (those within the top

10%) were enriched for genes harboring ASE-causing variants located within promoter region TF motifs (Fisher p-value = $2.5e-03$). As a second independent validation, we compared data from a published chromatin accessibility QTL (caQTL) analysis in LCLs to those genes whose regulatory difference score were in the top 10% (Supplementary Note S6.4) [17] and found that these genes having $R_j^{(G)}$ values among the top 10% were enriched for having caQTLs within motifs in their promoter regions (Fisher p-value = $1.4e-04$). This suggests that many of the predicted regulatory SNPs alter their associated regulatory networks by affecting chromatin accessibility. It is worth noting that our results are based only on the genotypes of two cell lines; we anticipate that using a larger number of genotyped cell lines with available ChIP-seq, caQTL, and ASE data would increase both the specificity and sensitivity of predicting genotype-mediated effects in gene expression.

Overall, these results indicate that EGRET is capable of synthesizing diverse sources of data to model gene regulatory processes and can predict genotype-associated patterns of gene regulation.

3.2 EGRET networks for a population of individuals identify cell type-specific disease associations

A growing body of work indicates that cell-type specific gene regulatory processes affect gene expression [18, 15] and do so in a manner dependent on an individual's genotype [19, 20, 21], resulting in changes that alter the structure of functional “communities” or “modules” comprised of TFs and genes, and are enriched for genes associated with tissue-specific biological processes [22]. Banovich and colleagues [17] had previously analyzed RNA-seq data derived from three cell types: lymphoblastoid cell lines (LCLs), induced pluripotent stem cells (iPSCs), and cardiomyocytes (CMs; differentiated from the iPSCs). They demonstrated that genes preferentially expressed in CMs were enriched for processes associated with coronary artery disease, and those enriched in LCLs were associated with immune-related conditions. Our working hypothesis was that these effects should be linked to cell type-specific regulatory processes affected by an individual's genetic background.

To test this, we constructed 357 individual-specific EGRET networks using expression, genotype, and eQTL data from 119 Yoruba individuals for all three cell types used in the Banovich *et al.* [17] study (Supplementary Note S7). We also constructed a baseline GRN for each cell type (Supplementary Note S7.1). We calculated TF disruption scores (defined in Supplementary Table S2) for each TF in each individual EGRET network to identify TFs whose regulatory influence was disrupted by variants. TF disruption scores ($d_{x_i}^{(TF)}$) were then scaled per individual and cell type to have a mean of zero and standard deviation of one, and are denoted $d_{x_i}^{(TF)'}$ (Supplementary Note S7.2). We then labelled TFs as associated with Crohn's disease (CD) and coronary artery disease (CAD) (Tables S5 and S6) based on annotation from the NHGRI-EBI GWAS catalog [23]. We tested to see if disease-associated TFs were more likely to have significant disruption scores in relevant cell types. Using a T-test, we found that TF disruption scores were significantly higher in cardiomyocytes (CMs) for TFs associated with CAD than were disruption scores for non-CAD related TFs ($p = 4.5256e-06$); this CAD enrichment was not observed in LCLs ($p = 0.99831$). Similarly, we found TF disruption scores in LCLs, but not CMs, were substantially higher for TFs linked to CD than for non CD-linked TFs ($p = 5.3374e-16$ in LCL networks,

$p = 1$ in CM networks; Table 2). This analysis leads to an important observation: genotype-mediated, disease-related TF disruptions are cell-type specific and can be identified using networks inferred by EGRET. Indeed, we find that the highest TF disruption scores for CAD TFs occur in CMs (Figure 3A) and that the highest TF disruption scores for CD TFs occur in LCLs (Figure 3B).

Further supporting this observation, the TF disruption signal in CAD is dominated in a subset of the study population by single a TF, ERG, which is a member of the erythroblast transformation-specific (ETS) gene family and known to be involved in angiogenesis [24]. In these individuals, the high TF disruption scores for CAD TFs in CMs are driven by the presence or absence of a mutation on chromosome 1 (chr1:201476815, an eQTL for CSRP1) that lies in the binding motif for the TF ERG in the promoter region of the gene CSRP1 (ENSG00000159176). While ERG is identified as CAD-related in the GWAS catalog, CSRP1 (alias CRP1) is not. However, CSRP1 is a known smooth muscle marker [25] and has been found by GTEx [11] to be highly expressed in smooth muscles, especially in arteries (Figure S4). CSRP1 has also been associated with the bundling of actin filaments [26], cardiovascular development [27], and with response to arterial injury [28]. Further, knockdown of CSRP1 in zebrafish caused cardiac bifida [29] and a frameshift mutation in CSRP1 has been linked to congenital cardiac defects in a large human pedigree [30]. The results of our EGRET analysis support a previously unreported mechanism of action for ERG in heart disease—that ERG regulates the expression of CSRP1 and that this regulation can be disrupted by genetic variation.

We also tested the hypothesis that the network effects of genetic variants have the potential to subtly change the modular structure of genotype-specific networks, altering the functional network modules active in an individual. ALPACA [22] is a method that compares the modular structure of two networks and identifies modules that differ between the networks. The resulting gene differential modularity (DM) scores indicate which genes have undergone the greatest change in their “modular environment.” We used ALPACA to compare the modular structure of the cell-type and individual-specific EGRET GRNs with the baseline GRN for the corresponding cell type, and calculated the DM score for each gene in each network (Supplementary Note S7.3, Figure S5).

Given that individual 18 had the greatest TF disruption score for ERG in CMs, we further investigated cellular processes predicted by EGRET to be variant-perturbed within this individual’s three cell-type specific EGRET networks. For each cell type we ranked this individual’s genes by their DM scores from highest to lowest in each cell type reflecting their predicted impact on altering the modular structure of each cell-type specific network. We used GORILLA (Supplementary Note S7.4; [31]) with these ranked lists to identify GO biological process functions associated with modules altered by the presence of specific genetic variants. Several GO terms relevant to CMs and cardiovascular functioning and development, including “regulation of actomyosin structure organization,” “prepulse inhibition,” “ephrin receptor signaling pathway,” “maintenance of postsynaptic specialization structure,” and “actin cytoskeleton reorganization” were enriched in CMs from this individual (Figure 4, Table S7) but not in their LCLs or iPSCs (Figure 4, Tables S8 and S9). For full enrichment results, see Figure S6, Figure S7, and S8, Figure S9). Further evidence of cell type-specific alteration of functional modules can be seen by examining the DM scores of disease-associated target genes (as annotated by the NHGRI-EBI GWAS

catalog [23]). Coronary artery disease genes with high DM scores in CMs had low DM scores in iPSCs and LCLs (Figure 5A). In contrast, genes associated with Crohn’s disease, which has a strong immune component, that had high DM scores in LCLs and low DM scores in iPSCs and CMs (Figure 5B).

EGRET also predicts dosage effects of regulatory SNP variants on network structure. Consider CSRP1, which we previously discussed as having a regulatory SNP in its promoter region that can affect binding of the transcription factor ERG. EGRET shows that the presence of a genetic variant in the promoter region of CSRP1 affects not only regulation by ERG (as seen by a substantial TF disruption score) but also the role that CSRP1 plays in altering the functional modules in cardiomyocyte GRN models. As seen by CSRP1’s DM scores in Figure S10, EGRET predicts that the genetic variant exerts its influence on network structure in a dosage-specific manner; individuals homozygous for the disrupting variant are predicted to exhibit the greatest impact on the modularity, those who are heterozygous to have an intermediate effect, and those homozygous for the wild-type to exhibit minimal or no effect on modularity.

Collectively, these results suggest that phenotype- and disease-associated variants can act through disruption of TF binding leading to regulatory changes that manifest themselves both through altered expression of specific target genes and the modification of GRN functional modular structure.

4 Discussion

One of the fundamental tenets of genetics is that genotype influences phenotype. For many traits, especially those related to human disease, this connection is not straightforward. The vast majority of phenotype-associated genetic variants are non-coding and have small effect sizes [32, 33] and a recent analysis found that most (71%-100%) 1-MB windows in the genome contribute to schizophrenia heritability [34]. This suggests that many variants must act in concert to produce complex trait phenotypes, but the mechanisms by which they exert their influence remains an open question. Functional genomics studies have provided some insights into roles of these variants: variants are enriched in regulatory elements [35, 36, 37], disease heritability tends to be enriched in tissues relevant to the disease [1], and TF binding plays an important role in explaining heritability of human traits [5]. Despite this progress at the population level, questions remain regarding the influence of an individual’s genotype on these regulatory processes. Answers to these questions will be important for translating population-level insights into clinically actionable information.

EGRET is the first method, to our knowledge, that directly addresses these issues. EGRET begins with a reference motif prior network based on mapping transcription factor binding sites to the regulatory regions of genes. EGRET extends this by modifying the reference motif network based on evidence that SNPs in a gene’s regulatory region may influence TF binding as well as gene expression. Subsequently, EGRET uses a previously developed message passing framework [6] to iteratively seek consistency between a genotype-altered regulatory network model, TF-TF protein-protein interaction data (acknowledging that TFs can form regulatory complexes), and gene co-expression data (based on the assumption that genes regulated by the same TFs are likely to exhibit correlated expression). EGRET then outputs edge weights for all TF-to-gene edges. These edge weights reflect the confidence of a reg-

ulatory relationship between a TF and gene, given an individual's genotype. We demonstrate EGRET using publicly available eQTL, gene expression, and PPI data, and show that the algorithm provides powerful insights regardless of whether or not the individual genotypes are sample matched with other data types.

We validated EGRET in two ways. In the first, we inferred genotype-specific gene regulatory networks for two genotyped cell lines and identified genes that differed in their TF-gene edges, meaning that the model predicts differences in binding of specific TFs to upstream regions of individual genes. When we cross-referenced EGRET's predictions with ChIP-seq data for these cell lines, we found concordance between the predictions and ChIP-seq data, demonstrating that EGRET was able to accurately identify different TF binding patterns and effectively altered the structure of the regulatory network. We also found that genes with high regulatory difference scores between the two cell lines—those predicted to be differently regulated by EGRET—were enriched for QTLs associated with chromatin accessibility and enriched for allele-specific expression, suggesting that the EGRET-predicted regulatory changes are likely to have broader regulatory effects.

Our second validation looked at three different cell types in 119 genotyped individuals. We found distinct cell type-specific and genotype-specific differences in the gene regulatory networks that were linked to disease. Most notable among these were regulatory differences associated with Crohn's disease in lymphoblastoid cell lines and others linked to coronary artery disease in the regulatory networks in cardiomyocytes. Not only were individual TF-gene connections disrupted, but these disruptions led to higher-order changes in the network community structure, reorganizing the network in ways that predict changes in cell type- and disease-specific functional network communities.

Taken together, these results from EGRET present a compelling picture of the way in which small-effect, non-coding SNPs work together to influence phenotype. These SNPs have the potential to subtly alter the binding of TFs to their target genes. The direct effect of these individual SNPs is to alter which TFs regulate specific genes. However, their indirect, and possibly more important effect, is to alter the structure and membership of functional communities in the overall regulatory networks. Indeed, it is known that even a small number of TF-gene regulatory edge additions or deletions can lead to significant changes in network modular organization [22].

EGRET is capable of inferring gene regulatory networks specific to an individual's genotype, synthesizing genetic and gene expression data in a way that, for the first time, allows verifiable, disease-associated regulatory changes to be inferred for individual research subjects. As such, EGRET has the potential to substantially advance our understanding of genetic effects on disease risk, development, and response to therapeutic interventions. Potential applications of EGRET are wide ranging. EGRET can be used to infer a specific gene regulatory network for any individual for whom genotype data are available, even without associated gene expression data—provided there is expression and eQTL data from a relevant cell type obtained from a sufficiently large population to infer accurate regulatory network models. This implies that EGRET can be used to retrospectively analyze large cohort GWAS studies to tease out mechanistic associations for phenotype-linked genetic variants, as well as in the context of new studies that seek to understand disease mechanisms and the regulatory role of non-coding genetic

variants.

Data and Code Availability

EGRET is available through the Network Zoo R package (netZooR v0.9; netzoo.github.io) with a step-by-step tutorial.

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6 Author Contributions

DW and JP developed the EGRET method, DW implemented, tested and applied the method, DW and JP interpreted the results, MBG assisted with software distribution and manuscript preparation, JQ and KG provided input into all stages of the project, JP conceived of the study.

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Figures

Figure 1: EGRET integrates multiple data types to construct individual-specific GRNs. (A) EGRET takes as input several data types (population-level inputs circled in blue, individual-specific inputs circled in orange) to construct individual-specific GRNs: an initial estimate of the binding locations of TFs in the form of a reference motif prior (M_{ij}), the beta values of eQTL associations between “eSNPs” and “eGenes” (β), the genetic variants (s) harbored by the individual in question, PPI data as an estimate of TF-TF co-operativity (P), and gene expression to estimate a gene co-expression matrix (C). (B) An individual’s genetic variants are used to modify the reference motif prior to produce an individual-specific EGRET prior (E) by penalizing motif-gene connections in which that individual carries a variant allele (A) in the relevant promoter-region motif such that the variant is an eQTL for the adjacent gene (β) and the variant is predicted by QBiC to affect TF binding at that location (q). (C) Message passing is used to integrate the co-expression (C) and PPI (P) networks with the EGRET prior (E) resulting in a final, unique GRN per individual (E^*). (D) Regulatory disruption scores can be calculated to quantify the extent to which an edge or node in the network is disrupted by variants. Edge disruption scores are calculated by subtracting a genotype-agnostic baseline network (B^*) from the individual’s EGRET network and taking the absolute value. TF or gene disruption scores are calculated taking the sum of the edge disruption scores around the TF or gene in question.

Figure 2: EGRET identifies variant-impacted TF binding disruptions. (A) Example of variant-disrupted RELA binding in K562 but not in GM12878. Positions of eQTLs (purple track), genetic variants (orange tracks), ChIP-seq binding regions (teal tracks), and genes (black track) are shown in the region of SLC16A9. (B) Example of variant-disrupted ARID3A binding in K562 but not in GM12878. Positions of eQTLs (purple track), genetic variants (orange tracks), ChIP-seq binding regions (teal tracks) and genes (black track) are shown in the region of PMS2CL. The eQTL track is labeled according to the TF motif in which the eSNP resides as well as the adjacent eGene.

Figure 3: **Disease-related TFs are disrupted in relevant cell types.** Scaled TF disruption scores $d_{x_i}^{(TF) '}$ are shown for 119 Yoruba individuals for TFs associated with (A) coronary artery disease (CAD) or (B) Crohn's disease (CD). Each point represents the scaled TF disruption score for a disease-related (CD or CAD) TF, for a given individual for a given cell type (LCL, CM or iPSC). Disease-related TFs were identified using the GWAS catalog [23]. Scaled TF disruption scores for CAD-related TFs are highest in the cardiac-related cell type, CMs. Scaled TF disruption scores for CD-related TFs are highest in the immune cell type, LCLs.

Figure 4: **Variant-disrupted gene regulation affecting network modularity is enriched for coronary/heart related functions in CMs for an individual with a CAD disruption signature.** GO terms enriched in genes with high DM scores for individual 18, the individual with the highest TF disruption score for ERG. Several GO terms related to coronary/cardiac function are enriched in highly ranked DM genes in CMs but not in LCLs and iPSCs. Point size corresponds to the the number of high-DM genes annotated with the corresponding GO term. For display purposes, several generic GO terms enriched only in CMs were omitted in this figure. The entire set of enriched GO terms can be seen in Figure S9, as well as Tables S7, S9 and S8

Figure 5: **Variant-disrupted disease genes affect the modularity of the individual's regulatory network in the relevant cell type.** Differential modularity (DM) scores indicate the extent to which a gene's modular environment in the network changes between the genotype-specific EGRET network and the genotype-agnostic network. (A) CAD-related genes with high DM scores in cardiomyocytes (CMs) have low DM scores in the other cell types; (B) CD-related genes with high DM scores in LCLs have low scores in the other cell types.

Tables

Table 1: Data types and sources used as input to EGRET. Note that the application of EGRET to the 119 Yoruba individuals uses the same motif and PPI priors as used in the cell line analysis.

Data type	Tissue/Genotype	Source
Genotype cell line comparison		
eQTLs	LCL	https://gtexportal.org/home/datasets [11]
Gene expression	LCL	https://gtexportal.org/home/datasets [11]
Genotype	GM12878	https://www.illumina.com/platinumgenomes.html [12]
Genotype	K562	https://www.encodeproject.org/files/ENCFF538YDL/ [13]
ChIP-seq	GM12878, K562	http://pedagogix-tagc.univ-mrs.fr/remap/ [14]
PPI	N/A	https://sites.google.com/a/channing.harvard.edu/kimberlyglass/home [15]
Motif	N/A	constructed using FIMO [9]
Population application: 119 Yoruba individuals		
eQTLs	LCL	http://eqtl.uchicago.edu/jointLCL/ [17]
eQTLs	iPSC/iPSC-CM	http://eqtl.uchicago.edu/yri_ipsc/ [17]
Gene expression	LCL	http://eqtl.uchicago.edu/jointLCL/ [17]
Gene expression	iPSC/iPSC-CM	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE107654 [17]
Genotype	Yoruba individuals	http://eqtl.uchicago.edu/yri_ipsc/ [17]

Table 2: T-test p-values of differences between the TF disruption scores of disease (CD or CAD related TFs, determined from the GWAS catalog) versus non-disease TFs in different cell types. CAD TFs have significantly higher TF disruption scores than non-CAD TFs in CMs, but not in LCLs. CD TFs have significantly higher TF disruption scores than non-CD TFs in LCLs, but not in CMs.

Disease	Cell type	P-value
CAD	LCL	0.99831
CAD	CM	4.5256e-06
CD	LCL	5.3374e-16
CD	CM	1

D Regulatory disruption scores

Edge disruption scores

$$d_{xij}^{(E)} = \left| \begin{array}{c} \text{TF}_i \text{ to } G_j \\ \text{TF}_i \text{ to } G_j \end{array} \right| = \left| E_x^* - B^* \right|$$

Gene disruption scores

$$d_{xj}^{(G)} = \sum \left| \begin{array}{c} \text{TF}_i \text{ to } G_j \\ \text{TF}_i \text{ to } G_j \end{array} \right| = \left| E_x^* - B^* \right|$$

TF disruption scores

$$d_{xi}^{(TF)} = \sum \left| \begin{array}{c} \text{TF}_i \text{ to } G_j \\ \text{TF}_i \text{ to } G_j \end{array} \right| = \left| E_x^* - B^* \right|$$

A

B

A**Disruption scores for CAD TFs** **B****Disruption scores for CD TFs**

A**Top CAD genes in CMs** **B****Top CD genes in LCLs**