Brief Report

Genome-Wide Association Study of White Blood Cell Counts in Patients With Ischemic Stroke

Nuria P. Torres-Aguila, MSc; Caty Carrera, MSc; Anne-Katrine Giese, MSc; Natalia Cullell, MSc; Elena Muiño, MD; Jara Cárcel-Márquez, MSc; Cristina Gallego-Fabrega, PhD; Jonathan González-Sánchez, MSc; María del Mar Freijo, MD; José Álvarez-Sabín, MD, PhD; Carlos Molina, MD, PhD; Marc Ribó, MD, PhD; Jordi Jimenez-Conde, MD, PhD; Jaume Roquer, MD, PhD; Tomás Sobrino, PhD; Francisco Campos, MD; José Castillo, MD; Lucia Muñoz-Narbona, PhD; Elena Lopez-Cancio, MD, PhD; Antoni Dàvalos, MD, PhD; Rosa Diaz-Navarro, MD; Silvia Tur, MD; Cristòfol Vives-Bauza, PhD; Gemma Serrano-Heras, PhD; Tomás Segura, MD; Jerzy Krupinski, MD, PhD; Raquel Delgado-Mederos, MD; Joan Martí-Fàbregas, MD, PhD; Laura Heitsch, MD; Laura Ibañez, PhD; Carlos Cruchaga, PhD; Natalia S. Rost, MD, PhD; Joan Montaner, MD, PhD; Jin-Moo Lee, MD, PhD; Israel Fernandez-Cadenas, PhD

Background and Purpose—Immune cells play a key role in the first 24h poststroke (acute phase), being associated with stroke outcome. We aimed to find genetic risk factors associated with leukocyte counts during the acute phase of stroke.

Methods—Ischemic stroke patients with leukocyte counts data during the first 24h were included. Genome-wide association study and gene expression studies were performed.

Results—Our genome-wide association study, which included 2064 (Discovery) and 407 (Replication) patients, revealed a new locus (14q24.3) associated with leukocyte counts. After Joint analysis (n=2471) 5 more polymorphisms reached genome-wide significance ($P<5\times10^{-8}$). The 14q24.3 locus was associated with acute stroke outcome (rs112809786, $P=0.036$) and with *ACOT1* and *PTGR2* gene expression. Previous polymorphisms associated with leukocyte counts in general-population did not show any significance in our study.

Conclusions—We have found the first locus associated with leukocyte counts in ischemic stroke, also associated with acute outcome. Genetic analysis of acute endophenotypes could be useful to find the genetic factors associated with stroke outcome. Our findings suggested a different modulation of immune cells in stroke compared with healthy conditions. **(*Stroke*. 2019;50:3618-3621. DOI: 10.1161/STROKEAHA.119.026593.)**

Key Words: genome-wide association study ■ humans ■ leukocytes ■ risk factors ■ stroke

Ischemic stroke (IS) is a complex disease. Even though there are several genes associated with stroke risk, little is known about the genetics behind stroke outcome. Only 2 genome-wide association studies (GWAS)[1,2] have found 2 loci associated with 3-month disability poststroke. The clinical complexity and multifactorial modulation of stroke outcome hinder the discovery of genetic associations. To avoid this problem, one strategy is to individually analyze internal

Received June 5, 2019; final revision received August 7, 2019; accepted September 5, 2019.

From the Stroke Pharmacogenomics and Genetics Laboratory, Hospital de la Santa Creu i Sant Pau Research Institute, Barcelona Spain (N.P.T.-A., E.M., J.C.-M., C.G.-F., J.G.-S., I.F.-C.); Neurovascular Research Laboratory, Vall d'Hebron Research Institute, Barcelona, Spain (N.P.T.-A., C.C., J.M.); Neurology Department, Massachusetts General Hospital, Boston (A.-K.G., N.S.R.); Stroke Pharmacogenomics and Genetics Laboratory, Fundació Mútua Terrasa, Hospital Universitari Mútua Terrassa, Spain (N.C., C.G.-F., J.G.-S.); School of Healthcare Science, Manchester Metropolitan University, Manchester, United Kingdom (J.G.-S., J.K.); Neurology Department, Hospital de Basurto, Bilbao, Spain (M.d.M.F.); Stroke Unit, Neurology Department, Hospital Universitari Vall d'Hebron, Barcelona, Spain (J.Á.-S., C.M., M.R.); Neurology Department, Institut Hospital del Mar d'Investigacions Mèdiques–Hospital del Mar Barcelona, Spain (J.J.-C., J.R.); Neurovascular Research Group, Institut Hospital del Mar d'Investigacions Mèdiques, Barcelona, Spain (J.J.-C., J.R.); Universitat Autónoma de Barcelona/Departamento de Ciencias Experimentales y de la Salud, Universitat Pompeu Fabra, Barcelona, Spain (J.J.-C., J.R.); Clinical Neurosciences Research Laboratory, Instituto de Investigación Sanitaria de Santiago de Compostela, Santiago de Compostela, Spain (T.S., F.C., J.C.); Neurosciences Department, Hospital Germans Trias I Pujol–Universitat Autónoma de Barcelona, Barcelona, Spain (L.M.-N., A.D.); Stroke Unit, Hospital Universitario Central de Asturias, Oviedo, Spain (E.L.-C.); Neuroscience Laboratory, Fundació Institut d'Investigació Sanitària Illes Balears, Mallorca, Spain (R.D.-N., S.T., C.V.-B.); Neurology Department, University Hospital of Albacete, Spain (G.S.-H., T.S.); Neurology Service, Hospital Universitari Mútua Terrassa, Spain (J.K.); Stroke Unit, Neurology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain (R.D.-M., J.M.-F.); Emergency Medicine (L.H.), Neurology Department (L.H., J.-M.L.), and Psychiatry Department (L.I., C.C.), Washington University School of Medicine, Saint Louis, MO; Stroke Research Program, Instituto de Biomedicina de Sevilla/Hospital Universitario Virgen del Rocío/Consejo Superior de Investigaciones Científicas/University of Seville (J.M.); and Department of Neurology, Hospital Universitario Virgen Macarena, Seville, Spain (J.M.).

The online-only Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/STROKEAHA.119.026593.

Correspondence to Israel Fernández-Cadenas, PhD, Stroke Pharmacogenomics and Genetics Laboratory, Hospital de la Santa Creu i Sant Pau Research Institute, Carrer de Sant Antoni Maria Claret, 167, 08025 Barcelona, Spain. Email israelcadenas@yahoo.es

factors of stroke (endophenotypes) associated with stroke outcome.

The acute phase of stroke (first 24 hours) dramatically influences the short and long-term outcome. During this phase, there is an important inflammatory and immune response. Immune cell levels, such as white blood cell counts (WBCc), have been associated with worse outcome.[3] Currently, there are several GWAS of WBCc performed in different populations[4,5] that have found >20 loci, but none have been related to stroke.

We aimed to find genetic risk factors associated with WBCc during the acute phase of IS due to its relevant role in poststroke outcome.

Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Population

The patients with IS included were >18 years old, had a measured neurological deficit on the National Institutes of Health Stroke Scale (NIHSS) within 6 hours poststroke onset, and underwent blood analysis with WBCc data within 24 hours poststroke onset. The outcomes studied were NIHSS at 24 hours (acute) and modified Rankin Scale at 3 months (long term). Written informed consent and ethical committee approval was obtained for all participants. Additional cohort details are in Methods in the online-only Data Supplement.

Genetic Analysis

Genotyping, imputation, and quality controls are described in Methods in the online-only Data Supplement. We performed the single-nucleotide polymorphism (SNP) association analysis using SNPTEST software, and the Joint analysis was performed with METAL software.

To study expression quantitative trait loci associated with the candidate SNPs, we performed in silico analyses with data from the Genotype-Tissue Expression Project. The statistical analyses were performed using SPSS software v17.0 (detailed in Methods in the online-only Data Supplement).

Results

In this study, 2132 European white IS patients were recruited in the Discovery cohort, and 407 American white patients were included in the Replication cohort. Detailed characteristics of the Discovery cohort are included in Table I in the online-only Data Supplement.

In the Discovery cohort, WBCc was independently associated with long-term and acute outcome (3-month modified Rankin Scale, $P=3.88\times10^{-4}$; 24-hour NIHSS, $P=6.81\times10^{-3}$). Besides, several variables were associated with WBCc in the univariate analysis (Table I in the online-only Data Supplement). After stepwise linear regression, age, diabetes mellitus, baseline NIHSS, and atherothrombotic stroke remained significant and were included as covariates in the genetic analyses.

In the Discovery GWAS, 68 participants were excluded for lacking some covariate; afterward, 2064 patients and 7 340 003 SNPs were analyzed. We found one locus in chromosome 14 associated with WBCc (Figure). Replication showed the same directionality as in the Discovery cohort for the 6 suggestive SNPs ($P<10^{-6}$) of the 14q24.3 locus, 3 of them with nominal significance ($P<0.05$; Table). In the Joint analysis, all 6 SNPs reached genome-wide significance ($P<5\times10^{-8}$; Table).

We performed an additional genetic association analysis in a subgroup of patients with data collected within the first 6 hours after IS, to test the influence of time collection. In this subcohort that represents 69.67% of the Discovery cohort of the study (n=1438), the 14q24.3 locus was associated with WBCc but did not reach genome-wide significance (Table II in the online-only Data Supplement). Additionally, to test whether the identified locus was associated with a specific cell type of WBCc, we performed genetic association analysis with the neutrophil and lymphocyte content for a subgroup of patients included in the Discovery cohort (neutrophil count, n=1199; lymphocyte count, n=793). The 14q24.3 locus was not significantly associated specifically with neutrophil or lymphocyte counts (Figure I in the online-only Data Supplement).

We tested the association of each genome-wide significant SNP in the Joint analysis with stroke outcome. For acute outcome, rs112809786 was associated with 24-hour NIHSS ($P=0.036$) and 4 other SNPs showed a trend ($P<0.1$; Table III in the online-only Data Supplement) with the same directionality of WBCc association (allele risk was associated with worse NIHSS score). However, for long-term outcome, no association was found (Table III in the online-only Data Supplement).

We searched previously described SNPs reported in GWAS of WBCc, in different ethnicities and conditions, in our GWAS of WBCc in patients with IS. Twenty-eight of 30 SNPs were present in our GWAS, but none was significantly associated with WBCc after Bonferroni correction ($P>0.002$; Table IV in the online-only Data Supplement).

In silico approaches showed that the 14q24.3 locus was associated with expression quantitative trait loci of *ACOT1* in adrenal gland tissue ($P=2.0\times10^{-5}$) and *PTGR2* in thyroid tissue ($P=1.3\times10^{-5}$). For *ACOT1*, minor alleles were associated with higher gene expression, whereas for *PTGR2*, minor alleles were associated with lower expression.

Discussion

We have described the first locus (14q24.3) associated with the WBCc during the acute phase of stroke, confirmed by the Joint analysis of 2 independent populations. Additionally, this locus was not specific to any cell type population (neutrophils or lymphocytes) and was not influenced by the time of collection, at least during the first 6 hours after stroke. In addition, previous SNPs reported to be associated with WBCc in other healthy populations and diseases were not consistently associated with WBCc in stroke, suggesting a different genetic modulation for leukocyte proliferation/activation. Furthermore, we confirmed the previously reported association of WBCc with stroke outcome: we found an independent association of WBCc with acute and long-term stroke outcome with a positive correlation, whereby higher WBCc was associated with higher NIHSS and modified Rankin Scale and was consequently associated with a worse outcome. Moreover, the rs112809786 SNP within the 14q24.3 locus was associated with acute stroke outcome, in the same directionality as WBCc

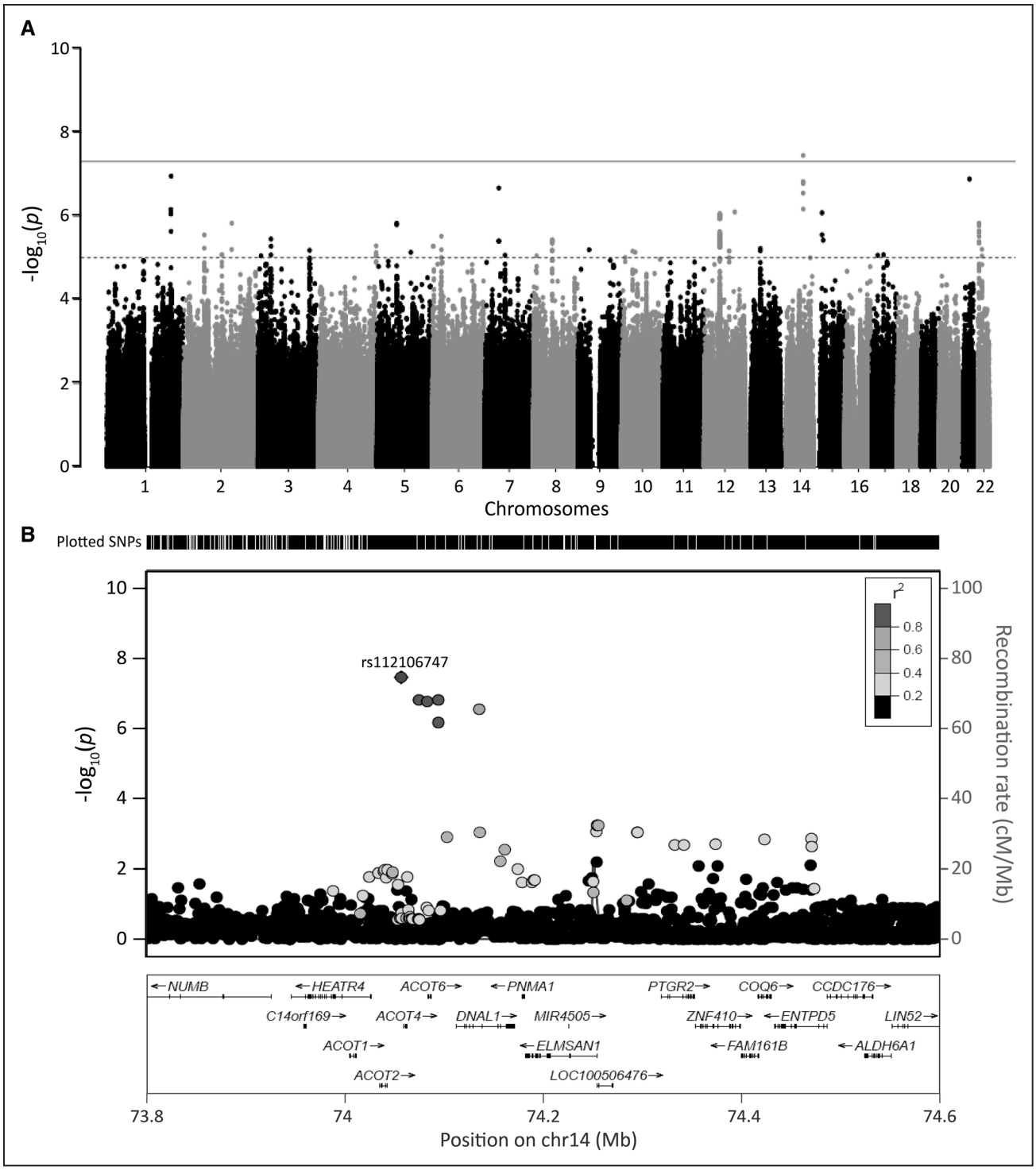

Figure. Results of the Discovery analysis. **A**, Manhattan plot of the Discovery analysis. Lines represent genome-wide significance ($P<5.0\times10^{-8}$) and suggestive threshold ($P<1.0\times10^{-5}$). **B**, Zoom plot performed on the LocusZoom portal of the 14q24.3 locus (rs112106747, top SNP). The *x* and *y* axes show chromosome location and negative logarithm to base 10 of *P* ($-\log_{10}$ [*P*]), respectively.

(the risk allele group, associated with high WBCc, had higher 24-hour NIHSS mean than the no-risk allele group). This finding supports the idea that individually analyzing endophenotypes could be an interesting strategy to find new genetic associations for stroke outcome. Unexpectedly, rs112809786 was not associated with long-term outcome. One reason could be that other factors that occur after the acute phase (infection, rehabilitation, etc) are influencing 3-month modified Rankin Scale. Our results suggested that the genetic factors associated with WBCc may have more influence on acute outcome than on long-term outcome.

Besides, in silico approaches revealed expression quantitative trait loci of *ACOT1* and *PTGR2* genes for 14q24.3 locus. *ACOT1*—an acyl-CoA thioesterase family gene member—is

Table. List of SNPs in the 14q24.3 Locus Associated With White Blood Cell Count

SNP	A1	A2	Discovery (n=2064)			Replication (n=407)		Joint (n=2471)	
			MAF	*P* Value	B±SE	*P* Value	B±SE	*P* Value	B±SE
rs112106747	G	A	0.016	3.57×10^{-8}*	0.67±0.12	0.061	0.43±0.23	8.05×10^{-9}*	0.62±0.1
rs113898499	A	G	0.016	1.54×10^{-7}	0.64±0.12	0.055	0.44±0.23	2.78×10^{-8}*	0.60±0.1
rs113492829	A	G	0.016	1.67×10^{-7}	0.63±0.12	0.058	0.44±0.23	3.16×10^{-8}*	0.59±0.1
rs112809786	A	G	0.017	6.89×10^{-7}	0.59±0.12	0.047	0.49±0.25	3.16×10^{-8}*	0.57±0.1
rs78476982	C	T	0.016	1.54×10^{-7}	0.64±0.12	0.018	0.59±0.25	8.01×10^{-9}*	0.63±0.1
rs74995185	A	G	0.016	2.86×10^{-7}	0.63±0.12	0.032	0.72±0.33	2.51×10^{-8}*	0.64±0.1

A1 indicates allele 1 (minor allele); A2, allele 2; B, β-coefficient for minor allele; MAF, minor allele frequency; and SNP, single-nucleotide polymorphism.
*Genome-wide significant *P* values.

described as being involved in the regulation of lipid metabolism by the PPAR (peroxisome proliferator-activated receptor)-α, which participates in the activation and proliferation cascade of some immune cells. Regarding *PTGR2*, it is described for the metabolism of prostaglandins and in the regulation of other PPAR family genes. Interestingly, *PTGR2* has been found downregulated in monocytes of patients with leukemia,[6] which is consistent with our in silico observation (14q24.3 alleles associated with higher WBCc were associated with lower *PTGR2* expression). Thus, *ACOT1* and *PTGR2* are candidate genes for regulating leukocyte proliferation and contributing to worse acute stroke outcome; however, further studies are necessary to confirm this potential association.

As a limitation, we could not exclude patients with conditions that may influence WBCc. However, this unbiased inclusion suggests that our findings could be applicable to any type of IS patients.

Acknowledgments

N.P. Torres-Aguila, C. Carrera, A.-K. Giese, and Dr Rost performed the data analysis, prepared the figures, and drafted a significant proportion of the manuscript. N. Cullell, Dr Muiño, J. Cárcel-Márquez, Dr Gallego-Fabrega, J. González-Sánchez, Dr Mar Freijo, Dr Álvarez-Sabín, Dr Molina, Dr Ribó, Dr Jimenez-Conde, Dr Roquer, Dr Sobrino, Dr Campos, Dr Castillo, Dr Lopez-Cancio, Dr Dàvalos, Dr Diaz-Navarro, Dr Tur, Dr Vives-Bauza, Dr Serrano-Heras, Dr Segura, Dr Krupinski, Dr Delgado-Mederos, Dr Martí-Fàbregas, Dr Heitsch, Dr Ibañez, Dr Cruchaga, Dr Montaner, and Dr Lee contributed to data acquisition and substantially edited the manuscript, giving their final approval. Dr Fernandez-Cadenas contributed to the study conception and design and to drafting the manuscript.

Sources of Funding

This study was supported by the Carlos III Health Institute, the European Regional Development Fund: Redes Temáticas de Investigacion Cooperativa en Salud, Invictus-Plus (RD16/0019/0002 and RD16/0019/0010), CPII17/00021, CPII17/00027, and CP14/00154; Fundació Marató de TV3 (76/C/2011); the National Institutes of Health (K23 NS099487 and R01NIH NS085419); the Spanish Ministry of Science and Innovation (PI051737, PI10/02064, PI12/01238, PI15/00451, and PI18/00022); and the Barnes-Jewish Hospital Foundation.

Disclosures

Dr Lee reports grants from Biogen outside the submitted work. Dr Heitsch reports grants from the National Institutes of Health NINDS K23, American Heart Association Career Development Grant, and Emergency Medicine Foundation Career Development Grant during the conduct of the study and personal fees from Genentech outside the submitted work. Dr Krupinski reports receiving personal fees for conferences from Boehringer, Bayer, and Ferrer. Dr Ribó reports personal fees and other fees from Anaconda Biomed and Cerenovus and personal fees from Medtronic, Stryker, and Apta Targets outside the submitted work. The other authors report no conflicts.

References

1. Mola-Caminal M, Carrera C, Soriano-Tárraga C, Giralt-Steinhauer E, Díaz-Navarro RM, Tur S, et al. PATJ low frequency variants are associated with worse ischemic stroke functional outcome. *Circ Res.* 2019;124:114–120. doi: 10.1161/CIRCRESAHA.118.313533
2. Söderholm M, Pedersen A, Lorentzen E, Stanne TM, Bevan S, Olsson M, et al; International Stroke Genetics Consortium, the NINDS-SiGN Consortium, and the Genetics of Ischaemic Stroke Functional Outcome (GISCOME) Network. Genome-wide association meta-analysis of functional outcome after ischemic stroke. *Neurology.* 2019;92:e1271–e1283. doi: 10.1212/WNL.0000000000007138
3. Nardi K, Milia P, Eusebi P, Paciaroni M, Caso V, Agnelli G. Admission leukocytosis in acute cerebral ischemia: influence on early outcome. *J Stroke Cerebrovasc Dis.* 2012;21:819–824. doi: 10.1016/j.jstrokecerebrovasdis.2011.04.015
4. Keller MF, Reiner AP, Okada Y, van Rooij FJ, Johnson AD, Chen MH, et al; CHARGE Hematology; COGENT; BioBank Japan Project (RIKEN) Working Groups. Trans-ethnic meta-analysis of white blood cell phenotypes. *Hum Mol Genet.* 2014;23:6944–6960. doi: 10.1093/hmg/ddu401
5. Jain D, Hodonsky CJ, Schick UM, Morrison JV, Minnerath S, Brown L, et al. Genome-wide association of white blood cell counts in Hispanic/Latino Americans: the Hispanic Community Health Study/Study of Latinos. *Hum Mol Genet.* 2017;26:1193–1204. doi: 10.1093/hmg/ddx024
6. Maffei R, Bulgarelli J, Fiorcari S, Bertoncelli L, Martinelli S, Guarnotta C, et al. The monocytic population in chronic lymphocytic leukemia shows altered composition and deregulation of genes involved in phagocytosis and inflammation. *Haematologica.* 2013;98:1115–1123. doi: 10.3324/haematol.2012.073080