BRIEF REPORT

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LPAR1 and *ITGA4* Regulate Peripheral Blood Monocyte Counts

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ABSTRACT: We recently mapped a quantitative trait locus for monocyte counts to chromosome 9q31 (rs7023923). Here we extend this work by showing with two independent approaches that rs7023923 regulates the expression levels of the nearby *LPAR1* gene ($P<0.0001$), specifically implicating this gene in monocyte development. Furthermore, we tested 10 additional loci identified in the original analysis for replication in 1,122 individuals and confirm that rs6740847 near the alpha-4-integrin gene (*ITGA4*) associates with variation in monocyte counts (combined $P=2.7\times10^{-10}$). This variant is in complete linkage disequilibrium ($r^2=1$) with a previously reported eQTL for *ITGA4* (rs2124440), indicating that this is the likely causal gene in the region. Our results indicate that rs7023923 and rs6740847 respectively upregulate *LPAR1* and downregulate *ITGA4* expression and this increases the number of monocytes circulating in the peripheral blood. Further studies that investigate the downstream mechanism involved and the impact on immune function are warranted.
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KEY WORDS: *LPAR1*; *ITGA4*; eQTL; GWAS; lysophosphatidic; *EDG2*

Genome-wide association studies (GWAS) have recently been used to identify the first genetic loci to explain a significant fraction of the variation in blood cell numbers among healthy individuals [e.g., Ferreira et al., 2009; Soranzo et al., 2009]. The expectation is that some of these loci may not only explain variation within the normal, healthy range but may also play an important role in disease, as demonstrated for a locus recently identified to regulate both eosinophil counts and asthma risk [Gudbjartsson et al., 2009]. However, despite this recent progress, the actual causal genes in many regions of confirmed association remain to be identified. Furthermore, only a small fraction of the variation between individuals can be accounted for by the identified loci, as is the case for most complex traits and diseases [Manolio et al., 2009], indicating that many more loci remain to be identified.

In a recent GWAS of hematology traits [Ferreira et al., 2009], we identified 11 loci with strong association ($P<10^{-5}$) with monocyte counts, including a region on chromosome 9q31 that reached genome-wide significance (rs7023923, $P=8.9\times10^{-14}$) and replicated unambiguously ($P=0.001$) in an independent panel. However, we did not identify the most likely causal gene underlying the 9q31 association nor did we attempt to follow-up the 10 remaining loci that did not reach genome-wide significance. In the present study, we report results from gene-expression experiments and association analyses conducted in additional samples that identify (1) the likely causal gene in the

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9q31 region and (2) a novel confirmed locus regulating monocyte counts in the general population.

We first sought to identify the causal gene underlying our previous reported association between monocyte counts and chromosome 9q31 [Ferreira et al., 2009]. Three genes are located within 250 kb of rs7023923, namely, the lysophosphatidic acid receptor 1 (*LPAR1*; MIM♯ 602282, also known as *EDG2*, 74 kb distant from rs7023923), olfactory receptor family 2 subfamily K (*OR2K2*, 114 kb) and *KIAA0368* (147 kb). We hypothesized that the SNP previously shown to associate with variation in monocyte counts (rs7023923) may also associate with variation in the expression levels of one of these three genes and this would be the most likely causal gene in the region.

To test this hypothesis, we analyzed gene-expression data obtained from whole blood with Illumina HumanHT-12 arrays for 48 pairs of monozygotic (MZ) twins as described in the Supp. Methods. After quality control, expression levels were available for transcripts ILMN_1701441 in *LPAR1*, ILMN_1745801 in *OR2K2* and ILMN_1847822 in *KIAA0368* (Supp. Table S1). Genotype data for rs7023923 were also available for each twin pair as described previously [Ferreira et al., 2009]. Linear regression considering additive single nucleotide polymorphism (SNP) effects was then used to test whether rs7023923 was associated with the expression of each of the three transcripts, considering the mean expression for each MZ pair. SNP rs7023923 was found to be a significant predictor of the expression levels of *LPAR1* ($P = 9.5 \times 10^{-6}$; Table 1) but not of *OR2K2* or *KIAA0368* ($P > 0.05$). This SNP explained 35% of the between-individual variation in *LPAR1* expression; the rs7023923T allele that associated with increased *LPAR1* expression also associated with increased monocyte counts. We replicated these results using a different technology (DeepSAGE, Supp. Methods) in 94 independent samples ($P = 5.4 \times 10^{-5}$, Supp. Fig. S1). Furthermore, results from a large study of the monocyte transcriptome [Zeller et al., 2010] strongly support our observation (Supp. Table S2). Thus, our results specifically implicate *LPAR1* in the regulation of monocyte counts.

Table 1. Association Between the Expression Levels of *LPAR1* Measured in Whole Blood with Illumina Expression Arrays[a] and rs7023923 Genotype, a SNP Previously Confirmed to Associate with Monocyte Counts [Ferreira et al., 2009]

Genotype	N	Mean	SD	Effect[b]	SE	h^2(%)[c]	P-value
CC	10	21.8	7.3				
CT	25	28.7	14.4	13.8	2.8	35.0	9.5×10^{-6}
TT	13	48.6	13.3				

[a]Expression of *LPAR1* was assessed by probe ILMN_1701441 that is present in the Illumina HumanHT-12 v3.0 Beadchip used.
[b]Effect corresponds to the slope from a linear regression of expression levels on the number of T alleles. Sex was not a significant predictor of the expression of the transcripts tested and so was not included as a covariate.
[c]h^2 represents the proportion of the between-individual variation in gene expression explained by the SNP.
N, sample size; SE, standard error.

Lysophosphatidic acid (LPA) is a ubiquitous extracellular growth factor produced from membrane phospholipids that exerts physiological effects through five high-affinity cognate receptors [Choi et al., 2010]. The first to be identified was the LPA1 receptor [Hecht et al., 1996], which is encoded by the *LPAR1* gene that we now implicate in monocyte development. This receptor is moderately expressed in many human tissues, including monocytes [Choi et al., 2010; Goetzl et al., 2000], and it has been suggested to mediate the capacity of LPA to suppress cellular apoptosis [Goetzl et al., 1999]. Together with our results, these data suggest that the rs7023923T allele increases the numbers of peripheral blood monocytes by increasing the

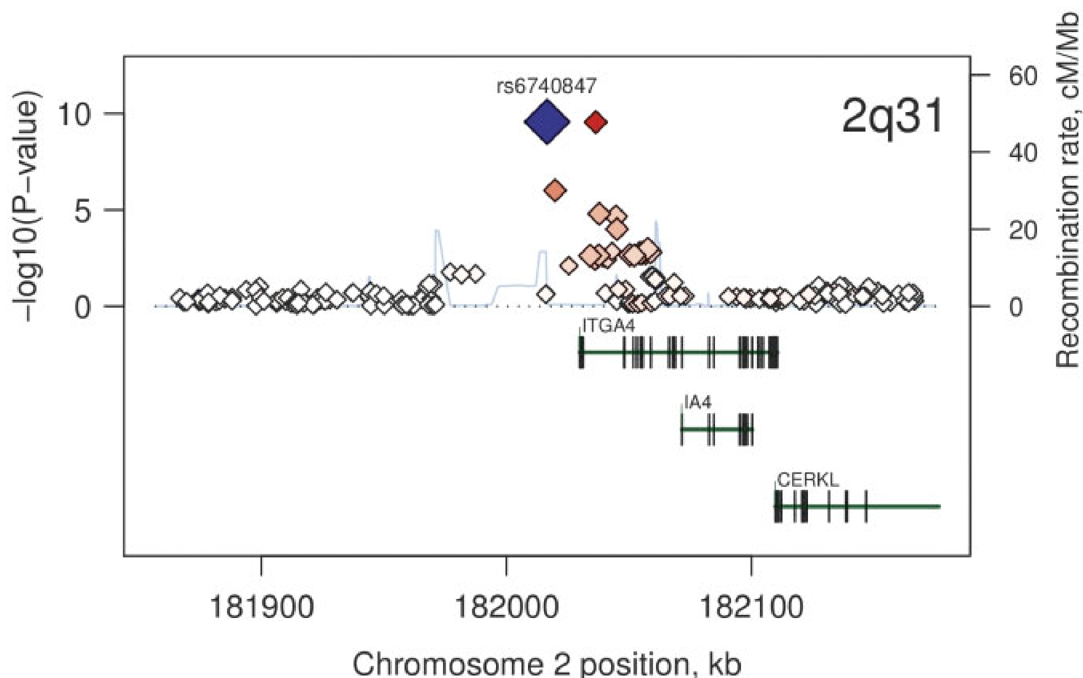

Figure 1. Regional plot of association between SNPs in chromosome 2q31 and monocyte counts. Results are shown for the combined analysis of the original GWAS panel ($N = 4{,}225$) and the new replication panel ($N = 1{,}122$) and include the most associated SNP (rs6740847, shown in blue, $P = 2.7 \times 10^{-10}$) as well as all available SNPs within 150 kb of rs6740847. The color of the remaining markers reflects the linkage disequilibrium (r^2) with rs6740847 (increasing red hue associated with increasing r^2). The recombination rate (second y axis) is plotted in light blue and is based on the CEU HapMap population. Exons for each gene are represented by vertical bars, based on all isoforms available from the March 2006 UCSC Genome Browser assembly.

surface expression of the LPA1 receptor, which in turn upregulates LPA signaling pathways that promote monocyte proliferation or survival.

Next, we followed-up the 10 top loci that did not reach genome-wide significance in our original GWAS of monocyte counts in a further 1,122 unrelated individuals ascertained from the general population and tested as described in the Supp. Methods. Of those 10 SNPs, only one located in chromosome 2q31 showed a convincing association with monocyte counts in the replication panel (rs6740847, uncorrected $P = 0.002$; Supp. Table S3). In the combined analysis of the original GWAS and replication panels ($N = 5{,}347$) the association with this locus was highly significant ($P = 2.7 \times 10^{-10}$). Thus, our results indicate that a novel locus that regulates monocyte counts is located on chromosome 2q31.

The rs6740847 variant is located 13 kb upstream from the transcription start site of the alpha-4-integrin gene (*ITGA4*; MIM♯ 192975; Fig. 1). We therefore tested whether rs6740847 was a significant predictor of *ITGA4* expression levels in the blood, but found no significant association with the specific probe for this gene available in the Illumina expression array used (Supp. Table S4) or for the nearby *CERKL* (MIM♯ 608381) gene (four probes, all with $P > 0.05$). We sought to confirm these negative results using deepSAGE analysis in 94 independent samples, but instead observed that the rs6740847G allele was associated with decreased *ITGA4* expression ($P = 0.02$; Supp. Fig. S2). No association was observed with *CERKL* (not shown). Furthermore, we subsequently noted that rs6740847 was in complete LD ($r^2 = 1$) with an eQTL for *ITGA4* in monocytes (rs2124440, $P = 2.2 \times 10^{-33}$; Supp. Table S5) recently identified by Zeller et al. [2010]. These results thus suggest that *ITGA4* is indeed the likely causal gene underlying the rs6740847 association with monocyte counts. The negative results observed in our microarray eQTL analysis likely reflect the low power provided by the analysis of 48 samples (57% for $\alpha = 0.05$).

Alpha-4-integrin (or very late activation antigen-4, VLA-4) is expressed on the cell surface of most circulating and tissue-resident mononuclear leukocytes, including monocytes [Hemler et al., 1990]. It preferentially binds VCAM-1 and fibronectin produced by stromal cells and this adhesive interaction is crucial for leukocyte homing and trafficking through the bloodstream [Kukreti et al., 1997; Oostendorp and Dörmer, 1996]. Indeed, blocking alpha-4-integrin [Issekutz, 1991; Papayannopoulou and Nakamoto, 1993] or disrupting VCAM-1 expression [Gurtner et al., 1995] led to a significant increase in the number of circulating leukocytes. These experimental observations are consistent with our results for rs6740847, which suggest that the G allele increases monocyte counts in the peripheral blood by decreasing *ITGA4* expression.

In summary, results from our analyses specifically implicate the *LPAR1* and *ITGA4* genes in the regulation of monocyte counts, with the two variants identified (rs7023923 and rs6740847) jointly accounting for 1.2% ($P = 0.0015$) of the between-individual variation in the replication cohort tested. Further studies are warranted that investigate the downstream molecular mechanisms involved and the potential impact of monocyte numbers in inflammation and the innate immune response.

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