



Genome-wide expression quantitative trait loci analysis in asthma

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Purpose of review

Expression quantitative trait loci (eQTL) mapping studies are the next most important step in genomics to identify susceptibility genes and molecular pathways involved in human diseases following the completion of genome-wide association studies (GWAS). This article reviews the emerging concepts in genetics of gene expression and the empirical value of eQTL mapping to refine GWAS asthma susceptibility loci.

Recent findings

eQTL mapping studies were paramount to reveal the *cis* and *trans* control of gene expression, the cell type and tissue specificity of eQTLs, and the pleiotropic nature of eQTL single nucleotide polymorphisms. A small number of eQTL studies were recently performed in tissues and cell types that are relevant for asthma and are used to interpret the biology underpinning GWAS loci including the most robust asthma susceptibility locus on 17q21.

Summary

The full potential of eQTL mapping studies is just starting to be revealed. Imminent progress is expected owing to the accelerating advances in sequencing technologies to map genetic variants and transcriptomes as well as the development of bioinformatics and computational algorithms to exploit integrative genomic approaches. A short-term challenge in the field of asthma is the creation of well powered eQTL datasets testing gene expression and other molecular phenotypes in disease-relevant tissues.

Keywords

asthma, eQTL mapping study, expression quantitative trait loci, genomics, genome-wide association studies

INTRODUCTION

Rapid advances in genomic technologies are constantly reshaping the tools and methodologies available to unlock the genetic architecture of human diseases. During the last decade, genome-wide association studies (GWAS) have revolutionized our ability to identify genetic variants reproducibly associated with human biological traits and diseases [1]. Extending GWAS to gene expression has allowed the identification of genetic variants, mostly single nucleotide polymorphisms (SNPs), regulating gene expression, that is, expression quantitative trait loci (eQTLs). eQTL mapping studies are now commonly used to understand basic processes of gene regulation and to aid in the interpretation of GWAS findings. This article aims to briefly review the basic principle underlying genome-wide eQTL mapping studies and describe recent advances in this area in the field of asthma.

EXPRESSION QUANTITATIVE TRAITS

Standard molecular techniques are used to measure messenger RNA (mRNA) transcript abundance in cells and tissues. Quantitative real-time PCR can detect and quantify small amounts of mRNA for selected candidate genes [2]. At the genome-wide scale, microarray technology has been refined during the last 2 decades to quantify the expression

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KEY POINTS

- Most of our knowledge on genetics of gene expression is derived from typing SNPs and microarray technology quantifying whole transcript abundance in readily available tissues.
- Many thousands of *cis*-acting and *trans*-acting eQTLs were found and akin to gene expression traits, eQTLs are very sensitive data influence by cellular and tissue context, environmental cues, and transcriptomic platforms.
- eQTL mapping studies are particularly relevant to identify functional variants and disease-causing genes underlying GWAS findings.
- eQTL analyses in LCLs suggested *ORMDL3* as the most likely causing gene mediating the risk of asthma on chromosome 17q21, but eQTL mapping in disease-relevant tissues pointed more strongly toward *GSDMA*.
- Further developments are needed to identify a wider spectrum of genetic variants (other than SNPs) that influence not only the abundance of gene expression but also the structure of transcripts as well as other molecular phenotypes.

of all known genes in a single experiment [3]. mRNA sequencing (RNA-Seq) is now gradually replacing microarrays with a better resolution of the transcriptome [4]. With these constantly progressing tools, we learned throughout the years that gene expression traits are highly sensitive and affected by both genetic and environmental factors [5]. For example, a recent genome-wide gene expression study [6] in human lung specimens revealed thousands of genes that were significantly altered by smoking. Considerable variability in gene expression phenotypes is also observed between populations [7]. Although environmental factors account for some of this inter-population variability, a large proportion is also explained by differences in allele frequencies [8,9].

GENETICS OF GENE EXPRESSION

eQTL mapping studies [10,11] in animal models and human cell lines have provide convincing evidence that gene expression phenotypes are under strong genetic contribution. In addition, these studies provide the means to examine the overall genetic complexity of gene expression traits. Thousands of genetic loci that influence gene expression have been identified. During the last decade, basic principles emerged from eQTL mapping studies. Some of these principles are described below and more comprehensive overviews are available elsewhere [5,12–14].

Gene expression phenotypes are under the influence of *cis* and *trans* control

cis and *trans* control refers to the physical distance between SNPs and regulated genes. SNPs located nearby the expressed transcripts are *cis*, whereas SNP and transcript separated by an arbitrary distance on the same chromosome or on different chromosomes are *trans*. There is no consensus on the exact physical distance to define *cis* and *trans*-eQTLs. So far, *cis*-acting variants were mapped anywhere within 50 kb [15]–2.5 Mb [16] windows that are usually centered on the transcription start site (TSS) or the probe interrogating transcript levels. Empirical evidence suggested that a large majority of regulatory SNPs are found in the vicinity of genes, more specifically within 100 kb of the TSS [14], which confirms the important regulatory elements in gene promoters. However, cumulatively *trans*-eQTLs were found to explain a significant part of the heritable variation in human gene expression [17,18].

Cell type and tissue specificity of *cis* and *trans*-eQTLs

eQTL mapping studies were typically performed in single tissue. However, more recent studies [11,16,19,20,21,22] evaluated eQTLs in multiple cell lines and/or tissues. eQTL mapping in fibroblasts, T cells, and lymphoblastoid cell lines (LCLs) derived from the same 75 individuals revealed that 69–80% of *cis*-eQTLs are cell type-specific [11]. Only 8.5% of genes with *cis*-eQTLs were shared among all three cell types. A similar study [16] performed in primary monocytes and B cells derived from 283 healthy individuals found 21.8% of *cis*-eQTLs and 7.6% of *trans*-eQTLs shared between the two cell types. In other studies [23,24], the eQTL mapping results were profoundly affected by the differentiation states of cells and following exposure the various stresses, suggesting that the regulatory landscape within a single cell type is context-dependent. The specificity of eQTL is also well recognized in human tissues. eQTL mapping in skin, fat, and LCLs derived from healthy female twins revealed that only 30% of eQTLs are shared among tissues [20]. However, an extended sample size of the same study including 856 female twins estimated a greater degree of tissue overlap of *cis* effects. More than 60% of *cis*-eQTLs were detected in multiple tissues suggesting less tissue specificity than previously estimated is observed when the study is well powered. However, *trans*-eQTLs remained largely tissue-dependent in the later study. *Trans*-eQTLs were also characterized by very low replication rates in other studies, arguing that

larger sample size will be required to reliably identify *trans*-regulatory effects [21²²]. More research will be needed to study the specificity of eQTLs in tissues of different biological states including developmental stages and tissues explanted from diseased and healthy individuals.

The strength of *cis* and *trans*-eQTLs

The proportion of the total variability in gene expression explained by a single eQTL SNP varies widely within studies, but average roughly at 10% across studies. In general, the effect size and density of eQTLs decreased as a function of distance from the TSS [14]. For example, for three tissues (skin, adipose tissue, and LCLs), *cis*-eQTL SNPs located less than 200 kb from the TSS were found to explain on average 8% of the variance in expression levels, whereas *cis*-eQTL SNPs located more than 200 kb from the TSS explained on average 4% of the variability in gene expression [21²²]. Shared eQTLs across cell types and tissues also have larger effect size and tend to cluster around the TSS [11,16²²]. In contrast, cell and tissue-specific eQTLs have smaller effects and are more widely distributed around the TSS. The direction of the allelic effect for eQTLs shared in different cell types are usually consistent [11], but cell type-dependent and tissue-dependent direction effects were also observed [16²²,22²³].

Single eQTL SNPs-regulated multiple genes and multiple eQTLs per gene

Given the coexpression of genes, it is not surprising to identify single eQTL SNPs regulating the expression of many neighboring genes. Multiple eQTLs are also found for a certain number of genes. For example, in the 856 female twins study described above, 3529, 4625, and 2796 *cis*-eQTLs were found in adipose tissues, LCLs, and skin, respectively. More than one independent *cis*-eQTLs were found for 14, 17, and 10% of these transcripts, respectively [21²²]. The functional effects of genetic variants are known to be cell type-dependent. For example, rs738289 was strongly associated with the expression of *MGAT3* in B cells, whereas it was associated with a neighbor gene, namely *SYNGR1*, in monocytes [16²²]. The same study also observed genes that were regulated by different genetic variants depending on the cell types. Studying eQTLs in disease-relevant cell types or tissues is thus important.

COMBINING EQTLs WITH GENOME-WIDE ASSOCIATION STUDIES RESULTS

During the last decade, GWAS were particularly successful to identify genes reproducibly associated

with diseases [1]. However, 88% of the GWAS-nominated SNPs are intronic or intergenic [25] suggesting that noncoding regions of the genome are responsible for most of the disease risk and some of this risk is likely to act through gene regulation. Many eQTL mapping studies in human were performed using peripheral blood leukocytes and LCLs. These are readily available tissues that can serve as proxies when disease-relevant tissues are not available [26]. As the field progresses, a larger number of tissues are being interrogated and eQTL datasets in human are now available in the liver [27,28], adipose tissue [19,21²²,28,29], skin [19], brain [30,31²³], lung [32²³], stomach [19], muscle [28], and sputum [33], among others [31²³]. Some of these tissues are particularly relevant to study asthma and allergic diseases and have been used to leverage previous GWAS on asthma and extend their functional meaning to gene expression.

eQTL MAPPING AND ASTHMA

The first GWAS of asthma was published in 2007 by the GABRIEL consortium [34]. Briefly, they genotyped more than 317 000 SNPs in 994 patients with childhood-onset asthma and from 1243 non-asthmatics, using family and case-control panels. Multiple markers on chromosome 17q21 were found to be strongly associated with asthma. This association was then replicated in 2320 individuals from a cohort of German children and in 3301 individuals from the British 1958 birth cohort. This region is now considered the most consistent locus associated with asthma [35,36]. The 17q21 genomic interval associated with asthma is relatively large (206 kb) and contains multiple genes. To find the culprit gene, the relationship between markers in the 17q21 locus and transcript levels in Epstein-Barr virus-transformed LCLs were studied in children in the asthma family panel used in the association study [34]. SNPs associated with childhood asthma were strongly associated with transcript levels of *ORMDL3*. This result suggests that genetic variants regulating *ORMDL3* expression are determinants of susceptibility to childhood asthma. This study demonstrated the power of combining whole-genome gene expression and genotyping data to identify susceptibility genes of asthma. This study also showed that eQTLs analysis of readily available tissue types such as LCLs can contribute to improve our molecular understanding of asthma and set the stage for other complex diseases.

A genome-wide survey of *cis*-eQTL was also performed in peripheral blood CD4⁺ lymphocytes in 200 young adults with asthma [15]. eQTL testing was restricted to variants mapping within 50 kb of

the transcript. Briefly, 7274 SNP-transcript combinations had false discovery rate less than 0.05 and involved 1585 unique genes (Table 1) [11,15,33, 16²²,32²³]. The *cis*-acting variants were found to be enriched with GWAS-nominated loci for immune-related diseases, but also at the similar extent to nonimmune-related diseases. This study performed in primary peripheral blood CD4⁺ lymphocytes confirmed SNPs regulating gene expression of *ORMDL3* on 17q21 observed in LCLs. However, the most significant SNP regulating *ORMDL3* was different and was also regulating the expression levels of a nearby gene, namely *GSDMB*. The coregulation of these two genes has been described [37] indicating a common disease allele with domain-wide *cis*-regulatory effect. It should be noted that, following the 2007 GABRIEL study [34], further analysis of the same samples taking advantage of the 1000 genomes project to increase the density of markers in the 17q asthma susceptibility locus as well as gene expression measurements from a different microarray platform suggested that *GSDMB* located in vicinity of *ORMDL3* is possibly the causal gene [38,39].

In white blood cell RNA samples collected from 743 Icelandic individuals, the GWAS variant associated with asthma was associated with the mRNA expression levels of both *ORMDL3* and *GSDMB* [40]. As observed in peripheral blood CD4⁺ lymphocytes, other sequence variants were most strongly associated with the expression of both genes. In cord blood mononuclear cells of 200 children, asthma risk allele on 17q21 were associated with increased *ORMDL3* and *GSDMA* gene expression [41]. However, these effects were only significant in cord blood samples stimulated with *Dermatophagoides pteronyssinus* (*ORMDL3* and *GSDMA*) or phytohemagglutinin (*GSDMA*). The expression of other genes located on 17q21, including *GSDMB*, was unaffected by the genotype status. Of interest, this study also observed that the asthma risk allele on 17q21 was associated with elevated IL-17 secretion in cord blood mononuclear cells.

We recently completed a large-scale eQTL mapping study [32²³] in human lung. Briefly, non-tumor lung parenchyma specimens were collected at three sites and combined into a meta-analysis. Following clinical, genotyping, and gene expression quality control, 1111 patients were included in the analysis. A total of 17 049 *cis*-eQTLs and 534 *trans*-eQTLs were detected (Table 1). SNPs that were found to regulate gene expression in the lung were enriched for association with asthma in the 2010 GABRIEL study [42]. The lung eQTL dataset is thus particularly valuable to refine previous GWAS on asthma. For instance, we were able to identify the

Table 1. Expression quantitative trait loci mapping studies in asthma-relevant human tissues and cell types

Tissues/cells	Gene expression arrays	Mapping	Distance/interval to define cis-eQTL	Sample size	# of cis-eQTLs	# of trans-eQTLs	References
T cells	Illumina WG-6 v3	Gene-centric	2 Mb window of TSS	75	430 genes	na	Dimas et al. [11]
Primary blood CD4 ⁺ lymphocytes	Illumina Human-Ref8 v2	Gene-centric	Within 50 kb of the transcripts	200	7274 (1585 genes)	na	Murphy et al. [15]
Sputum	Affymetrix Human U133 Plus2	Gene-centric	Within 50 kb of TSS	131	4315 (1086 genes)	na	Qiu et al. [33]
Primary monocytes	Illumina Human HT-12 v4	Genome-wide	Within 2.5 Mb of the probe	283	55 797 (8791 genes)	1409 (120 genes)	Fairfax et al. [16 ²²]
Primary B cells	Illumina Human HT-12 v4	Genome-wide	Within 2.5 Mb of the probe	283	44 511 (8154 genes)	295 (94 genes)	Fairfax et al. [16 ²²]
Lung	Custom Affymetrix	Genome-wide	Within 1 Mb of the probeset	1111	17 049 transcripts	534 transcripts	Hao et al. [32 ²³]

eQTLs, expression quantitative trait loci; TSS, transcription start site.

most likely causal gene and SNPs on chromosome 17q21. In the lung eQTL study, SNPs at the locus were associated with the expression levels of four genes, *ORMDL3*, *GSDMA*, *GSDMB*, and *CRKRS*, indicating that these genes are coregulated as previously proposed [37,40]. However, the most compelling eQTL on 17q21 was observed with *GSDMA*, suggesting that the risk allele at this locus mediate its effect by modulating the expression levels of *GSDMA* in the lung. The risk allele (rs7216389-T) for asthma on 17q [34] was associated with lower expression of *GSDMA* (Fig. 1). However, the strongest eQTL SNP on 17q was rs3859192 located in intron 6 of *GSDMA* (Fig. 1). This polymorphism has also been associated with asthma [34,42]. Many additional SNPs on 17q were strongly associated with *GSDMA*. Whether rs3859192 is the causal SNP remained to be determined.

The later study demonstrates the value of lung-specific eQTLs to refine previous GWAS hits for asthma. However, one important drawback of tissue-specific eQTLs is the heterogeneity of cell composition. The specific cell type(s) driving the eQTLs cannot be identified without additional functional work. It is also known that many of the cell type-specific eQTLs are hidden by other cell types present in the tissues and for which the eQTLs are absent. For instance, eQTL effects observed in B cell and monocytes isolated from whole blood were frequently absent when RNAs from peripheral blood mononuclear cells from the same individuals were analyzed [16²²]. Alternatively studying cell type-specific eQTL is hampered by isolation techniques and gene expression profiles that may not necessarily reflect the in-vivo cellular context. A combination of both disease-relevant tissues

and cell types will be needed for continual progress in the field.

INTEGRATIVE GENOMIC APPROACHES

eQTL mapping is now part of a larger set of tools and approaches in genomics to elucidate the genetic architecture of complex diseases. As we progress, more studies will combine different approaches to reach this goal. A new set of asthma susceptibility genes were recently found using integrative genomic approaches [43]. A GWAS for lymphocyte count was conducted in 462 individuals. In parallel, a genome-wide gene expression study in LCLs of 95 individuals selected from the extreme tails of the lymphocyte count distribution was performed. Genes were ranked by differential expression between individuals with high and low lymphocyte count. Then, the best *cis*-eQTLs mapping within 150kb of the top-ranked genes were identified. There was an enrichment of these *cis*-eQTLs associated with lymphocyte count for the top 193 genes whose expression levels were most correlated with lymphocyte counts. Similarly, asthma GWAS *P*-values for these *cis*-eQTL SNPs derived from GABRIEL [42] and EVE [44] consortia were smaller to what would be expected by chance, suggesting that a subset of these genes play a role in asthma.

eQTL data also provide the opportunity to reanalyze GWAS data by prescreening for functional SNPs or adding informative weights. A recent study [45²³] reanalyzed previous GWASs on asthma by incorporating weights to LCLs eQTL SNPs and found additional SNPs associated with asthma. Further research is needed to refine the best weighting scheme, but this eQTL weighting approach is

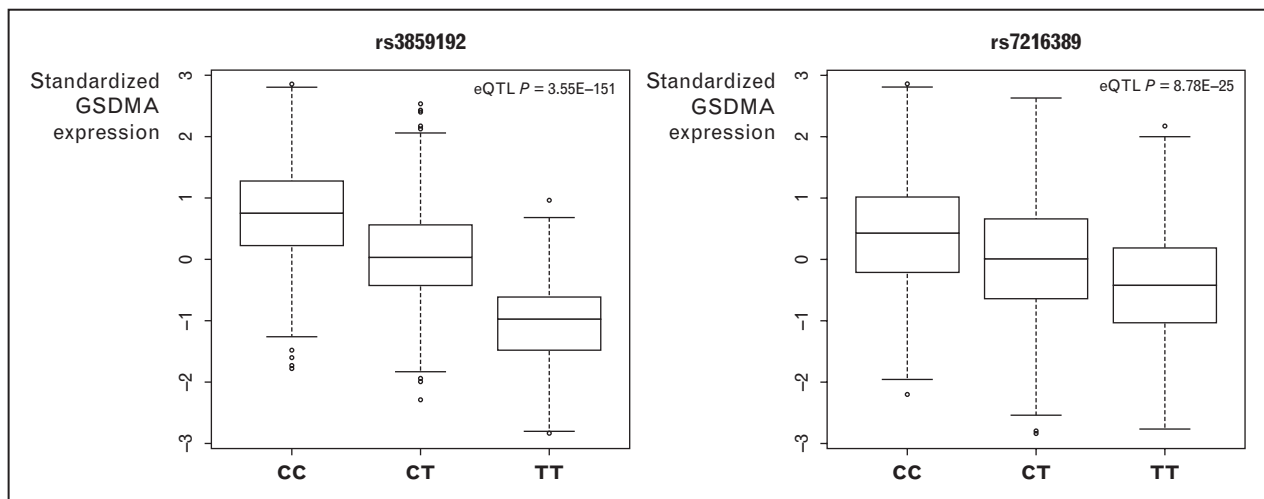


FIGURE 1. Boxplots of gene expression levels for *GSDMA* in 1111 human lung specimens according to genotype groups CC, CT, and TT for SNPs rs3859192 (left panel) and rs7216389 (right panel). This figure was taken from Hao *et al.* [32²⁴].

promising and cheap as it can be applied to existing GWAS and eQTL datasets. This approach is yet to be performed with eQTL information derived from disease-relevant tissues.

Development in bioinformatics and computational algorithms will facilitate data integration and leverage the existing and new eQTL datasets. For instance, an approach was described to identify biological pathways enriched for eQTL loci associated with diseases [46]. By taking advantage of known biological pathways found in Kyoto encyclopedia of genes and genomes [47] and eQTL SNPs discovered in disease-relevant tissues, pathway-dependent eQTLs can be identified and later test for enrichment for small *P*-values derived from GWAS. A new statistical framework was also recently proposed to identify disease-causing genes by combining GWAS and eQTL results [48²]. The new computational algorithm, named Sherlock, was used to identify possible disease-causing genes for Crohn disease and type 2 diabetes. These bioinformatics approaches are yet to be utilized in the field of asthma. However, datasets are available including large-scale GWAS on asthma [42,44] and disease-relevant eQTL studies [15,32²,33]. Advances in this area seem obvious in the near future.

FUTURE DEVELOPMENT

So far, the majority of eQTL mapping studies have assayed gene expression by microarrays, and results from these studies have shaped most of what we currently know about eQTLs. As RNA-Seq become more affordable, a larger number of eQTLs are likely to be discovered [49,50]. On top of providing more accurate quantification of gene expression levels, RNA-Seq can identify variants responsible for alternative splicing and allele-specific expression. Similarly, eQTL mapping studies were mostly restricted to one form of genetic variants, that is, SNPs. Structural variants were also found to explain variability in gene expression [51,52]. In addition, low-frequency and rare regulatory variants are also likely to play a significant role [21²]. Accordingly, the field of eQTL mapping will evolve with improved technologies to map genetic variants and the transcriptome. Deeper molecular phenotyping of cells and tissues will also transform the field. Progress in genomics and proteomics provides new tools to interrogate different molecular phenotypes at the genome-wide scale including gene and protein expression, protein states, metabolite levels, epigenetics marks, and so on. As costs of using these technologies continue to decline, eQTL mapping studies will be carried out in extended multidimensional genomic and proteomic datasets [31²,53].

Combined evidence suggested that functional genetics variants are more likely to be uncovered in disease-relevant tissues. For asthma, such tissues mean a large variety of tissues and cell types. Genome-wide eQTL datasets are now available for lung parenchyma [32²] and sputum [33] as well as for specialized cell types involved in the pathogenesis of asthma including CD4⁺ lymphocytes [15], monocytes and B cells [16²], and T cells [11] (Table 1). Major effort will be needed to collect additional lung specimens from patients with and without asthma. Microdissection of the different components of the lung is likely to be essential including bronchi, epithelium, smooth muscle, and parenchyma. Similarly, many specialized cell types would be relevant to study including among others epithelial cells, pneumocytes, alveolar macrophages, and smooth muscle cells. Accordingly, collecting the relevant biological material to conduct well powered eQTL mapping studies in asthma will be a significant challenge for the next few years.

CONCLUSION

eQTL mapping is now a standard genomic approach to elucidate the mechanisms underlying disease pathogenesis. Most of the knowledge on functional variants affecting gene expression was derived from readily available tissues, mostly blood leukocytes and LCLs. Important evolving concepts have emerged from these studies including the large number of eQTLs discovered and their cumulative impact on gene expression, the *cis* and *trans*-acting control on gene expression, the tissue and cell type specificity of eQTLs, and the pleiotropic nature of expression variants acting on multiple genes and different genes depending on tissues/cell types. Results from eQTL studies were particularly useful to provide functional meaning to GWAS, and the field of asthma is no exception. However, only a small number of eQTL studies were performed in tissues and cell types that are relevant for asthma. Larger collections of lung specimens should be prioritized for the next few years in order to complete well powered eQTL studies. In parallel, advances in sequencing technologies and bioinformatics will allow more comprehensive evaluation of the transcriptome as well as the wide spectrum of rare and common genetic variants regulating gene expression and pathways altered in asthma. The collection of well characterized patients with asthma coupled with multidimensional genomic approaches is likely to uncover new biological signals underlying disease.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 579–580).

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