**LBAR1** 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 monocyte. 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 \(P = 2.7 \times 10^{-18}\)). 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.


**KEY WORDS:** LPAR1; ITGA4; eQTL; GWAS; lysoosphatidic; EDG2

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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 hematologic 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, \(P = 8.9 \times 10^{-14}\)) 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...
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 x 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 x 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.

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

<table>
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<tr>
<th>Genotype</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Effectb</th>
<th>SE</th>
<th>h^2(%)c</th>
<th>P-value</th>
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<tr>
<td>CC</td>
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<td>21.8</td>
<td>7.3</td>
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<td>25</td>
<td>28.7</td>
<td>14.4</td>
<td>13.8</td>
<td>2.8</td>
<td>35.0</td>
<td>9.5 x 10^{-6}</td>
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<tr>
<td>TT</td>
<td>13</td>
<td>48.6</td>
<td>13.3</td>
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aExpression of LPAR1 was assessed by probe ILMN_1701441 that is present in the Illumina HumanHT-12 v3.0 Beadchip used.
bEffect 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.
ch^2 represents the proportion of the between-individual variation in gene expression explained by the SNP.

N, sample size; SE, standard error.
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 Dorner, 1996]. Indeed, blocking alpha-4-integrin [Isssekutz, 1991; Papayannopoulou and Nakamoto, 1993] or disrupting VCAM-1 expression [Gurtner GC, Davis V, Li H, McCoy MJ, Sharpe A, Cybulsky MI. 1995. Targeted disruption of the murine VCAM1 gene: essential role of VCAM-1 in chorioamnion fusion and placentation. J Biol Chem 265:14005–14014] can decrease 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.

Acknowledgments

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