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A Common Cortactin Gene Variation Confers Differential Susceptibility to Severe Asthma

Shwu-Fan Ma, PhD^{1,†}, Carlos Flores, PhD^{1,†,‡}, Steven M. Dudek, MD¹, Dan L. Nicolae, PhD¹, Carole Ober, PhD², and Joe G.N. Garcia, MD^{1,*}

¹Department of Medicine, University of Chicago, Chicago, IL 60637, USA

²Department of Human Genetics, University of Chicago, Chicago, IL 60637, USA

Abstract

Genomic regions with replicated linkage to asthma-related phenotypes likely harbor multiple susceptibility loci with relatively minor effects on disease susceptibility. The 11q13 chromosomal region has repeatedly been linked to asthma with five genes residing in this region with reported replicated associations. Cortactin, an actin-binding protein encoded by the *CTTN* gene in 11q13, constitutes a key regulator of cytoskeletal dynamics and contractile cell machinery, events facilitated by interaction with myosin light chain kinase; encoded by *MYLK*, a gene we recently reported as associated with severe asthma in African Americans. To evaluate potential association of *CTTN* gene variation with asthma susceptibility, *CTTN* exons and flanking regions were re-sequenced in 48 non-asthmatic multiethnic samples, leading to selection of nine tagging polymorphisms for case-control association studies in individuals of European and African descent. After ancestry adjustments, an intronic variant (rs3802780) was significantly associated with severe asthma (odds ratio [OR]: 1.71; 95% confidence interval [CI]: 1.20-2.43; $p = 0.003$) in a joint analysis. Further analyses evidenced independent and additive effects of *CTTN* and *MYLK* risk variants for severe asthma susceptibility in African Americans (accumulated OR: 2.93, 95% CI: 1.40-6.13, $p = 0.004$). These data suggest that *CTTN* gene variation may contribute to severe asthma and that the combined effects of *CTTN* and *MYLK* risk polymorphisms may further increase susceptibility to severe asthma in African Americans harboring both genetic variants.

Keywords

CTTN; *MLCK*; cytoskeleton; SNP; asthma

Introduction

Asthma is a common airway inflammatory disorder affecting 7% of the US population [Mannino et al., 2002]) characterized by airflow obstruction and bronchial hyper-responsiveness, often associated with atopy. Incomplete twin concordance and patterns of

*Correspondence to: Joe G. N. Garcia, MD, Section of Pulmonary and Critical Care Medicine, Department of Medicine, University of Chicago, 5841 S. Maryland Avenue, W604, Chicago, IL 60637, Telephone: 773-834-3163, Facsimile: 773-702-4427, jgarcia@medicine.bsd.uchicago.edu.

†These authors contributed equally to this work.

‡Current address: Unidad de Investigacion, Hospital Universitario N.S. de Candelaria, Santa Cruz de Tenerife, Spain; CIBER de Enfermedades Respiratorias, Spain.

Electronic Database Information: The URLs for the software used here are as follows:

EPIDAT 3.0, <http://dxsp.sergas.es>

Simple Interactive Statistical Analysis, <http://www.quantitativeskills.com/sisa>

UCSC Genome Browser, <http://genome.ucsc.edu>

inheritance in families have suggested that asthma and related phenotypes have considerable and complex genetic components [Ober, 2005]. Genome-wide linkage analyses have revealed at least 20 broadly defined major asthma susceptibility regions, several of which have been replicated [Wills-Karp and Ewart, 2004]. Candidate gene association studies have indicated that many regions contain multiple susceptibility loci with relatively small effects on disease risk [Ober and Hoffjan, 2006]. Chromosome 11q13, a region replicated across various studies [Wills-Karp and Ewart, 2004], contains at least five asthma-related genes identified in two or more association studies.

Histopathological studies of asthmatic airway tissue samples typically demonstrate persistent airway tissue inflammation, irreversible airway structural changes [Moore and Peters, 2006] and vascular permeability consistent with tissue inflammatory changes and both angiogenic and microvascular remodeling processes [Orsida et al., 1999; McDonald, 2001]. Although the majority of putative asthma susceptibility genes are related to immune function or therapeutic target pathways [Ober and Hoffjan, 2006], it has become apparent that vascular genes (involved in regulation of both angiogenesis and inflammation) as well as genes governing pro-fibrotic processes (cell hypertrophy, cell adhesion and migration), are also relevant to asthmatic inflammatory lung injury, tissue remodeling and pathogenesis. Asthma susceptibility genes implicated in airway tissue remodeling include *ADAM33* [Van Eerdewegh et al., 2002; Kedda et al., 2006], *EGFR* [Wang et al., 2006], *ITGB3* [Weiss et al., 2005; Thompson et al., 2007], *SELP* [Bourgain et al., 2003], *TGFB1* [Pulley et al., 2001; Hoffjan et al., 2004; Silverman et al., 2004], and *VEGFR2* [Park et al., 2006]. Cytoskeletal genes provide dynamic regulation of cell shape, cell motility and adhesion and are involved in remodeling processes during asthma and are up-regulated in asthmatic peripheral blood lymphocytes [Hansel et al., 2005] and in asthmatic airway smooth muscle cells [Benayoun et al., 2003]. We recently reported that a non-synonymous variation in the myosin light chain kinase gene (*MYLK*), a central regulator of cellular contraction, is strongly associated with severe asthma in African Americans [Flores et al., 2007], a finding subsequently validated by our report in an Afro-Caribbean asthmatic cohort from Barbados [Gao et al., 2007].

Cortactin, an F-actin binding multidomain scaffold protein, is involved in cortical actin assembly and dynamic actin cytoskeleton rearrangement [Cosen-Binker and Kapus, 2006]. We have previously described the interaction between cortactin and MLCK [Dudek et al., 2002], and cortactin participates in diverse cellular processes, including lung vascular barrier regulation [Dudek et al., 2004], cell motility (overexpression enhances migration of both fibroblasts and endothelial cells and promotes metastasis in human cancers [Daly, 2004]), intercellular junction assembly [Cosen-Binker and Kapus, 2006], and leukocyte adhesion and transmigration [Tilghman and Hoover, 2002; Johansson et al., 2004; Yang et al., 2006]. Additionally, current evidences suggest that cortactin is critical for the angiogenic lipid factor sphingolipid sphingosine 1-phosphate (S1P), found in elevated concentrations in the airways of asthmatic (but not control) subjects after segmental antigen challenge [Ammit et al., 2001], in mediating lymphocyte chemotaxis and transmigration [Rovietto et al., 2004; Brinkmann and Baumruker, 2006], and for the enhancement of endothelial and vascular smooth muscle cell proliferation and migration [Black and Johnson, 2002; Sawicka et al., 2003; Lee et al., 2006a].

Cortactin is encoded by the *CTTN* gene (~ 38 kilobases [kb]), located in close proximity to previously identified asthma-related genes (*FCER1B*, *CC16*, *GSTP1*, *GPR44* and *IL18*) in the 11q13 linkage region [Ober and Hoffjan, 2006]. Based on the linkage evidence and the biological plausibility for its implication in immunoregulation and angiogenesis, we explored the potential association of *CTTN* gene variants with susceptibility to asthma. We sequenced *CTTN* to identify novel polymorphisms, selected nine SNPs for genotyping a

multiethnic case-control sample collected in Chicago, and identified a significant association with severe asthma with an intronic *CTTN* polymorphism. In addition, we observed that a recently reported susceptibility variant of *MYLK*, the gene encoding MLCK, coupled to the *CTTN* polymorphism increases the risk for severe asthma in African Americans (~ three fold).

Methods

Study populations and clinical evaluation

Unrelated asthma cases (110 European Americans and 192 African Americans) were recruited in the Chicago area as part of the Collaborative Study on the Genetics of Asthma protocol [CSGA, 1997]. A brief clinical description of these samples has been reported [Ober et al., 2000; Lester et al., 2001]. Controls (209 European-Americans and 193 African Americans) consisted of adult individuals with negative personal and first-degree relative family history for asthma. Cases and controls reported at least three grandparents who were either of European or African American ancestry. No medical history was taken, and no medical testing was performed on control individuals. Twenty seven European Americans and 103 African Americans asthmatics were diagnosed with severe asthma on the basis of nocturnal symptoms, prescribed use of either inhaled or oral steroids, a forced expiratory volume measurements (FEV_1) < 60% predicted value at any time and time related reversibility (> 15% increase in baseline FEV_1 after treatment). This study was approved by the Institutional Review Board and written informed consent was obtained from all participants. Five samples (three European American controls and two African American cases, one of them with severe asthma) were excluded from subsequent analysis [Flores et al., 2007] since their self-reported ancestry did not match molecular ancestry determined by the genotypes of 30 microsatellite markers selected to be informative on individual European, Asian and African ancestries [Amundadottir et al., 2006].

CTTN Polymorphism Discovery

DNA samples from 48 unrelated non-asthmatic individuals (19 African and 29 European Americans) were used to search for common variations in the *CTTN* gene. DNA sequencing protocols and polymorphism identification were performed as previously described [Gao et al., 2006]. Primer pairs used are described in Table AI (Appendix A). The genomic sequence NM_005231 corresponding to *CTTN* isoform a, the longest transcript of the gene, was used as the reference sequence. Sequence variation is reported following established guidelines [Antonarakis, 1998].

Genotyping

Genotyping was conducted by means of TaqMan™ allelic discrimination assays on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) except for SNPs rs7131282 and rs34959377, that were genotyped using the SNaPshot® multiplex kit (Applied Biosystems, Foster City, CA). Briefly, amplification of SNP-containing fragments were performed in two separate PCR reactions (AmpliTaq Gold™ DNA Polymerase with 2.5 mM Mg^{2+} , 10% DMSO and 0.1 μ M of primers 5'-TGGAGTTATGTGGTGGAAAC-3' and 5'-GGGAAGAGAACACAGAGAAA-3' for rs7131282, and 5'-GTATTCTCTGAACCCTTGGGA-3' and 5'-CTGAGGCTGCTCTTAAACTG-3' for rs34959377 respectively). The SNaPshot® single base extension reaction was carried out simultaneously for both SNPs using 1 μ M of primers 5'-CCCTGCAGAGATGCGG-3' for rs7131282 and 5'-CCCCCGGGAAGTCAAGGGAACG-3' for rs34959377 based on manufacturer's recommendations. All genotype calls, excepting for rs7131282 and rs34959377 (genotyped by SNaPshot® reactions), were performed automatically by the SDS software (Applied Biosystems, Foster City, CA) based on discriminating plots (95%

confidence) from the ratio between the allele probe fluorescent intensities after normalization. Genotyping was blind to the ethnic background of the sample or case and control status. Approximately 10% of the samples were genotyped in duplicate to monitor genotyping quality.

Statistical Analysis

Departures from Hardy-Weinberg equilibrium (HWE) were tested by means of an exact test. The multiple-marker selection algorithm, haplotype r^2 , included in TagIT 3.03 software [Weale et al., 2003] was used to select a set of tagging SNPs (tSNPs) satisfying a minimum performance of $r^2 > 0.90$. This algorithm is known to maintain the tSNPs prediction accuracy while tending to increase tagging efficiency by selecting fewer tSNPs than other existing methods [Ding and Kullo, 2007]. A SNP-dropping-with-re-sampling method [Ahmadi et al., 2005] was used to verify that the expected properties of the tSNP set ensured a minimum performance of $r^2 > 0.85$ in both populations. SNP associations were tested by means of an additive model using the Armitage trend test. SPSS 14.0 (SPSS Inc., Chicago, IL) was used for multiple logistic regression analysis, including use of individual ancestries based on 30 microsatellites as covariates [Flores et al., 2007] when appropriate, to estimate the odds ratios (OR) with their respective 95% confidence interval (CI). EPIDAT 3.0 was used for the Mantel-Haenszel stratified analysis and to estimate the effects of the two risk variants in *CTTN* and *MYLK* genes, the latter using dominant models for both SNPs due to the small sample sizes. A non-additive effect for gene-gene interaction was tested by means of SNPstats [Sole et al., 2006]. Individual SNP associations were adjusted for multiple testing as implemented in the Simple Interactive Statistical Analysis web site [Sankoh et al., 1997], using the Šidák procedure taking into account the composite linkage disequilibrium (LD), which works well in capturing the LD correlation among SNPs [Zaykin et al., 2006], as a correlation estimator. In addition, we used two replication samples to reduce the likelihood of type I error. *P*-values were not corrected for the two conditions tested due to the non-independent nature of both phenotypes. The patterns of LD were explored using Haploview 3.32 [Barrett et al., 2005].

Results

SNP discovery and tagging SNP (tSNP) selection

A total of 27 *CTTN* variants, all in Hardy-Weinberg equilibrium, were identified by re-sequencing 9,746 base pairs in each sample with each variant consisting of a single base change (SNP), with the exception of variation number 27 (ss76859860) which demonstrated a single base insertion (Table I). These SNPs generate an overall occurrence of one SNP every ~375 bp, similar to the average rate (one *per* 348 bp) reported in a re-sequencing survey of 106 genes [Cargill et al., 1999]. Ten SNPs were entirely novel, two SNPs predicted an amino acid change, and 4 SNPs were not previously validated by frequency. *CTTN* SNPs 1, 2, 8, 13 and 27 showed a MAF < 5% in both populations whereas the previously described *CTTN* SNPs 1 (rs11825246) and 2 (rs11825335) demonstrated allelic frequencies of ~2-3% in Yoruba Nigerians (YRI) in the newly released HapMap [The International HapMap Consortium, 2005]. Linkage disequilibrium (LD) plots suggested a broad heterogeneity of LD in the region with strong and moderate values clustering in the 3' end of the gene in both populations, also extending to the 5' end in European Americans (Fig. 1). After excluding SNPs with MAF < 5%, a total of 6 and 7 SNPs were sufficient to provide an adequate coverage of *CTTN* gene variation in European and African Americans, respectively. Nine cosmopolitan tSNPs, providing adequate coverage in both populations, were selected for association studies. These tSNPs tagged common SNPs (MAF ≥ 5%) in YRI subjects and in samples from Utah residents with ancestry from northern and Western Europe (CEU) with slightly increased performances than those achieved in the re-sequenced

samples. Additionally, a total of 4 and 7 tSNPs from HapMap provided adequate coverage of *CTTN* gene variation in CEU and YRI, respectively, congruent with our estimates for European- and African Americans.

***CTTN* gene variants in disease susceptibility**

All SNPs displayed average automatic quality call values over 99% confidence. Among the successfully retyped samples, no discrepancies were found with the original genotypes for any of the SNPs, giving an estimated overall discordance rate of 0.0% (95% CI: 0.0-0.9%). Additionally, all *CTTN* tSNPs were in HWE in both panels (Table BI, Appendix B). The minor allele frequencies by population, SNP information, and *p*-values for association with asthma and severe asthma are shown in Table II. No SNP was associated with susceptibility to asthma in either European or African Americans, however, an intronic SNP (rs3802780) showed a significant uncorrected association with severe asthma in African Americans (OR: 1.62, 95% CI: 1.08-2.42). When adjusted for multiple testing, this association trended towards significance ($p \sim 0.08$) and strikingly, this was the only SNP with a significant uncorrected association in European Americans (OR: 2.21, 95% CI: 1.07-4.57), despite the small number of severe asthmatics in this panel. Nevertheless, an adjustment for multiple testing also drove this association towards borderline significance ($p \sim 0.09$). We noted that HWE *p*-value for this SNP was nearly significant in European American controls. However, quality measures for this SNP revealed satisfactory results since genotyping was simultaneous and blind to affection status or ethnicity and non-significant HWE *p*-values were found for other sample groups, automatic quality call was 99.45%, completion rate was 97%, and no differences for the rate of completion proportions were found between controls and severe asthmatics ($p = 0.32$). Thus, systematic errors in genotyping do not appear to account for these results. To rule out potentially spurious associations due to population stratification, proportions of ancestry estimated from 30 unlinked microsatellites available from these samples [Amundadottir et al., 2006] were considered for adjustments. Estimated individual ancestries were not significantly different between African American controls and severe asthmatics [Flores et al., 2007], and the results remained unchanged when included in a logistic regression model (OR: 1.61, 95% CI: 1.07-2.42, $p = 0.022$). In European Americans, both European (0.98 ± 0.054 in controls vs. 0.99 ± 0.021 in severe asthmatics, *t*-test $p = 0.015$) and Asian (0.014 ± 0.049 in controls vs. 0.00 ± 0.006 in severe asthmatics, *t*-test $p = 0.001$) ancestries differed slightly but significantly between controls and severe asthmatics. However, the observed SNP association in European Americans remained unchanged in a logistic regression model (OR: 2.62, 95% CI: 1.22-5.63, $p = 0.014$). A stratified Mantel-Haenszel test ($p = 0.0022$) or a joint logistic regression model for the two populations taking into account the estimated ancestries ($p = 0.003$) further showed a strong association of this SNP with severe asthma. The rs3802780 A allele was associated with risk in both populations (joint OR: 1.71, 95% CI: 1.20-2.43) (Table III). A sliding window haplotype analysis performed as we previously described [Flores et al., 2007] failed to reveal significant results for any sample comparison (not shown).

Combined effects of *CTTN* and *MYLK* risk variants in severe asthma

The *CTTN* SNP rs3802780 was found to be significantly associated with severe asthma in the same African American case-control samples in which we previously reported the association ($p = 0.005$) of a non-synonymous *MYLK* coding SNP (rs9840993) with severe asthma [Flores et al., 2007]. As we have previously described strong biological interaction between these gene products [Dudek et al., 2002; Dudek et al., 2004], we explored the combined effects of both genes on severe asthma in African Americans. Other than an additive accumulation of risk from the alleles at both genes (Figure 2), interaction effects were not detected under any model tested (p -value > 0.10). However, African Americans carriers of at least one risk allele at both genes had almost a three-fold increase risk for

severe asthma (OR: 2.93, 95% CI: 1.40-6.13, $p = 0.004$) compared to non-risk allele carriers at both genes.

Discussion

Although the TENOR [Dolan et al., 2004] and the ENFUMOSA [2003] multi-centric studies have provided new epidemiological information regarding severe or difficult-to-treat asthma, the pathogenic mechanisms leading to this condition remain unclear. The structural changes in the airways accompanying asthma, a consequence of an airway injury/repair driven by chronic inflammation [Orsida et al., 1999; McDonald, 2001; Black and Johnson, 2002], are collectively termed airway remodeling with epithelial changes, mucus gland and goblet cell hyperplasia, subepithelial fibrosis, airway smooth muscle cell hypertrophy and hyperplasia, and vascular remodeling. Remodeling is most prominent in severe asthma and contributes to the development of persistent bronchial hyperresponsiveness, chronic airflow obstruction and lack of responsiveness to corticosteroid treatment [Holgate and Polosa, 2006]. Twin studies have suggested that asthma severity exhibits a hereditary component [Sarafino and Goldfeder, 1995] and, congruently, several genes have been associated with disease severity [Ober and Hoffjan, 2006]. Firmly replicated susceptibility genes, such as *ADAM33* and *TGFBI*, encode molecules which participate in the remodeling processes (rather than in immunological aspects), are highly expressed in airway tissues/cells of severe asthmatics [Minshall et al., 1997; Balzar et al., 2005; Lee et al., 2006b; Foley et al., 2007], and demonstrate significant association with severe asthma exacerbations [Pulley et al., 2001; Jongepier et al., 2004; Kedda et al., 2006; Mak et al., 2006].

Despite the evidence provided by biochemical studies, the exact role of cortactin in asthma remains ill-defined but likely involve participation in processes such as cell migration, angiogenesis and immunoregulation thereby contributing to the complex cellular and molecular events involved in remodeling [Jeffery, 2001]. Consistent with this concept, re-sequencing *CTTN* gene and genotyping 9 tSNPs in case-control samples of diverse ancestry revealed a SNP (rs3802780) significantly associated with severe asthma, where remodeling is suggested to be the major determinant for the persistent and uncontrolled phenotypic characteristic [Holgate and Polosa, 2006]. Despite this persuasive pathobiologic rationale, we fully recognize that individually, our results are only modestly significant for *CTTN* effects in asthma and did not survive strict correction for multiple testing (adjusted p -values 0.09). However, the consistent association of the identical SNP with the same phenotype in asthma in two independent case-control samples of African and European American descent provides strong evidence for a modest but potentially significant role of *CTTN* gene in severe asthma. We also recognize the limitations of our reduced sample size studied. As a result, our results must be interpreted with caution since statistical power was not adequate to detect associations of variants with weak-to-moderate effects in severe asthma, particularly in European Americans (power < 30%). Additional limitations of our study is the utilization of controls in which asthma was not clinically excluded thereby failing to allow for evaluation of potential confounding factors that may alternatively explain the association. Moreover, it can be argued that the inclusion of control samples not clinically characterized would tend to favor null hypothesis (no association between variants and phenotypes) due to the potential inclusion of subjects with undiagnosed asthma, reducing the power of the study even further (on average <4% as estimated by Kurz et al., [2006]). It remains unlikely, however, that any of controls subjects had undiagnosed asthma (undiagnosed severe asthma is unquestionably highly unlikely), particularly given that no first degree relative had asthma in this over 18 years old cohort. We reduced the likelihood of false positives by adjusting for the proportions of ancestry estimated from 30 unlinked microsatellites that were selected for being informative for distinguishing between European, African and Asian ancestries [Amundadottir et al., 2006]. Although not

empirically evaluated for their individual assignment ability, similar numbers of less informative microsatellites have demonstrated an assignment accuracy of >99% among European and African Americans [Yang et al., 2005]. However, since these markers were not selected for their ability to detect substructure within continental regions [Price et al., 2008], another limitation of the study is that modest levels of population stratification cannot be discarded that may have biased the results.

Although rs3802780 is intronic, the G allele is conserved across several (but not all) mammalian species (rhesus monkey, dog, armadillo and opossum) as assessed by the UCSC Genome Browser. In order to explore if other variant with apparent functional consequences in *CTTN* or in flanking genes may explain the association of this SNP with severe asthma, we examined the LD patterns of common (MAF \geq 5%) variation in our re-sequenced samples and in the \sim 400 kb region around *CTTN* (including the two flanking genes, *PPFIA1* and *SHANK2*, the latter encoding a multidomain scaffolding protein which, similar to MLCK, binds directly to the SH3 domain of cortactin [Redecker et al., 2001; McWilliams et al., 2004]) in CEU and YRI HapMap samples. Since rs3802780 did not exhibit moderate-to-strong LD levels to SNPs outside of *CTTN* gene, this suggested that the association with severe asthma was most likely to be directly due to the *CTTN* gene (Figure C1, Appendix C). Maximum r^2 values with flanking regions were 0.26 with rs3781649 in YRI and 0.68 with rs3781646 in CEU, both SNPs located in *PPFIA1* gene. Seven additional *CTTN* SNPs in high LD with rs3802780 ($r^2 \geq$ 0.85) and common to both populations were: rs643301, rs482438, rs1198236, rs548687, rs611216, rs592501, and rs1198234. Of these, only rs643301 was a coding SNP but only predicted a synonymous change (Tyr486Tyr). The other three coding SNPs in *CTTN* showed weak levels of LD with the associated SNP in both HapMap samples (rs2298397 was the only other coding SNP available and showed MAF \geq 5% in CEU; $r^2 =$ 0.07) and the re-sequencing samples (maximum r^2 values were 0.26 with the novel SNP ss76859857 in African Americans, 0.18 with the novel SNP ss76859859 in European Americans). Thus, non-synonymous *CTTN* SNPs were weakly correlated with rs3802780, and functional consequences of the SNPs in strong LD with it in either the *CTTN* gene itself or in flanking genes were not apparent.

Finally, we also demonstrated that severe asthma risk for African Americans increases by three-fold in carriers of at least one risk allele at both rs3802780 and rs9840993, a previously described severe asthma susceptibility polymorphism in *MYLK* gene [Flores et al., 2007]. Although these two proteins physically interact in cellular environments [Dudek et al., 2002], an effect due to gene-gene interaction was not detected. Rather, our results suggest that variation in multiple genes in this pathway have an additive effect on risk for severe asthma. However, according to our own estimations following Gauderman [2002], larger sample sizes would be required to derive a firm conclusion pertaining to the gene-gene interaction.

In summary, motivated by published positional and biological evidence, we studied the association of 9 carefully selected tSNPs of *CTTN* gene with susceptibility to asthma in multi-ethnic case-control samples and identified a promising association with the intronic SNP rs3802780 in severe asthma. Differences for the expression level of this gene have been reported between HapMap YRI and CEU cell lines [Storey et al., 2007; Zhang et al., 2008]. However, our data indicates that this SNP *per se* is not contributing to disparities in asthma severity between ethnic groups [El-Ekiaby et al., 2006]. Additionally, the combined effect of both rs3802780 and rs9840993, a previously described susceptibility SNP in the *MYLK* gene in African Americans, was much greater than the effects of either individual SNP. These observations, combined with reduced LD in other variants in nearby regions in reference samples [The International HapMap Consortium, 2005], support our hypothesis that the *CTTN* gene contains severe asthma susceptibility polymorphisms. We can not yet

conclude whether this association is truly positive and if it is directly determined by rs3802780 or by other gene variant(s), the focus of additional future studies to confirm this association.

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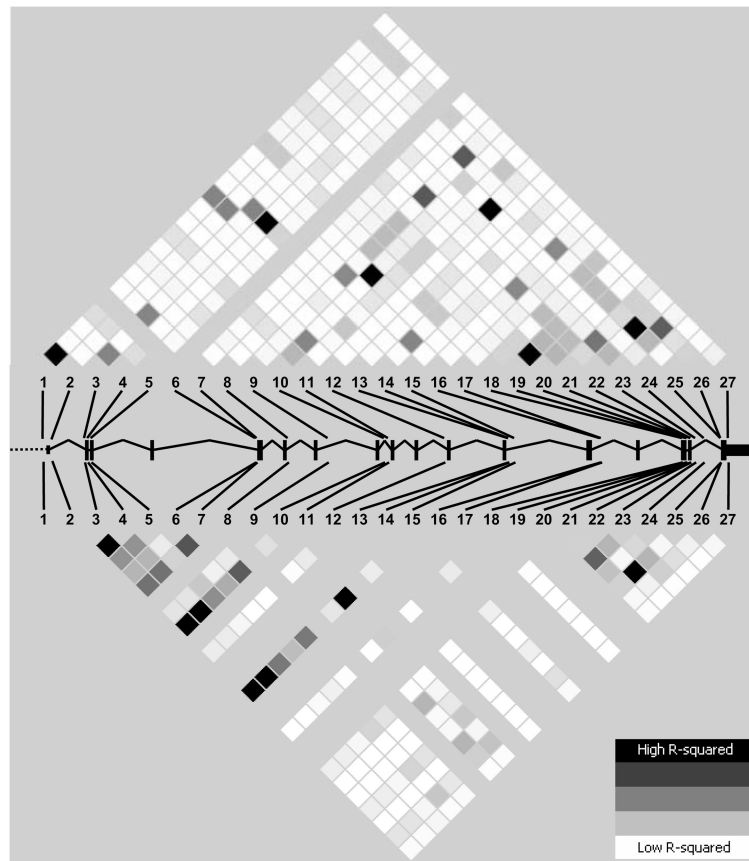


Figure 1. Linkage disequilibrium patterns (measured by r^2) in African (upper panel) and European Americans (lower panel). Boxes denote the approximate location of *CTTN* gene exons. Polymorphisms are numbered as in Table I.

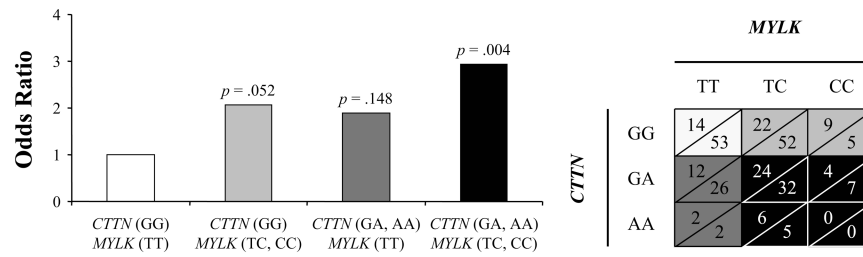


Figure 2. Combined effects of *CTTN* (rs3802780) and *MYLK* (rs9840993) genotypes in severe asthma in African Americans. Histograms indicating the odds ratios for the different genotype combinations for the *CTTN* rs3802780 G/A and *MYLK* rs9840993 T/C compared to the reference non risk genotypes for both SNPs. Genotype counts in cases (above the diagonal) and controls (below the diagonal) are also shown.

Table 1

Summary of variation (tSNPs in bold) in the CTTN gene exons and flanking intronic regions in European and African Americans.

SNP #	Position ^a	Location (effect) ^b	rs/ss #	Flanking (5')	Flanking (3')	Val ^c
1	69928894	IVS1-125C>G	rs11825246	-	-	?
2	69929306	IVS2+191C>C	rs11825335	-	-	?
3	69931037	IVS2-15C>T	rs2298396	-	-	?
4	69931123	c.72C>T (p.Thr24Thr)	rs2298397	-	-	?
5	69931234	IVS3+96C>T	rs569732	-	-	?
6	69938246	IVS5-50C>T	rs34053053	-	-	?
7	69938257	IVS5-39T>C	rs7932550	-	-	?
8	69939481	IVS7+10G>A	ss76859851	GGTAAGACGC	AAAGGTGCAG	-
9	69940999	IVS8+122G>A	rs1198234	-	-	?
10	69943894	IVS9+284G>A	rs35617256	-	-	?
11	69944090	IVS9-62A>G	ss76859852	TGAAAAACAT	CTTTCCACCT	-
12	69946558	IVS11-136C>T	rs12802851	-	-	?
13	69949352	IVS13+197C>T	ss76859853	ACCTTTTAGA	GCAGCATGGT	-
14	69949368	IVS13+213C>T	ss76859854	ATGGTGCAGC	GTTTCAGTGG	-
15	69949575	IVS13+420G>A	rs3781650	-	-	?
16	69952996	IVS14+43C>A	ss76859855	TCCCTGGGAC	TGTGCCGAGG	-
17	69953003	IVS14+50G>A	ss76859856	GACCTGTGCC	AGGGGATTGG	-
18	69956879	c.1291C>G (p.Leu431Val)	ss76859857	CAAGGCAGAG	TGAGCTACAG	-
19	69957113	IVS16+81G>T	rs11825631	-	-	?
20	69957275	IVS16+243G>A	ss76859858	GCAGAGGAAAG	GAGGGTTTCA	-
21	69957407	c.1451G>A (p.Ser484Asn)	ss76859859	TTAGAGGACA	CACCTACGAT	-
22	69957414	c.1458C>T (p.Tyr486Tyr)	rs643301	-	-	?
23	69957575	IVS17+103G>A	rs17160866	-	-	?
24	69957890	IVS17+418A>G	rs34959377	-	-	?
25	69958640	IVS17-140G>A	rs3802780	-	-	?
26	69958655	IVS17-125T>C	rs7131282	-	-	?
27	69958933	g.*18_19insC	ss76859860	GCCTCCCCC	GGAGCTGCGC	-

^aChromosome position according to NCBI build 36;

^bAs in CTTN isoform a;

^cValidation status:

^dfrequency available,

^eunknown or by 2-hit as in dbSNP build 126.

Table II

Genotyped SNPs, MAFs and unadjusted *p*-values for association with asthma and severe asthma in the two populations.

SNP ^a	Location (effect) ^b	rs/ss #	Position (Build 36)	African Americans				European Americans					
				MAF		<i>p</i> -value		MAF		<i>p</i> -value			
				Controls (N=193)	Asthma (N=190) ^c	Severe (N=102) ^c	CAd	CS ^e	Controls (N=206) ^c	Asthma (N=110)	Severe (N=27)	CAd	CS ^e
5	Intron 3	rs569732	69931234	0.19	0.20	0.19	0.663	0.962	0.40	0.47	0.50	0.086	0.157
7	Intron 5	rs7932550	69938257	0.16	0.17	0.14	0.684	0.488	0.22	0.24	0.18	0.491	0.524
11	Intron 9	ss76859852	69944090	0.28	0.25	0.27	0.412	0.766	-	-	-	-	-
12	Intron 11	rs12802851	69946558	0.07	0.05	0.04	0.112	0.133	0.19	0.20	0.16	0.649	0.632
19	Intron 16	rs11825631	69957113	0.34	0.30	0.31	0.377	0.591	-	-	-	-	-
21	Exon 17 (Ser484Asn)	ss76859859	69957407	0.19	0.15	0.16	0.219	0.415	-	-	-	-	-
24	Intron 17	rs34959377	69957890	0.11	0.11	0.09	0.871	0.426	0.18	0.20	0.17	0.639	0.904
25	Intron 17	rs3802780	69958640	0.22	0.27	0.30	0.118	0.020	0.21	0.26	0.35	0.172	0.028
26	Intron 17	rs7131282	69958655	0.47	0.44	0.43	0.433	0.282	0.20	0.23	0.15	0.516	0.395

^aAs in Table I;

^bAs in CTTN isoform a;

^cExcluding outlier individuals determined in Flores et al. [2007];

^dControl vs. asthma comparison;

^eControl vs. severe asthma comparison. Statistically significant *p*-values in bold.

Effects of rs3802780 in severe asthma adjusted for individual ancestries in European and African Americans individually and in a joint analysis.

Table III

Sample ^a	GG	Genotype	GA	AA	Sample OR (95% CI)	p-value	Joint OR (95% CI)	p-value
African Americans								
Severe asthma	48 (48.5%)	42 (42.4%)	9 (9.1%)					
Controls	112 (60.5%)	66 (35.7%)	7 (3.8%)		1.61 (1.07-2.42)	0.022		
European Americans								
Severe asthma	10 (43.5%)	10 (43.5%)	3 (13.0%)					
Controls	116 (59.5%)	75 (38.5%)	4 (2.0%)		2.62 (1.22-5.65)	0.014	1.71 (1.20-2.43)	0.003

^aExcluding outlier individuals determined in Flores et al. [2007]