2002;100(7):2292-302.
15. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937-51.
16. Malcovati L, Porta MG, Pascutto C, Invernizzi R, Boni M, Travaglino E, et al. Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol. 2005;23(30):7594-603.
17. Malcovati L, Germing U, Kuendgen A, Della Porta MG, Pascutto C, Invernizzi R, et al. Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol. 2007;25(23):3503-10.
18. Patnaik MM, Lasho TL, Finke CM, Gangat N, Caramazza D, Holtan SG, et al. WHO-defined 'myelodysplastic syndrome with isolated del(5q)' in 88 consecutive patients: survival data, leukemic transformation rates and prevalence of JAK2, MPL and IDH mutations. Leukemia. 2010;24(7):1283-9.
19. Chee CE, Steensma DP, Wu W, Hanson CA, Tefferi A. Neither serum ferritin nor the number of red blood cell transfusions affect overall survival in refractory anemia with ringed sideroblasts. Am J Hematol. 2008;83(8):611-3.
20. Kantarjian H, O'Brien S, Ravandi F, Cortes J, Shan J, Bennett JM, et al. Proposal for a new risk model in myelodysplastic syndrome that accounts for events not considered in the original International Prognostic Scoring System. Cancer. 2008;113(6):1351-61.
21. Passamonti F, Cervantes F, Vannucchi AM, Morra E, Rumi E, Pereira A, et al. A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). Blood. 2010;115(9):1703-8.
22. Tefferi A, Siragusa S, Hussein K, Schwager SM, Hanson CA, Pardanani A, et al. Transfusion-dependency at presentation and its acquisition in the first year of diagnosis are both equally detrimental for survival in primary myelofibrosis-prognostic relevance is independent of IPSS or karyotype. Am J Hematol 2009;85:14-17.
23. Elena C, Passamonti F, Rumi E, Malcovati L, Arcaini L, Boveri E, et al. Red blood cell transfusion-dependency implies a poor survival in primary myelofibrosis irrespective of International Prognostic Scoring System and Dynamic International Prognostic Scoring System. Haematologica. 2011;96(1):167-70.
24. Passamonti F, Rumi E, Elena C, Arcaini L, Merli M, Pascutto C, et al. Incidence of leukaemia in patients with primary myelofibrosis and RBC-transfusion-dependence. Br J Haematol 2010; in press.
25. Tefferi A, Mesa RA, Pardanani A, Hussein K, Schwager S, Hanson CA, et al. Red blood cell transfusion need at diagnosis adversely affects survival in primary myelofibrosis-increased serum ferritin or transfusion load does not. Am J Hematol. 2009; 84(5):265-7.

Determinants of platelet count in humans

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An adequate supply of circulating platelets is essential to maintain vascular integrity and to facilitate thrombus formation at sites of vascular injury. The normal platelet count in humans ranges from 150×10^9/L to 400×10^9/L. Given that platelets have a circulating lifespan of around 10 days, and that about one third of platelets are sequestered in the spleen at any time, approximately 100×10^9 of these small anucleate cells must be released from mature megakaryocytes into the circulation each day in order to maintain a normal platelet count. A constant balance is, therefore, required between thrombopoiesis, and platelet consumption and senescence. Thrombopoietin is the primary humoral regulator of megakaryocyte differentiation and platelet number under steady state conditions.[1] It is synthesized in the liver and kidney and mediates its effects through its receptor c-Mpl which is present on megakaryocyte and platelet membranes.[1] Levels of thrombopoietin are controlled via binding to, and internalization into, cells expressing the receptor. When platelets and megakaryocytes are decreased in number, less thrombopoietin is removed from plasma, and the thrombopoietin level rises, while when platelet numbers increase, more thrombopoietin is cleared from the plasma and the thrombopoietin level falls again.[2] In addition to the thrombopoietin (*THPO*) and c-Mpl (*MPL*) genes, a large number of genes encoding membrane glycoproteins, cytoskeletal components and proteins involved in transcription, cell cycle regulation and signaling have been demonstrated to participate in the differentiation of pluripotent stem cells to mature platelet-shedding megakaryocytes.[3]

Defined as a platelet count less than 150×10^9/L, thrombocytopenia is usually an acquired disorder. Causes include increased platelet consumption, splenomegaly, drugs or infection-mediated bone marrow suppression, and bone marrow failure. Increasingly, however, inherited forms of thrombocytopenia, caused by mutations in genes encoding proteins involved in the differentiation of megakaryocytes and platelet production, which can result in autosomal dominant, autosomal recessive, and X-linked recessive forms of inherited thrombocytopenia, are being recognized.[3]

Thrombocytosis, defined as a platelet count exceeding the upper limit of the normal range ($>400\times10^9$/L), is associated with an increased risk of thrombosis.[4] Primary thrombocytosis can be either inherited or acquired and is caused by alterations targeting hematopoietic cells (e.g. *MPL*, *THPO* or *JAK2* mutations) while secondary thrombocytosis is due to external factors such as chronic inflammation or cancer.[4]

While much has been done to elucidate the molecular mechanisms controlling megakaryocyte differentiation and platelet production and to identify defects in the genes encoding the relevant proteins in patients with inherited thrombocytopenia, the factors that determine platelet count in the normal population are less well characterized. In a study published in this issue of the journal, Biino *et al.* addressed this issue by carrying out a cross-sectional investigation of platelet counts among 12,517 inhabitants of ten villages in the Ogliastra region of

Eastern Sardinia.[5] This sparsely populated, mountainous province in Sardinia is unusual in that it has one of the most genetically homogeneous populations in Europe. The population has remained small and isolated throughout its history. Despite this, sub-populations in the region show a strong demarcation in their genetic pools, possibly due to different founders, to the extent that the small populations have divergent genetic histories even where they inhabit neighboring villages.[6] In their study, Biino *et al.* found that platelet count was higher in women, and that the prevalence of thrombocytopenia was higher among men (4.8%) than among women (3.2%). Interestingly, the distribution of platelet counts varied markedly between villages and the prevalence of thrombocytopenia ranged from 1.5% to 6.8% in the different villages studied. The thrombocytopenia was, in general, mild and asymptomatic and was not associated with other cytopenias or conditions known to be associated with thrombocytopenia. Furthermore, it showed a negative correlation with a mild form of thrombocytosis which ranged in prevalence from 0.9% to 4.5% between villages. Platelet counts were shown to decrease progressively with age, with the consequence that thrombocytopenia was more common among the elderly, while thrombocytosis was more frequent among younger people. Biino *et al.* concluded that the predispositions to platelet counts above or below the normal range are inherited traits.[5]

There is considerable evidence that the platelet count is largely determined by genetic factors. Monozygotic twins show a higher level of concordance in platelet count than dizygotic twins.[7] Also, differences in platelet count have been described in populations of different ethnic origin that share the same environment.[8] Longitudinal studies demonstrate considerable stability of steady state platelet counts. For example, in their analysis of serial platelet counts from 3,789 subjects, Buckley *et al.* showed that the repeatability of the platelet count was 0.871 in males and 0.849 in females, indicating that heritability of platelet count is high,[9] and while Biino *et al.* found that the heritability of platelet count was 54% in their study, figures of up to 87% have been reported in previous studies.[5,9]

So what genetic factors are responsible for determining platelet count? Despite the evidence that thrombopoietin and c-Mpl are essential for platelet production, there is little evidence for their genes having effects on the variation in platelet count in the normal population. In a genome-wide association study performed to identify quantitative trait loci for platelet count in inbred mouse strains, genotyping of mice with platelet counts at the extremes of the distribution led to the identification of two quantitative trait loci on chromosomes 1 and 11, neither of which appeared to be associated with known genes that regulate platelet production.[10] More interesting, perhaps, was the absence of quantitative trait loci in the region of the *Thpo* or *Mpl* genes, or those genes encoding interleukin-11, nuclear factor erythroid-derived 2 (Nf-e2), Gata-1 or Fog-1 all of which are important in platelet production.[10]

The first quantitative trait locus influencing platelet count in humans was identified by Soranzo *et al.* who performed a genome-wide association study for mean platelet volume, also a highly heritable trait, which is inversely correlated with platelet count, in the healthy population.[11] They identified a single nucleotide polymorphism, rs342293, on chromosome 7q22.3 which was significantly associated with mean platelet volume and platelet count in healthy subjects, though the effect on platelet count was entirely explained by its association with volume. The single nucleotide polymorphism occurred in an intergenic region that did not contain any genes, suggesting that regulation of mean platelet volume and platelet count are probably mediated through *cis*- or *trans*-regulatory effects. Interestingly, all six genes in a 1 Mb interval centered on the single nucleotide polymorphism were expressed in megakaryocytes and the platelet transcript levels of one of these, *PIK3CG*, varied according to genotype for rs342293, while those of a second gene, *PRKAR2B*, showed a non-significant correlation with genotype.[11] Both *PIK3CG* and *PRKAR2B* are credible candidates for contributing to mean platelet volume and platelet count. *PIK3CG* encodes the γ-chain of PI3/PI4-kinase which is responsible for the synthesis of phosphatidylinositol-3,4,5-trisphosphate (PIP3), which is essential for the initiation of megakaryopoiesis and proplatelet formation.[12] *PRKAR2B* encodes the β-chain of cAMP-dependent protein kinase, which has an antagonistic effect on megakaryopoiesis and proplatelet formation by reducing the release of intracellular calcium through the phosphorylation of ITPR3, the receptor for PIP3.[13]

More recently, Soranzo *et al.* detected 12 loci which

Table 1. Single nucleotide polymorphisms contributing to a common haplotype located at 12q24 and associated with platelet count.

SNP	Location	Gene	Protein	Alleles	PLT raising allele
rs3184504	Arg262Trp	*SH2B3*	SH2B adaptor protein 3	C/T	T
rs4766578	Intron	*ATXN2*	Ataxin 2	A/T	T
rs10774625	Intron			G/A	A
rs653178	Intron			T/C	C
rs11065987	Intergenic			A/G	G
rs17696736	Intron	*NAA25*	N α-acetyltransferase 25, NatB auxiliary subunit	A/G	G
rs17630235	3' region	*TRAFD1*	TRAF-type zinc finger domain containing 1	G/A	A
rs11066188	Intron	*C12orf51*	Probable E3 ubiquitin-protein ligase	G/A	A
rs11066301	Intron	*PTPN11*	Protein tyrosine phosphatase, non-receptor type 11	A/G	G
rs11066320	Intron			G/A	A

Data summarized from Soranzo et al.[14] *SNP: single nucleotide polymorphisms; PLT: platelet count.*

were reproducibly associated with mean platelet volume in 13,943 samples from six European population-based studies.[14] Four of these had been found to be associated with mean platelet volume in earlier studies by the group. Nine of the 12 loci were also associated with platelet count, the mean platelet volume-raising alleles being linked with a decrease in platelet count, though in all nine cases the single nucleotide polymorphisms were found to exert their effects on platelet count through the mean platelet volume. Three further loci which independently influenced platelet count were detected in the same study. An association signal at 6p21.3 (rs210135) centered on the *BAK1* gene which encodes a pro-apoptotic protein that regulates steady state platelet lifespan.[15] It was suggested that a locus at 9p24.1-p24.3 (rs385893) may regulate the expression of the proximal *JAK2* gene which is a key regulator of megakaryocyte maturation.[4,14] Two further single nucleotide polymorphisms, rs11065987 and rs11066301, map to a 1.6 Mb region located at 12q24, which contains 15 genes. Further investigation of the region identified ten single nucleotide polymorphisms, which formed a common haplotype spanning the length of the associated interval, all of which displayed significant association with platelet count (Table 1). These include a non-synonymous single nucleotide polymorphism, rs3184504, in the gene *SH2B3*, which encodes Lnk, an adaptor protein that mediates growth and maturation of megakaryocytes through its role in regulating cross-talk between integrin and cytokine signaling pathways.[16] Interestingly, this single nucleotide polymorphism was also associated with risk of coronary artery disease.[14] In addition, seven intronic single nucleotide polymorphisms within four genes (*ATXN2*, *NAA25*, *C12orf51* and *PTPN11*), one intergenic single nucleotide polymorphism and one single nucleotide polymorphism in the 3' region of the *TRAFD1* gene contributed to the 12q24 haplotype (Table 1).

In addition to genetic factors, there is evidence that gender, age and seasonal factors play a role in determining platelet count. Gender-dependent differences in the distribution of normal platelet counts have been reported in several studies, with higher counts being observed in females than in males, as was observed by Biino *et al.*[5,17] The inter- and intra-individual components of the variance in platelet count are similar in males and females, suggesting that the gender-dependent differences are most likely due to differences in hormone profiles. Megakaryocytes and platelets express steroid hormone receptors.[18] Differences in hormonal profiles between subjects might, therefore, be expected to result in different platelet phenotypes. Of interest is the finding that estrogen stimulates differentiation of $CD34^+$ cells and modulates expression of estrogen receptors α and β *in vitro*.[19] Furthermore, estradiol has been demonstrated to trigger proplatelet formation in megakaryocytes. Indeed, one of the targets in megakaryocytes for NF-E2, a transcription factor that is essential for proplatelet formation, is the gene for 3β-hydroxysteroid dehydrogenase which is essential for the formation of estradiol.[20]

An explanation for the age-dependent decline in platelet count observed by Biino *et al.* and others is still awaited.[5] It has been suggested that it may be due to reduced hematopoietic stem cell reserve in aging individuals.[8] It is also possible that the age-dependent decline in platelet number reflects epigenetic changes in the megakaryocyte genome, such as hypomethylation of genes that determine platelet count or changes in histone acetylation, which lead to differences in gene expression as we get older. The seasonal factors that in one longitudinal study accounted for 2% of the overall variance in platelet count, and caused a peak in platelet count during autumn and winter months, also remain to be clarified, though it is possible that this reflects the increased prevalence of viral infections during the winter months, since infection has been established to increase platelet count.[9] Thus, while considerable progress has been made in identifying the genes that determine platelet count in humans, several challenges remain, not least of which will be to determine the impact of epigenetic programming on platelet count. The value of populations such as those of the province of Ogliastra in facilitating greater understanding of this area cannot be overestimated.

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References

1. Kaushansky K. Determinants of platelet number and regulation of thrombopoiesis. Hematology Am Soc Hematol Educ Program. 2009; 147-52.
2. Nagata Y, Shozaki Y, Nagahisa H, Nagasawa T, Abe T, Todokoro K. Serum thrombopoietin level is not regulated by transcription but by the total counts of both megakaryocytes and platelets during thrombocytopenia and thrombocytosis. Thromb Haemost. 1997;77 (5):808-14.
3. Drachman JG. Inherited thrombocytopenia: when a low platelet count does not mean ITP. Blood. 2004;103(2):390-8.
4. Skoda RC. Thrombocytosis. Hematology Am Soc Hematol Educ Program. 2009;159-67.
5. Biino G, Balduini C, Casula L, Cavallo P, Vaccargiu S, Parracciani D et al. Analysis of 12,517 inhabitants of a Sardinian geographic isolate reveals that predispositions to thrombocytopenia and thrombocytosis are inherted traits. Haematologica. 2011;96(1):96-101.
6. Fraumene C, Petretto E, Angius A, Pirastu M. Striking differentiation of sub-populations within a genetically homogeneous isolate (Ogliastra) in Sardinia as revealed by mtDNA analysis. Hum Genet. 2003;114(1):1-10.
7. Whitfield JB, Martin NG. Genetic and environmental influences on the size and number of cells in the blood. Genet Epidemiol. 1985;2(2):133-44.
8. Segal JB, Moliterno AR. Platelet counts differ by sex, ethnicity, and age in the United States. Ann Epidemiol. 2006;16(2):123-30.
9. Buckley MF, James JW, Brown DE, Whyte GS, Dean MG, Chesterman CN, Donald JA. A novel approach to the assessment of variations in the human platelet count. Thromb Haemost. 2000(3);83:480-4.
10. Cheung CC, Martin ICA, Zenger KR, Donald JA, Thomson PC, Moran C, et al. Quantitative trait loci for steady-state platelet count in mice. Mamm Genome. 2004;15(10):784-97.
11. Soranzo N, Rendon A, Gieger C, Jones CI, Watkins NA, Menzel S, et al. A novel variant on chromosome 7q22.3 associated with mean platelet volume, counts and function. Blood 2009;113(16):3831-7.
12. Hawkins PT, Stephens LR. PI3Kgamma is a key regulator of inflammatory responses and cardiovascular homeostasis. Science. 2007;318(5847):64-6.
13. Wojcikiewicz RJ, Luo SG. Phosphorylation of inositol 1,4,5-trisphosphate receptors by cAMP-dependent protein kinase. Type I, II, and

III receptors are differentially susceptible to phosphorylation and are phosphorylated in intact cells. J Biol Chem. 1998;273(10):5670-7.
14. Soranzo N, Spector TD, Mangino M, Kühnel B, Rendon A, Teumer A, et al. A genome-wide meta-analysis identified 22 loci associated with eight hematological parameters in the HaemGen consortium. Nat Genet. 2009;41(11):1182-90.
15. Mason KD, Carpinelli MR, Fletcher JI, Collinge JE, Hilton AA, Ellis S, et al. Programmed anuclear cell death delimits platelet life span. Cell. 2007;128(6):1173-86.
16. Takizawa H, Eto K, Yoshikawa A, Nakauchi H, Takatsu K, Takaki S. Growth and maturation of megakaryocytes is regulated by Lnk/Sh2b3 adaptor protein through crosstalk between cytokine- and integrin-mediated signals. Exp Hematol. 2008;36(7):897-906.
17. Butkiewicz AM, Kemona H, Dymicka-Piekarska V, Matowicka-Karna J, Radziwon P, Lipska A. Platelet count, mean platelet volume and thrombocytopoietic indices in healthy women and men. Thromb Res. 2006;118(2):199-204.
18. Khetawat G, Faraday N, Nealen ML, Vijayan KV, Bolton E, Noga SJ, Bray PF. Human megakaryocytes and platelets contain the estrogen receptor beta and androgen receptor (AR): testosterone regulates AR expression. Blood. 2000;95 (7):2289-96.
19. Bord S, Frith E, Ireland DC, Scott MA, Craig JIO, Compston JE. Estrogen stimulates differentiation of megakaryocytes and modulates their expression of estrogen receptors alpha and beta. J Cell Biochem. 2004;92(2):249-57.
20. Nagata Y, Yoshikawa J, Hashimoto A, Yamamoto M, Payne AH, Todokoro K. Proplatelet formation of megakaryocytes is triggered by autocrine-synthesized estradiol. Genes Dev. 2003;17(23):2864-69.

In mixed hematopoietic chimerism, the donor red cells win

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Allogeneic hematopoietic stem cell transplantation (HSCT) was first observed to correct the thalassemias and hemoglobinopathies over three decades ago.[1,2] Since then, over 3000 transplants for these disorders have been performed worldwide, and allogeneic HSCT currently remains the only proven curative therapy for these highly morbid and life-limiting diseases. In these settings, engraftment of donor-derived cells following HSCT serves to replace dysfunctional cells of the red cell lineage. For over a decade, it has been recognized that a subset of patients transplanted for these disorders intriguingly demonstrate stable and durable co-existence of nucleated donor cells with host cells and that this chimeric state is associated with transfusion-independence and the lack of continued clinical manifestations of their disease.[3,4] Now, in a study presented in this issue of the journal, Andreani *et al.*[5] definitively demonstrate that patients with long-lasting stable mixed hematopoietic chimerism (3 with thalassemia and 1 with sickle cell disease), including mixed chimerism of marrow erythroid progenitors, expressed a 2- to 5-fold enrichment of donor-derived mature erythrocytes in the peripheral blood.

It is important to put this study in the context of previous work. Data from the current study along with those of previous studies[3,6] are indeed similar. In the bone marrow, the percentage of donor myeloid cells correlates with that of erythroid cells, consistent with the current understanding of myelo- and erythropoiesis deriving from common myelo-erythroid progenitors.[7] Furthermore, in the peripheral blood the percentage of donor leukocytes is similar to that in the marrow; however, the percentage of donor red cells is much higher (Figure 1). This enrichment in donor peripheral red cells has also been observed in children with sickle cell disease after myeloablative transplants.[4] The observation of full replacement by donor-derived mature red blood cells occurring within these mixed chimeras provides an understanding of the dramatic functional improvements observed in these patients following allogeneic HSCT.

These observations support the long-held notion that erythroid precursors in the thalassemias and hemoglobinopathies are at a competitive disadvantage for generating mature red blood cells capable of exiting the marrow. The current data from Andreani *et al.*[5] are highly convincing as they were derived from patients exhibiting long-term (>3 years) stable chimerism who did not require red blood cell transfusions. The effects measured

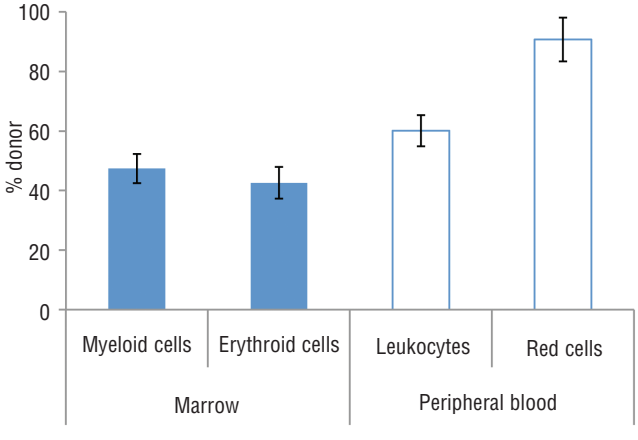

Figure 1. Comparison of marrow and peripheral blood donor chimerism. Results of two prior studies[3,18] along with those of the study by Andreani *et al.*[5] are summarized here. There were 16 samples of marrow myeloid cells, 13 of marrow erythroid cells, 31 of peripheral blood leukocytes, and 4 of peripheral blood red cells. The error bars refer to standard errors of the mean.