

Letter to the Editor

Promoter polymorphisms in *CHI3L1* are associated with asthma

To the Editor:

Increased expression of the glycoprotein Chitinase-3-like-1 (*CHI3L1*) gene has been associated with cancers, autoimmune diseases, and chronic inflammatory conditions such as asthma

(reviewed in Coffman¹). Asthma patients have a strong upregulation of YKL-40, the protein encoded by *CHI3L1*, in the airway epithelium and alveolar macrophages.² The levels of YKL-40 have been shown to be genetically regulated by the single nucleotide polymorphism (SNP) rs4950928; however, the data remain controversial. In a genome-wide association study, *CHI3L1* was found to be a susceptibility gene for asthma, bronchial

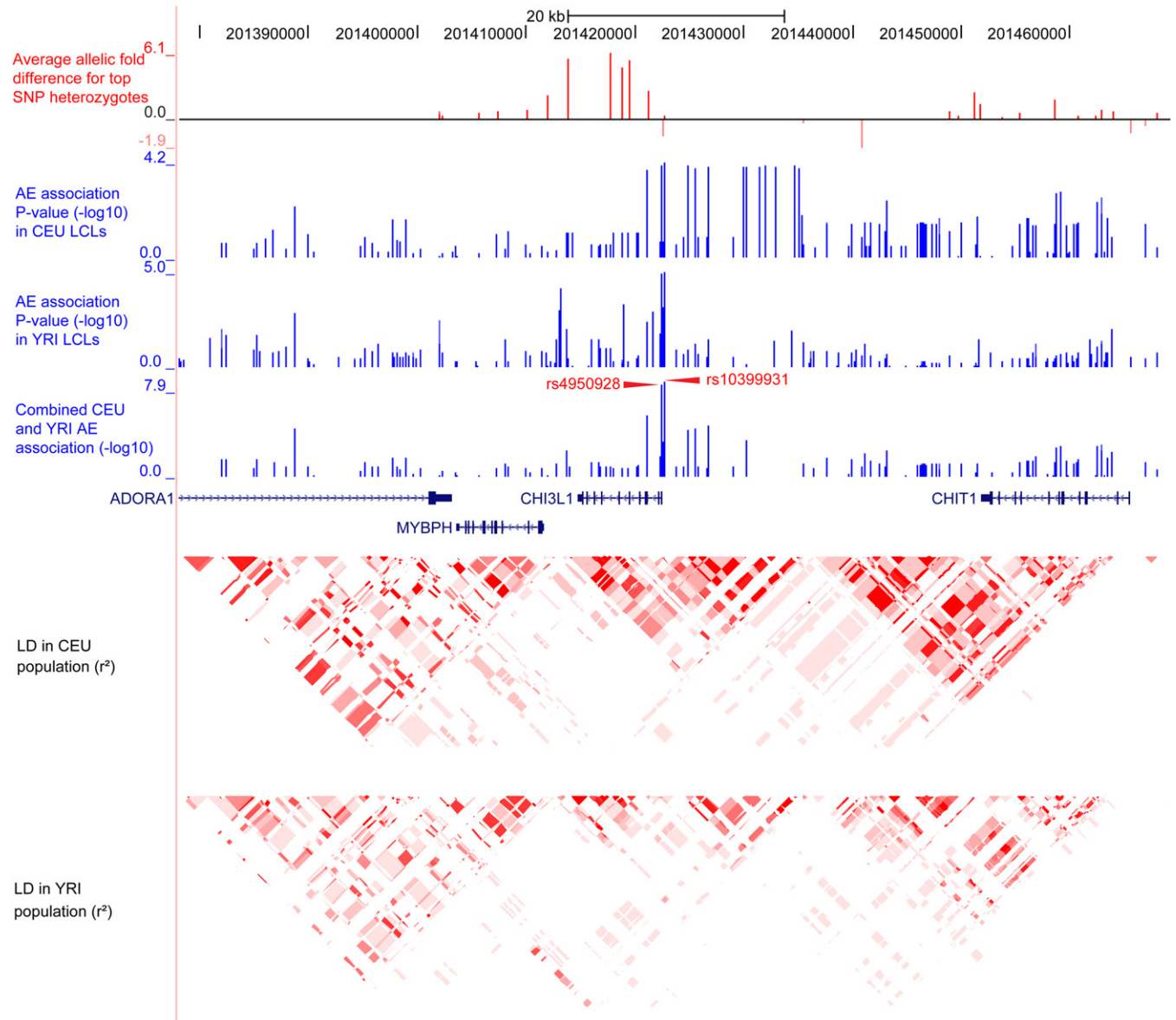


FIG 1. Functional analysis of the *CHI3L1* gene. AE mapping for *cis*-regulatory variation in *CHI3L1* locus using CEU and YRI population panels of lymphoblastoid cells. Coordinates (hg18) for locus shown on top; top track with vertical red bars show average phased allelic expression for rs10399931 heterozygotes indicated as fold-difference between alleles with up to 6-fold difference (C-allele overexpressed) observed for an expressed SNP in the primary transcript. Each of the red bars corresponds to independently measured population heterozygote ratio (average heterozygote ratio in DNA–average heterozygote ratio in RNA) at different expressed SNPs as described previously.⁵ The 3 blue tracks indicate the $-\log_{10}(P)$ value of the AE association across all SNPs tested in CEU, in YRI, and combined. Top SNP associations are highlighted in the combined mapping track. The location of transcripts is shown below this and bottom graphs show linkage disequilibrium maps (r^2). LCLs, Lymphoblastoid cell lines.

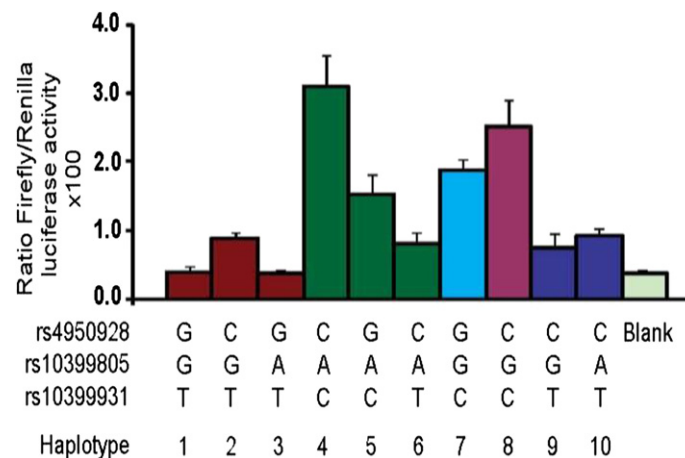


FIG 2. Gene reporter assays for the *CH13L1* 0.5-kb promoter haplotypes containing 3 SNPs (rs4950928, rs10399805, and rs10399931). Relative luciferase activity of the promoter haplotypes was measured following transient transfection in Jurkat cells. The empty promoterless pGL3-Basic vector was used as negative control. Results are expressed in a ratio of Firefly/Renilla activity multiplied by 100. In Jurkat cells, rs10399931 has the strongest effect on promoter activity. *P* values were calculated from 4 replicates with unpaired Student *t* test (for rs10399931, Hap02 vs Hap08 *P* < .0001; Hap01 vs Hap 07 *P* < .0001; for rs4950928, Hap07 vs Hap08 *P* = .02; Hap03 vs Hap10 *P* < .0001).

hyperresponsiveness, and reduced lung function. In relation to asthma, the causal SNP was -131 C → G (rs4950928) in the promoter region of *CH13L1*.³ By contrast, the rs4950928 G allele, and not the C allele, was shown to be associated with asthma in Danish.⁴

We have developed a tool that generates a genome-wide map of *cis*-regulatory SNPs based on interindividual variation in allelic expression (AE).⁵ Our approach measures the AE of intragenic SNPs in the HapMap lymphoblastoid cell lines using the Illumina 1M BeadChip Array. The genotypes and phase information for HapMap SNPs for each individual is publicly available (www.hapmap.org). By using normalized and AE scores for each SNP and then averaging across all measured SNPs within a transcript for every individual, we can test AE as a quantitative trait against local SNPs.^{5,6}

To investigate the differential expression observed in *CH13L1*,^{3,7} we combined AE data from Caucasian (CEU)⁵ and from unpublished genome-wide data set for Yoruban African (YRI) HapMap individuals (Ge et al⁵ and unpublished data) to look for causal variants. We validated the differential AE observed on high-density genotyping chips in both populations by using low-throughput sequencing. In CEU, a relatively long (>20-kb) region from the first intron of *CH13L1* extending to distally (5') of the gene shows strong linkage disequilibrium (LD), but in YRI the proximal promoter SNPs are not in significant LD with other variants, allowing fine-mapping of functional effects.

The strongest association explaining the differential expression of *CH13L1* (±50 kb) was observed for rs10399931 in both populations: in CEU, it explained 44% of *cis*-regulatory variance (*P* = 6.27×10^{-5}), and in YRI, it explained 48% of the variance (*P* = 9.35×10^{-6}). When combined across populations, this SNP showed strong evidence (*P* = 1.31×10^{-8} ; meta-analysis *P* value from Fisher combined test) for altering *cis*-regulation in human lymphoblastoid cell lines. We noted that the previously identified functional variant (rs4950928) explaining differential expression of *CH13L1* was also highly significant (Fisher combined *P* = 2.17×10^{-8}) but showed lower association in both populations. Given

the strong LD structure between Caucasian and African population for these top SNPs (Fig 1), we are not able to fine-map the signal with absolute confidence to rs10399931 alone. We therefore conclude that rs4950928 and rs10399931 are equally likely to modulate *CH13L1* expression. We then downloaded normalized full-transcript *CH13L1* expression scores from CEU and YRI lymphoblasts by using publicly available data sets RNA-Seq in both populations^{8,9} and exon-array data in YRI.¹⁰ The expression scores for *CH13L1* were not reported for CEU RNA-Seq⁸ data set; however, by both methods in the YRI population, we observed further evidence for functional importance of rs10399931, which indicates that in YRI this SNP is the strongest determinant for total expression variation as determined by regression analyses of total expression scores against rs10399931 genotypes using PLINK (*P*_{RNA-seq} = 3.14×10^{-7} and *P*_{ExonArrays} = 1.13×10^{-3} , respectively).

To assess the regulatory potential of rs10399931 and of the previously studied rs4950928, we subcloned haplotype-specific constructs for the *CH13L1* promoter (chr1:201422460-201423203) into a pGL3 vector containing a firefly luciferase reporter gene (Promega) as previously described.^{6,11} We tested 10 constructs in different human cell lines—cervical cancer (HeLa), choriocarcinoma (Jeg3), hepatocellular liver carcinoma (HepG2), osteosarcoma (MG-63), and T-cell lymphoblast-like (Jurkat)—and assessed them for haplotype-specific differences (see www.regulatorygenomics.org for all transfection assays). The transient transfections in Jurkat cells showed a strong allele-specific effect for the rs10399931 SNP (3.71 [2.89-4.69]-fold difference for its C/T alleles; *n* = 6), whereas the rs4950928 had a smaller impact (2.01 [1.35-2.47]-fold difference for its C/G alleles; *n* = 6) (Fig 2). The rs10399805, an SNP occurring frequently in the same haplotype, had no impact at all. No consistent genotype-dependent effects for these SNPs were observed in any of the other cell lines tested (data not shown). Among the haplotypes, the SNP rs4950928 appeared to modulate the primary *cis*-regulatory effect of rs10399931; this suggests a functional link between the SNPs in addition to the LD observed.

Finally, we tested the association of the rs4950928 and rs10399931 SNPs with asthma in a Saguenay-Lac-Saint-Jean asthmatic familial collection, which comprises 253 asthmatic probands and 1022 relatives of which 36.6% are also affected by asthma. Approval was obtained from the Centre de Santé et de Services Sociaux de Chicoutimi. Informed consent from the parents of participating children and the child's assent were obtained prior to their enrolment in the study. SNP genotyping and family-based association test analysis was performed as previously shown.⁶ The family-based association test revealed a positive asthma association with the C allele of rs10399931 ($P = .0036$; $Z = 2.91$) and with the C allele of rs4950928 ($P = .0122$; $Z = 2.51$), both of which are associated with an increase in *CHI3L1* expression. These data are in agreement with previous findings showing that the G allele of rs4950928 has been associated with decreased YKL-40 expression levels.⁷

In this study, we have confirmed the association of rs4950928 with the AE of *CHI3L1* but have also shown the presence of a second functional SNP (rs10399931). Because of the strong LD structure in Caucasian and African populations for these top SNPs, we were not able to fine-map the signal with absolute confidence to one of these SNPs alone. We therefore conclude that rs4950928 and rs10399931 are equally likely to modulate *CHI3L1* expression and susceptibility to asthma.

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