

Research article

## Platelet count and Interleukin 6 Gene polymorphism in healthy subjects

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### Abstract

**Background:** Interleukin 6 (IL-6) is thought to play important roles in the development of reactive thrombocytosis caused by inflammation by its stimulatory effect on megakaryocytopoiesis. A G/C polymorphism of the IL-6 gene at position -174 has been found to be associated to different transcription rates. Specifically, subjects with the CC genotype showed lower plasma IL-6 levels compared with GC or GG subjects. Given this difference in transcription rates of IL-6 we speculated on different platelet count according to this IL-6 polymorphism.

**Methods:** The G/C polymorphism of the IL-6 gene at position -174, serum IL-6 concentration and platelet count were prospectively analyzed in 59 (25 women) consecutive healthy subjects.

**Results:** Subjects who were homozygotes for the C allele at position -174 of the IL-6 gene (Sfa NI genotype) showed significantly lower platelet count than carriers of the G allele, despite similar age, sex, body mass index and proportion of smokers ( $205400 \pm 44088$  vs  $239818 \pm 60194$ ,  $p = 0.047$ ). This was in parallel to differences in peripheral white blood cell count ( $5807 \pm 1671$  vs  $6867 \pm 1192 \times 10^9/\text{ml}$ ,  $p = 0.01$ ).

**Conclusion:** This is the first description, to our knowledge, of a genetical influence on basal platelet counts, which appears to be partially dependent on a polymorphism of the IL-6 gene, even in the absence of inflammation.

### Background

Interleukin-6 (IL-6) is a pleiotropic cytokine involved in the regulation of the acute phase reaction, immune responses, and hematopoiesis. Earlier studies have found that IL-6 is a potent thrombopoietic factor in vivo in mice acting on maturational stages in megakaryocytopoiesis and promoting platelet production [1]. Human IL-6 was also found as a direct promoter of maturation of meg-

karyocytes in vitro [2]. In recent reports, circulating IL-6 appeared to be an active mediator involved in the regulation of thrombopoiesis during clonal and reactive thrombocytosis [3,4]. However, at the present moment, there is no evidence that IL-6 is required for normal thrombopoiesis in humans.

A polymorphism in the 5' flanking region of the *IL-6* gene alters the transcriptional response to stimuli such as endotoxin and interleukin-1. This G/C polymorphism of the *IL-6* gene at position -174 has been found to be associated to different plasma *IL-6* levels in healthy volunteers [5]. Given the role of *IL-6* in megakaryocytopoiesis we aimed to study the G/C polymorphism of *IL-6* in relation with the blood platelet count.

## Subjects and Methods

### Subjects

Fifty-nine healthy subjects (25 women) were prospectively studied. All subjects were of caucasian origin from Catalonia (northern part of Spain). Inclusion criteria were: 1) absence of any systemic disease, 2) absence of any infections in the previous month. None of the subjects were taking any medication (including glucocorticoids or estrogens) or had any evidence of metabolic disease. Liver disease and thyroid dysfunction were specifically excluded by biochemical workup. The protocol was approved by the Hospital Ethics Committee, and informed consent was obtained from each subject.

**Platelet count** was determined by routine laboratory tests (Coulter Electronics, Hialeah FL).

**Serum interleukin-6** levels were measured using a commercial immunoassay (MEDGENIX *IL-6* EASIA™, BioSource Europe S.A., Zoning Industriel B-6220, Fleunes, Belgium), with coefficients of variation lower than 6%. The minimum detectable doses of *IL-6* was 0.094 pg/ml.

### Restriction fragment length polymorphism (RFLP)-*IL-6* gene analysis

The SfaNI (restriction enzyme) polymorphism is due to a replacement of G by C at position 174, and primers were designed to amplify the promoter region of *IL-6* gene. The primers used in the PCR were: 5' TGACTTCAGCTT-TACTCTTTGT 3' and 5' CTGATTGGAAACCTTAT TAAG 3'. The reaction was carried out in a final volume of 50 µL containing 1.5 mmol/L of MgCl<sub>2</sub>, 0.2 mmol/L of each dNTP (Boehringer Mannheim™, Mannheim, Germany), 0.2 mmol/L of each primer and 2.5 unit of Taq polymerase (Gibco BRL). DNA was amplified during 35 cycles with an initial denaturation of 10 minutes at 94°C and a final extension of 10 m at 72°C. The cycle program consisted in 1 minute denaturation at 94°C, 1 minute and 35s annealing at 55°C and 1 m extension at 72°C. PCR products were digested with SfaNI restriction enzyme at 37°C overnight and electrophoresed on a 2% agarose gel. SfaNI RFLP was detected by ethidium bromide staining.

DNA was extracted from cellular blood components by the salting-out method. The polymerase chain reaction

was used to detect the *IL-6* SfaNI RFLP. The identified genotypes were named according to the presence or absence of the enzyme restriction sites, so SfaNI (G/G), (G/C), and (C/C) are homozygotes for the presence of the site (140/58 bp), heterozygotes for the presence and absence of the site (198/140/58 bp), and homozygotes for the absence of the site (198 bp), respectively. The frequency of the alleles in a large sample of subjects was C: 0.55, G: 0.45. The X<sup>2</sup> value of a goodness-of-fit statistics to test for Hardy-Weinberg equilibrium was 1.3 (p = 0.52, df = 1).

### Statistical analysis

Descriptive results of continuous variables are expressed as mean ± SD. Comparison of variables between groups of subjects was performed using unpaired t test.

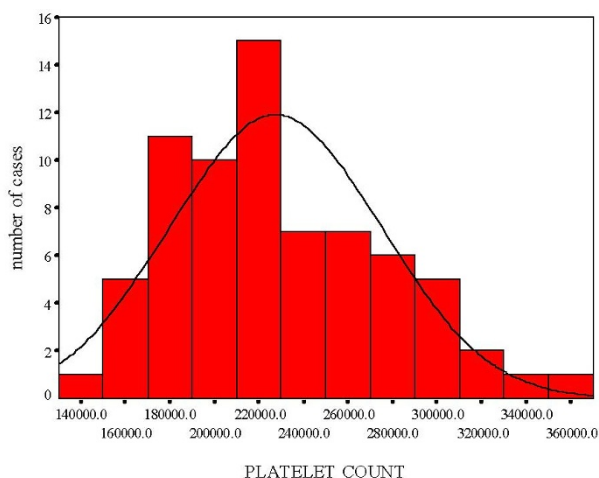
## Results

Clinical and biochemical characteristics of the subjects at the time of entry into the study are shown on Table 1. The samples from 59 subjects were divided into two groups on the basis of the *IL-6* genotype. Forty-four subjects had a G at position -174 of the *IL-6* gene, 29 heterozygotes (C/G) and 15 homozygotes (G/G). Fifteen subjects were homozygotes for the presence of C at this position.

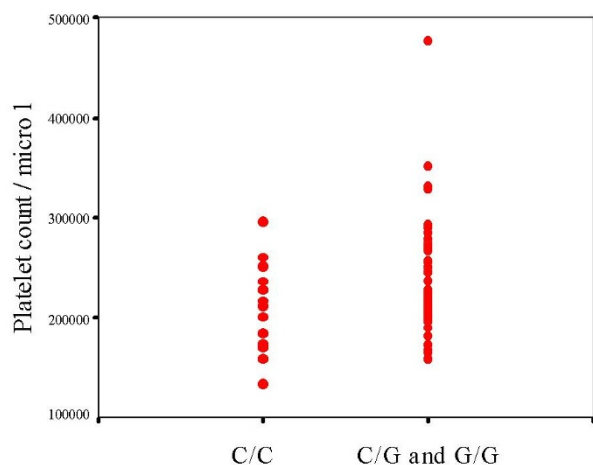
**Table 1: General characteristics of the Study Subjects**

Variable	C/C	G/C and GG	P
N	15	44	-
Men/women	8/7	26/18	NS
Age (years)	36.4 ± 10.6	40.4 ± 8.5	NS
Body mass index (Kg/m <sup>2</sup> )	26.07 ± 5.1	26.8 ± 4.8	NS
Smokers (yes/no)	4/11	12/32	NS
Platelet count (/µl)	205400 ± 44088	239818 ± 60194 <sup>a</sup>	0.04
Median (range)	201.000 (135000-298000) <sup>b</sup>	223.500 (159000-477000) <sup>c</sup>	
WBC (× 10 <sup>9</sup> /ml)	5807 ± 1671	6867 ± 1192	0.01
Hematocrit (%)	40.4 ± 5.4	42.2 ± 4.8	NS
Circulating <i>IL-6</i> (pg/ml) <sup>*</sup>	6.8 ± 1.7	10.3 ± 6.9	0.07

C/C, C/G and G/G define the allele polymorphism of the promoter of interleukin-6 gene. Data are provided as mean ± SD. \* *IL-6*, circulating interleukin 6, which was determined in 30 subjects, 10 of whom with the C/C allele; WBC, peripheral white blood cells. a. After excluding the outlier, the differences remained significant at a p = 0.046. b. There was only one subject below the lower normal range of 150.000 platelets/µl and 6 between 160.000-185.000/µl. c. The maximum platelet level is 353.000/µl after excluding the outlier. The normal range, mean and median of platelet counts are 230.970, 220500 (150.000-400.000) /ml.



**Figure 1**  
Histogram showing the normal distribution of platelet count.



**Figure 2**  
Peripheral platelet count according to interleukin-6 genotype. After excluding the outlier, the differences remained significant at a  $p = 0.046$ .

Platelet count showed a normal distribution (Figure 1). Subjects who were homozygotes for the C allele, associated to lower plasma levels of IL-6 in a recent study [5], were similar in age, sex, body mass index and proportion of smokers in comparison with carriers of the G allele (Table 1). The former subjects showed significantly lower platelet count than carriers of the G allele (mean difference -34.418 platelets, with a Levene's test for equality of Variances:  $F = 0.74$ ,  $p = 0.39$ , indicating a similar variance for platelet count in both groups (Table 1) (Figure 2).

## Discussion

When comparing constructs of the 5' flanking region of IL-6 in a luciferase reporter vector transiently transfected into *HeLa* cells, the -174C construct showed lower expression than the -174 G construct [5]. This different transcription rate was supported by the *in vivo* observation that IL-6 levels were lower in normal subjects with the CC genotype [5]. We have found a tendency towards lower levels of IL-6 in CC homozygotes using a different assay. Unlike other cytokines, IL-6 is unusual, in that its major effects take place at sites distinct from its origin and are consequent upon its circulating concentrations. For this reason, it is called the endocrine cytokine [6]. For instance, increased circulating levels of IL-6 and C-reactive protein have been recently associated with mortality in healthy older persons [7]. Serum IL-6 concentration is tightly regulated and circulates in proportion to different metabolic abnormalities such as lipid levels [8], insulin resistance and blood pressure [9].

The importance of this observation is that it is the first description of a molecular influence of basal platelet counts. The findings of the present study suggest that subjects with the G allele would be prone to a procoagulant state. This matter is of considerable importance in atherosclerosis. It has been speculated that IL-6 might play a key role in the development of coronary disease through a number of metabolic, endothelial and procoagulant mechanisms [7,8,9,10]. In fact, circadian interrelationships among levels of plasma fibrinogen, blood platelets and serum interleukin 6 have been recently reported [11]. Although basal interleukin 6 levels were not statistically different between genotypes in this report ( $p = 0.07$ ), the study of circadian IL-6 levels will shed light on differences of transcription rate attributed to *IL-6 gene* polymorphism. The effect of IL-6 on platelets, fibrinogen activation and coagulation would lead to a procoagulant state, as demonstrated in an experimental model of endotoxemia [12]. In fact, the asymptomatic homozygous subjects with the G allele showed an 11% greater carotid artery intima-media thickness than CC homozygous for the C allele, as indicative of early atherosclerosis [13]. Furthermore, diabetic patients with vascular disease show significantly increased platelet count [14]. We have hypothesized that inflammation is inherent to insulin resistance of type 2 diabetic patients that contribute to their chronic diabetic complications [15]. Given that patients with the G allele also showed significantly higher area under the curve of glucose after an oral glucose tolerance test in a recent study [16], a relationship between concomitant higher glucose levels and platelet count attributed to *IL-6 gene* polymorphism cannot be excluded.

Under physiological conditions, IL-6 would play a role to regulate homeostasis by maintaining the level of platelets and leukocytes. In fact, we have also described that the *IL-6* -174 gene polymorphism was associated to peripheral white blood cell count in healthy subjects [16], specifically neutrophils and lymphocytes, reinforcing the potential importance of IL-6 in hematopoiesis [17]. Peripheral white blood cell count constitutes another risk factor for atherosclerosis [18]. Elevated serum IL-6 levels cannot be excluded, however, to constitute an anti-inflammatory attempt [17] to fight against a chronic inflammatory process (atherosclerosis), being the enhanced hematopoiesis a byproduct.

### Conclusion

A polymorphism of the *IL-6* gene influences the platelet count in healthy volunteers even in the absence of inflammation. Whether this polymorphism leads to differences in reactive thrombocytosis merits further research.

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### Competing interests

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