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Mapping gene-environment interactions at regulatory polymorphisms

Insights into mechanisms of phenotypic variation

Joseph C. Maranville,[1] Francesca Luca,[1] Matthew Stephens[1,2] and Anna Di Rienzo[1,*]

[1]Department of Human Genetics; The University of Chicago; Chicago, IL USA; [2]Department of Statistics; The University of Chicago; Chicago, IL USA

Genetic effects on gene regulation make a substantial contribution to phenotypic diversity, yet their mechanisms remain elusive. Here, we discuss the potential insights to be gained from mapping gene-environment interactions at regulatory polymorphisms (i.e., genetic variation that affects gene expression under specific environmental conditions). We highlight a novel statistical method to identify specific patterns of gene-environment interaction at these regulatory polymorphisms. Reviewing its application to a study that mapped gene expression in the presence and absence of glucocorticoids, we discuss the mechanistic insights that this approach provides.

Key words: regulatory variation, transcription factor binding, gene-environment interaction, polymorphism, transcriptional response

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*Correspondence to: Anna Di Rienzo;
Email: dirienzo@bsd.uchicago.edu

Introduction

Genetic variation influences phenotypic diversity through effects on underlying molecular processes. Broadly, polymorphisms can, at the DNA level, either modify a protein's amino acid sequence, truncate the protein, or disrupt the regulation of gene expression. It has become increasingly clear over the last decade that regulatory polymorphisms (i.e., those that disrupt gene regulation) make a substantial contribution to phenotypic variability. Genome-wide mapping experiments for many phenotypes have found a large number of non-coding polymorphisms, which are also significantly more likely to be associated with variation in transcript levels than random polymorphisms.[1,2] To date, relatively little is known about the mechanisms by which these genetic variants modify gene expression, and even less is known about how these changes in expression impact the phenotype. Association mapping of transcript levels, usually called expression quantitative trait locus (or eQTL) mapping, is an effective way of identifying loci harboring regulatory polymorphisms. However, an association between a genetic variant and expression provides little mechanistic information. For instance, an eQTL could reflect any number of regulatory mechanisms, from effects on transcription factor binding leading to altered transcriptional efficiency to variable mRNA decay rates. Furthermore, these mechanisms could vary substantially across cell types and environmental conditions. This is a major problem for efforts to connect eQTLs to specific phenotypes. An individual's (or cell's) phenotype could depend heavily on how a gene is regulated under a set of specific conditions (e.g., the specific cell type or a given physiological environment), but not depend on how the same gene is regulated in other contexts.

Identifying the conditions under which regulatory polymorphisms exert their effects on gene expression could provide insights into the mechanisms underlying variation in transcript levels and, ultimately, phenotype. For example, if a polymorphism affects expression only when a certain transcription factor is active, it likely interacts (directly or indirectly) with that factor. The insights could extend beyond regulatory mechanisms to help connect polymorphisms to specific phenotypes. For example, a regulatory polymorphism that affects expression only in

Table 1. Studies interrogating interactions between regulatory polymorphisms and environmental/treatment conditions

	Species	Tissue/cell type	Treatment/environmental variable	Study design
Li et al. 2006[6]	*C. elegans*	whole animal	temperature	paired measures from genetically identical whole organisms grown at different temperatures
Smith and Kruglyak 2008[7]	*S. cerevisiae*	-	carbon sources	paired measurements on yeast grown with different carbon sources
Smirnov et al. 2009[8]	*H. sapiens*	lymphoblastoid cell lines	ionizing radiation	paired measurements on cells treated in vitro
Ruden et al. 2009[26]	*D. melanogaster*	whole animal	lead	unpaired measurements from flies given different food supplies
Idaghdour et al. 2010[27]	*H. sapiens*	peripheral blood	geography	unpaired measurements from humans living in different environments
Romanoski et al. 2010[9]	*H. sapiens*	aortic endothelial cell lines	oxidative stress	paired measurements on cells treated in vitro
Viñuela et al. 2010[28]	*C. elegans*	whole animal	age	paired measures from genetically identical whole organisms of differing ages
Maranville et al. 2011[13]	*H. sapiens*	lymphoblastoid cell lines	glucocorticoids	paired measurements on cells treated in vitro
Barreiro et al. 2012[11]	*H. sapiens*	dendritic cells	*M. tuberculosis* infection	paired measurements on cells treated in vitro

the presence of a specific drug is likely to influence overall patient response. Here, we discuss new insights to be gained from mapping variation in gene expression levels in the presence and absence of a perturbation, for example a physiological stimulus to the cell.

Approaches to Mapping Gene-Environment Interactions at Regulatory Polymorphisms

Interactions between regulatory variants and treatment conditions can be identified by genetic analysis of transcript levels in the presence and absence of a specific perturbation. To this end, several studies have compared transcript levels across experimental conditions in genetically distinct yeast strains. Testing for statistical interactions between genetic background (e.g., strain) and treatment condition, these studies have identified genetic effects on gene expression that depend on available nutrients,[3] the presence of copper sulfate[4] and temperature.[5] This study design can identify genes that are affected by condition-specific regulatory variants, but cannot identify these variants. Instead, mapping variation in transcript levels under multiple perturbations or environments (interaction eQTL mapping) can be used to identify specific regulatory variants that affect gene expression in a condition-specific manner. This approach has been successfully applied to a map eQTLs that interact with a variety of conditions in multiple species and cell types (see **Table 1**). For example, Li et al.[6] measured transcript levels under multiple experimental conditions (temperatures) in *C. elegans* recombinant inbred lines and mapped interaction eQTLs by testing for statistical interactions between temperature and genetic markers.

Li et al.[6] measured transcript levels on the same individual (i.e., recombinant inbred line) under each treatment, termed "paired measurements." Sample pairing can be leveraged to reduce noise and confounding due to unmeasured factors. This is because many non-genetic factors that affect expression levels are likely to have similar effects across treatment conditions, and therefore, will not affect the change in expression between these conditions. (An obvious exception to this would be non-genetic factors that influence response). Pairing allowed Li et al.[6] to incorporate a fixed "line" effect on expression. The more common approach has been to map some measure of the change in expression (e.g., $\log_2$ fold change). This approach directly tests for genetic variants that are associated with the magnitude of change in expression levels in each individual when a perturbation is introduced. This approach was first used to map eQTLs that affect response to different carbon sources in yeast,[7] and was later used to map eQTLs that affect transcriptional response to ionizing radiation in human lymphoblastoid cell lines (LCLs),[8] and response to oxidized phospholipids in human primary aortic endothelial cell lines.[9]

Mapping change in expression can only test if genetic effects on expression differ between treatment conditions and cannot distinguish differing configurations of genetic effects across conditions. For example, an eQTL that is functional only in the presence of a perturbation (e.g., a drug) would be indistinguishable from an eQTL that is functional only in the absence of a perturbation (e.g., a control) (**Fig. 1**). These patterns could be important from a mechanistic perspective. For example, the molecular features and phenotypic implications of an eQTL that acts only when a transcription factor is active may be different from those of an eQTL that acts only when a transcription factor is inactive. Recently, Grundberg et al. (2011) used an alternative approach to map eQTLs that interact with various stimuli in human osteoclasts.[10] Instead of

contrasting patterns of expression between stimulated samples and untreated controls, the authors performed eQTL mapping separately in each of three treatment conditions (dexamethasone, PGE_2, BMP-2) and compared the results, identifying interaction eQTLs as those that showed significant associations between genotype and expression in one treatment condition and not in another. Barreiro et al. used a similar approach to identify eQTLs that interact with *Mycobacterium tuberculosis* infection in human dendritic cells.[11] While this analytical approach can distinguish differing patterns of genetic effects across conditions (e.g., eQTL only in treated samples vs. eQTL only in untreated samples), it fails to exploit sample pairing. Moreover, it is not trivial to identify, with statistical confidence, genetic effects that appear in one condition and not in another. Direct comparisons of significance measures between eQTL mapping experiments in differing treatment conditions can suffer from false positives due to the incomplete power of the test in each condition. For example, an eQTL with equal effects in both conditions could be significantly associated with variation in transcript levels in one condition (i.e., a true positive), but, due to incomplete power, not in the other (i.e., a false negative). This would then be falsely identified as an interaction eQTL. [This concept was recently discussed with regard to tissue-specific eQTLs by Ding et al. Barreiro et al. addressed this issue by relaxing the significance threshold required for eQTLs to replicate across treatment conditions].

We previously developed a novel Bayesian framework that harnesses sample pairing and directly tests for specific configurations of genetic effects across treatment conditions.[13] This method jointly models genetic effects across conditions, allowing us to control the overall false discovery rate for interaction eQTLs and increase power by testing for plausible patterns of interaction between genotype and treatment conditions. This method may be especially useful for mapping interaction eQTLs that follow each of a set of specific and mechanistically informative patterns. Furthermore, this method can be generally applied to genetic analysis of any phenotype measured under two conditions in paired samples. Below we discuss some insights gained from applying this method to an experiment we performed to identify glucocorticoid interaction eQTLs.

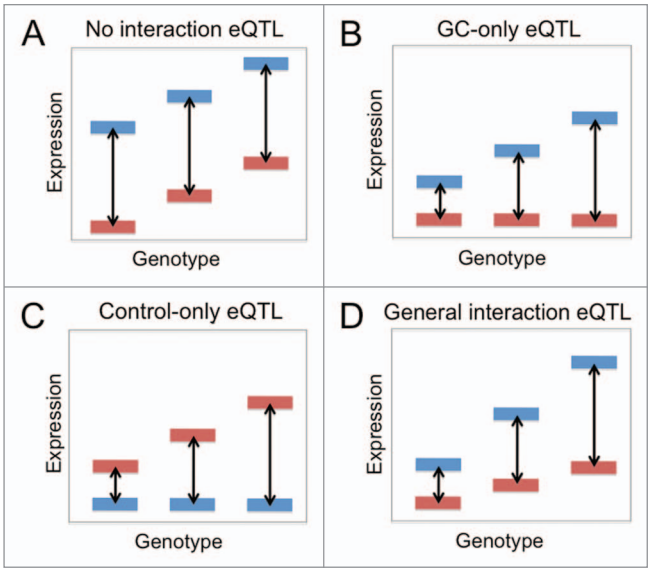

Figure 1. Associations between change in expression and genotype reflect interactions between genotype and treatment conditions, distinguishing them from (A) genotypic effects that act identically across treatment conditions, even at differentially expressed genes. Associations between change in expression and genotype could, however, reflect multiple distinct patterns of interaction, such as (B) genotypic effects on expression only in the presence of treatment (e.g., GCs), (C) genotypic effects on expression only in the absence of treatment (e.g., control), or (D) genotypic effects on expression that are present in both treatment conditions but differ in magnitude or direction. Horizontal bars represent mean expression for individuals from each of three genotype classes. Bar color indicates treatment condition.

Glucocorticoid Interaction eQTL Mapping

Glucocorticoids (GCs) are steroid hormones with widespread physiological effects, generally related to the neuroendocrine response to external stress. GCs are also commonly used as pharmaceuticals to treat a variety of inflammatory conditions (e.g., asthma), autoimmune diseases (e.g., ulcerative colitis), and certain types of cancer (e.g., acute lymphoblastic leukemia). GCs also have a well-characterized mechanism of action, making them an ideal perturbation for interaction eQTL mapping. GCs exert their effects on target cells mainly by activating a transcription factor called the glucocorticoid receptor (GR), which is otherwise inactive. The GR can either directly regulate gene expression or interfere with the activity of other transcription factors (e.g., NFκB and AP1). Treatment with GCs, therefore, corresponds to activating a specific transcription factor (GR) and inactivating a set of other transcription factors that are antagonized by GR. The relatively simple and well-characterized molecular biology of GC response enabled us to formulate mechanistic hypotheses about the patterns of genetic effects we observed across treatment conditions. Regulatory variants that affect expression only in the presence of GCs are, therefore, likely to influence both patient response to GC treatment and phenotypes that are regulated by endogenous GCs. To identify GC interaction eQTLs, we measured the expression of 13,232 genes in paired aliquots, one treated with the synthetic GC dexamethasone (dex) and one treated with the vehicle for dex (EtOH) as a control, in a panel of 114 densely genotyped HapMap B-lymphocytes transformed with Epstein-Barr Virus (EBV), commonly known as lymphoblastoid cell lines (LCLs).[13]

We began our mapping efforts by taking the $\log_2$ fold change in expression as a measure of response. We then asked if polymorphisms in the genes that encode the GR or its interacting transcription

factors were associated with variation in transcriptional response across genes. Even though we had a set of strong candidate loci, potentially increasing power compared with uninformed, genome-wide scans for trans-acting eQTLs, we found no evidence for associations at these genes. In contrast, we found the strongest evidence of interaction eQTLs when we limited our analysis to SNPs within 100 kb of each gene. This suggested to us that variation in response depends heavily on cis-acting regulatory variants that interact with GC treatment. Following the example of Smith and Kruglyak,[7] we then performed a post hoc examination of the configuration of genetic effects across treatments. At a qualitative level, we found that most interaction eQTLs identified by mapping the $\log_2$ fold change appeared to be only active in the presence of GCs. The remainder represented eQTLs that appeared to be active only in the absence of GCs or eQTLs with genetic effects in both conditions, but with different effect sizes. In order to move past this post hoc analysis, we developed a Bayesian framework to directly test different models of interaction eQTLs following the patterns above (and to distinguish them from eQTLs that do not interact with treatment) using a single statistical framework.

Using this framework, we again found that most interaction eQTLs have effects on expression only in the presence of GCs (termed GC-only eQTLs). These eQTLs are consistent with a specific set of regulatory mechanisms. Namely, they may influence the binding of transcription factors that are only active in the presence of GC treatment. This is most likely the GR, but could also involve cooperating transcription factors[14] or transcription factors that are not present until GR activates their transcription. Direct regulation by GR homodimers tends to lead to upregulation of transcription.[15] Consistent with this scenario, we found that genes affected by GC-only eQTLs tended to be upregulated by GCs. Furthermore, we found examples of GC-only eQTLs that overlapped with predicted GR binding site (e.g., rs10816772 for *C9orf5*, p for motif match = 6.8×10^{-3}).

We also found evidence of interaction eQTLs with an effect on expression only when GCs are absent (termed control-only eQTLs). These interaction eQTLs are compatible with a set of different and more complex regulatory mechanisms. Given GR antagonism of other transcription factors, this pattern is not unexpected and could simply reflect polymorphisms that disrupt the binding of these inhibited factors. For example, a regulatory variant that affects NFκB binding in the absence of GCs may no longer affect expression if GR inhibits NFκB's regulatory activity. Indeed, we found examples of control-only eQTLs that overlapped with predicted binding sites for such transcription factors (rs10449143 for *FBXL6*, matrix and core similarity for NFκB >0.9). GR is generally thought to antagonize transcriptional activators, causing downregulation of target genes after GC treatment. This mechanism does not seem to explain all of the control-only eQTLs we found, as there were equal numbers of upregulated and downregulated genes (4 of each). Additional mechanisms must also play a role, potentially including regulatory variants that affect binding sites for transcription factors that are displaced through competition with GR.

Interestingly, our Bayesian framework did not find evidence of interaction eQTLs with effects that were present in both treatment conditions, but that differed in size (termed general-interaction eQTL). This observation is consistent with the binary nature of GC action, where relevant transcription factors are either activated (e.g., GR) or inactivated (e.g., NFκB) in the presence of GCs. It is possible that these types of interaction eQTLs do exist, but are very rare. If this is the case, our statistical approach could fail to detect this class of interaction eQTLs. This is because our method uses estimates of the overall proportion of genes showing a particular type of eQTL in order to weigh the evidence of that model at each gene (i.e., experiment-wide proportions are used to set prior probabilities). This is a generally desirable property of the method, as these patterns are likely to be difficult to distinguish from noise. In other words, we may have less power to identify a subtle change in genotypic effect size following treatment than we do to identify a genotypic effect that either appears or disappears completely following treatment.

If indeed general interaction eQTLs do exists, it may be useful to speculate about their mechanistic implications. A possible example occurs at the *MS4A7* gene where the $\log_2$ fold change analysis identified an interaction eQTL that appears to effect expression levels in both conditions. The effect, while in the same direction, is slightly larger in the presence of GCs. This observation could reflect a complex mechanism where the GR acts to increase the relative contribution of the regulatory factor whose activity (e.g., binding) is altered by genetic variation. Consider a gene whose transcription is driven by the joint activity of NFκB and another transcription regulatory complex, which we will refer to as TF. Suppose that when GR antagonizes NFκB at this gene, TF can still drive transcription at some slower rate. A genetic variant that disrupted TF binding, however, could become a stronger predictor of expression levels when TF was the only relevant regulator (i.e., in the presence of GCs).

We also found an interaction eQTL for *DNAJC5G* that showed an effect on expression in both conditions, but of opposing directions (termed opposite effect eQTL). These eQTLs are a special class of general interaction eQTLs and, if true, they are likely to be due to complex mechanisms. One could speculate that these eQTLs represent a subset of regulatory variants that disrupt the function of transcription factors that are antagonized by the GR. While some such variants may have no effect on expression in the presence of GCs (causing control-only eQTLs), other such variants may have opposing effects on expression in GC-treated samples. This scenario could involve a transcriptional complex that has activating effects in the absence of GCs, but is repressive after GC treatment. If this complex is antagonized by the GR, the allele that increases the activator binding affinity would also increase expression in the absence of GCs, however, the same allele will still increase the strength of binding of the complex, which is now repressive (as opposed to simply non-functional).

The mechanisms outlined above represent specific cases of the possible relationships between regulatory variation and changes in the activity of corresponding regulators (e.g., transcription factor binding). The mechanistic implications of patterns of condition-specific eQTLs depend on the molecular biology of the experimental perturbation. Using information on the regulatory molecules that mediate response to changing conditions, interaction eQTL mapping can be used to test for patterns suggestive of specific mechanistic models. Furthermore, combining these analyses with additional experimental data (e.g., ChIP-seq) could eventually reveal the precise molecular mechanisms that underlie condition-specific effects on gene expression at these variants.

Interaction eQTL mechanisms can be expressed within a more general framework where transcript levels depend on the activity of the regulatory machinery. The relationship between transcript levels and, for example, the strength of a transcription factor binding to the chromatin may follow a sigmoid function, similar to the kinetics of enzymatic functions that require cooperative action of multiple activators; in this case, small changes in binding have a less marked effect on transcript levels when there is either very weak or very strong binding (reviewed in ref. 16, **Fig. 2**). Therefore, the effect of genotype on transcript levels depends on which part of the sigmoid function the two alleles inhabit (this is especially true if the allelic effect on binding is relatively small). Genotype could have a substantial effect on transcript levels if one or both alleles are in the linear portion of the function (e.g., the middle), but no effect if both alleles are in the same nonlinear portion of the function (i.e., very weak or strong binding). In turn, treatment condition could affect where alleles fall on this function through its strong influence on overall binding activity. For example, GC treatment causes the GR to switch from its inactive form sequestered in the cytoplasm (i.e., little to no binding) to a potent transcription factor (i.e., strong binding). A genetic variant in a GR binding site would have little to no effect on transcript levels in the absence of GCs. As discussed above, if GC treatment increases binding so that at least one allele is in the linear portion of the sigmoid function, this would create a GC-only eQTL. Note that GC treatment could also increase GR binding to a high enough level that both alleles result in the same transcript levels. There could, therefore, be many genetic variants that disrupt GR binding, but have little to no effect on transcript levels in either GC-treated or untreated samples. Depending on the shape of the sigmoid function, the relationship between transcript levels and binding may be flat for most of the function space. This scenario would predict a small fraction of interaction eQTLs, especially if a single treatment dose is used, similar to what we[13] and others[10] have observed even though a large number of genes are differentially expressed. We note that it may, therefore, be preferable to map some measure of gene expression over a range of treatment doses (e.g., a dose response curve) in order to achieve a more comprehensive survey of interaction eQTLs.

While we have focused our discussion on cis-eQTLs, environmental interactions at trans-eQTLs are also likely to make important contributions to overall cellular response and corresponding phenotypic diversity. Without consideration of interaction with environment, the relative contribution of cis- vs. trans-eQTLs to variation in gene expression is currently unclear. Estimates differ across species and among studies in the same species. Many studies in model organisms have found that trans-eQTLs make a large contribution to variation in expression, potentially even greater than cis-eQTLs.[17,18] Studies in humans have found conflicting evidence for widespread trans-eQTL effects, with some studies reporting a large number[19,20] and others finding a relatively small number of trans-eQTLs.[21–23] Following a similar pattern, studies of transcriptional response in model organisms have generally estimated a greater contribution from trans-compared with cis-eQTLs.[6,7] In humans, similar to baseline expression, the relative importance of environmental interactions at cis- vs. trans-eQTLs is ambiguous. This relationship may, in fact, vary depending on the environmental conditions under consideration. For example, we found no significant evidence of trans-eQTLs that interacted with GC treatment. In contrast, Smirnov et al.

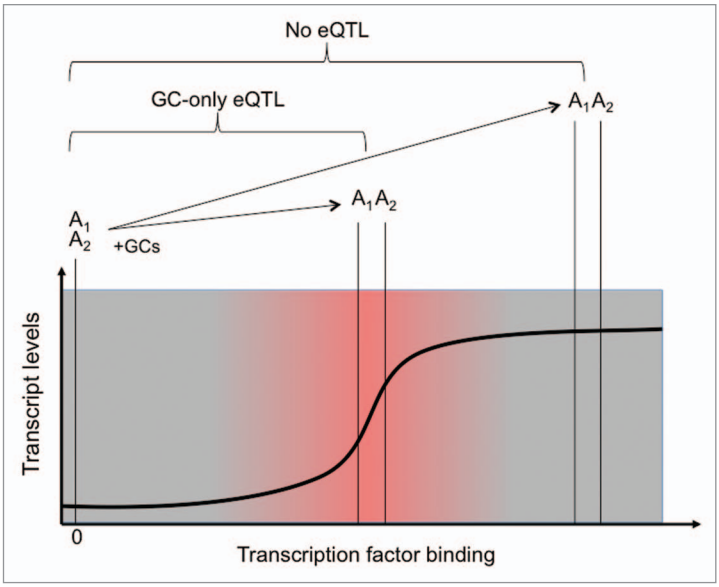

Figure 2. Transcript levels likely vary in relation to regulatory activity (e.g., transcription factor binding) according to a sigmoid function like the one depicted. Using GC treatment and consequent GR binding as an example, we illustrate how differences in binding between alleles post-treatment can generate an allelic effect on transcript levels only in GC-treated samples (GC-only eQTL) or remain irrelevant to transcript levels post-treatment (no eQTL). The range of transcription factor binding levels where subtle genotypic effects on binding would also impact transcript levels is indicated in red.

found many more interactions with ionizing radiation at trans- than at cis-eQTLs. Evaluating the relative contribution of cis- and trans-eQTLs is complicated by differences in power because the genomic search space is much larger for trans-eQTLs. Further complicating the situation, evolutionary constraints due to pleiotropy may cause trans-eQTLs to have on average smaller genetic effects than cis-eQTLs. This could explain the discrepant results between studies in model organisms and those in natural populations, such as those performed in humans. Even if effects on individual target genes tend to be smaller, trans-eQTLs could still make a major contribution to cellular and organismal responses due to their cumulative effects over many genes. Therefore, we need improved methods for identifying trans-eQTLs and assessing their interactions with environmental conditions.

Future Prospects: Connecting Interaction eQTLs to Phenotype

In addition to insights into regulatory mechanisms, interaction eQTL mapping can help connect regulatory polymorphisms to specific phenotypes. For example, overlaying our GC interaction eQTLs with results from genome-wide association studies for various diseases, we found that a GC-only eQTL (rs762421) for the autoimmune regulator gene (*AIRE*) was also associated with risk of Crohn disease. Downregulation of *AIRE* expression seems to depend on the presence of the rs762421-G allele, which is also associated with greater susceptibility to Crohn disease. This observation could suggest a role for glucocorticoids or the glucocorticoid receptor activity (or "GR activity") (e.g., cellular response to cortisol) in the etiology of Crohn disease, potentially involving the expression of *AIRE*. Generally, overlaying interaction eQTLs with genome-wide association results could reveal novel connections between treatment conditions (and related biological processes) and diseases.

Furthermore, interaction eQTLs can be used as candidates for association studies of related phenotypes. This could help narrow the search for associated variants by providing a set of candidate variants that may be especially likely to influence a particular phenotype, potentially leading to great improvements in power. For example, when we performed a focused scan of the GC interaction eQTLs we identified, we found that a GC-only eQTL (rs6870205) at the *TNIP1* gene was also associated with response to GC therapy in a sample of asthma patients (Bonferroni-corrected p value = 0.037). This variant would not have been indentified in a scan testing all (both interaction and non-interaction) eQTLs (Bonferroni-corrected p value = 1), much less in a genome-wide association scan. Furthermore, studies that map variation in transcript levels without GC treatment, (e.g., one of the many published studies on eQTL mapping without any treatment[24]) would not have identified this variant as an eQTL at all. Using a similar approach, Grundberg et al.[10] identified an additional variant associated with asthma patient response to GC treatment and Barreiro et al. identified a variant associated with host susceptibility to pulmonary tuberculosis.

Although eQTLs that interact with specific treatment conditions may be more likely to affect corresponding organismal phenotypes (as observed by Barreiro et al.), we note that these phenotypes could be influenced also by non-interacting eQTLs. This is especially true if the relationship between gene expression and organismal phenotype is not linear. For example, consider a situation where asthma patients show symptoms only when expression of a particular GC target gene is below a specific threshold level. Suppose that this gene is affected by an eQTL and is upregulated by GC treatment, but the eQTL does not interact with treatment. Before GC treatment, patients have different expression levels depending on genotype. For all individuals, however, expression is below the threshold, so they all show similar asthma symptoms. After GC treatment, expression increases to the same extent across patients. For patients with genotypes associated with higher expression before treatment, the gene may now be expressed above the threshold level leading to clinical response and alleviation of symptoms. For individuals with genotype associated with the lowest expression levels, however, the increased expression levels may still be below the threshold leading to treatment failure. Indeed, the recently reported association between a non-interacting cis-eQTL for *GLCCI1* and GC response in asthma patients[25] may reflect a threshold effect as the one outlined above. We note, however, that this eQTL could be misclassified as non-interacting because of incomplete power or because it interacts with GCs under different conditions (e.g., dose, duration of treatment, cell type).

Even greater benefits could be gleaned from directly combining phenotype and interaction eQTL mapping. Expression data with and without a carefully chosen perturbation could be collected in parallel with phenotypic information (i.e., on the same individuals). This type of data would allow researchers to jointly model genetic effects on expression in specific conditions and on phenotypic outcomes. Furthermore, taking multiple measurements under different conditions in the same individuals would allow us to apply tests relying on conditional independence and directly interrogate the role of gene regulation in specific contexts as an intermediate to specific phenotypes. Additional levels of biological information could also be included (e.g., hormone levels, cellular phenotypes) ultimately uncovering all the links in the chain of mechanisms that connects genotype to phenotype.

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