



## Human-platelet-antigen and neutrophil-antigen gene frequency in the Italian population determined by polymerase chain reaction with sequence specific primers

In our study we evaluated the genotype and gene frequency of human platelet antigen in the Italian Caucasian population by polymerase chain reaction sequence specific primers. DNA typing is an alternative tool or a possible replacement of serologic typing in immune mediated disorders and prenatal diagnosis.

Sir,

Platelets and neutrophil antigen systems play important roles in immune-mediated disorders, since the respective alloantibodies and autoantibodies mediate immune thrombocytopenia and granulocytopenia.

Serologic typing for human platelet antigen (HPA) and neutrophil-antigen (NA) systems is well developed but good quality sera are available only in a few specialized laboratories while DNA based methods now afford a way round the limitations of the serologic technique.<sup>1</sup>

In the Italian Caucasian population only the distribution of HPA-1 dialleles has been described<sup>2-5</sup> and there are few data describing the distribution of NA systems among the Caucasian population.<sup>6,7</sup>

We studied the genotype and gene frequency of HPA-1,2,3,5 and NA1 and NA2 systems by polymerase chain reaction sequence specific primers (PCR-SSP) in 200 randomly selected healthy volunteer blood and bone marrow donors, belonging to the Italian Caucasian population.

We evaluated the frequency of HPA and NA system genotypes and from these data obtained the gene frequencies with standard error (Table 1).

All the alleles of the HPA-1,2,3,5 systems are present in the Italian Caucasian population with the gene frequency of alleles HPA-1a, 2a, 3a, 5a being  $0.86 \pm 0.017$ ,  $0.87 \pm 0.016$ ,  $0.72 \pm 0.022$ ,  $0.785 \pm 0.02$ , which is greater than the gene frequencies of HPA-1b, 2b, 3b, 5b ( $0.14 \pm 0.017$ ,  $0.13 \pm 0.016$ ,  $0.28 \pm 0.022$  and  $0.225 \pm 0.02$  respectively). The homozygous genotypes have a frequency of 78, 76, 53, 58.5% for HPA-1a/a, 2a/a, 3a/a, 5a/a. This is more frequent than heterozygous patterns HPA-1a/b, 2a/b, 3a/b, 5a/b (16, 22, 38 and 40%) and homozygous HPA-1b/b, 2b/b, 3b/b, 5b/b (6, 2, 9, 1.5%) respectively. The genotype patterns evaluated in the Italian population overlap those observed in other Caucasian populations but a few differences emerge: in the Asian population HPA-1a/a, HPA-2a/a, HPA-5a/a are the most commonly present patterns, occurring with a percentage of 99, 95 and 93, respectively, whereas in the Italian population the frequencies are 78, 76, 58.5 respectively.<sup>2,8</sup>

The NA system is represented by the two alleles NA1 and NA2. Here the gene frequency of NA2 ( $0.692 \pm 0.023$ ) is more frequent than that of NA1 ( $0.282 \pm 0.022$ ). In particular the homozygous NA1 genotype (10.5%) occurs at a lower rate than the heterozygous NA1/NA2 (35.5%) or homozygous NA 2

**Table 1. Distribution of HPA and NA genotypes and gene frequencies observed in the Italian caucasian population.**

Genotype	(%)	Gene	gF±se
HPA- 1a/1a	78	HPA-1a	0.86±0.017
HPA- 1a/1b	16	HPA-1b	0.14±0.017
HPA- 1b/1b	6		
HPA- 2a/2a	76	HPA-2a	0.87±0.016
HPA- 2a/2b	22	HPA-2b	0.13±0.016
HPA- 2b/2b	2		
HPA- 3a/3a	53	HPA-3a	0.72±0.022
HPA- 3a/3b	38	HPA-3b	0.28±0.022
HPA- 3b/3b	9		
HPA- 5a/5a	58.5	HPA-5a	0.785±0.02
HPA- 5a/5b	40	HPA-5b	0.225±0.02
HPA- 5b/5b	1.5		
NA 1	10.5	NA-1	0.282±0.022
NA 1/2	35.5	NA-2	0.692±0.023
NA 2	54		

gF: gene frequency; se: standard error.

pattern (54%), the last being the most represented genotype in several Caucasian populations. We found that gene NA2 is more frequent in the Italian population (0.692) which is in accordance with the reported NA phenotype in other white populations (European and North American),<sup>6,7</sup> in contrast with the gene frequency of 0.65 and 0.68 for NA1 and 0.30 and 0.31 for NA2 reported for the Japanese and Chinese populations, respectively.<sup>9</sup>

PCR-SSP applied to the typing of HPA and NA systems is thus an available tool, and a way to replace serologic typing, especially in patients who are severely thrombocytopenic and neutropenic, and in prenatal diagnosis. The simplicity and accuracy of this method for platelet and granulocyte investigation in patients and donor recommend its widespread application.

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### Key words

Human-platelet-antigen, neutrophil-antigen, genotyping, Caucasians

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### Cell cycle analysis in the diagnosis of Fanconi's anemia

Previous studies have demonstrated a cell cycle disturbance in Fanconi's anemia (FA), with a G2 block. Different methods for diagnosis of FA by flow cytometry have been proposed. We describe

here a new, highly sensitive and specific approach that utilizes cell cycle analysis after incubation with phytohemagglutinin and melphalan.

Sir,

The diagnosis of Fanconi's anemia (FA) is classically founded on the demonstration of an increased chromosomal sensitivity to alkylating agents, such as diepoxybutane (DEB).<sup>1,2</sup> Previous studies have demonstrated the possibility of diagnosing FA by flow cytometric analysis, showing a block of cell cycling in the G2 phase.<sup>3-5</sup> However, the experimental conditions of the test have not been clearly defined and different methods have been proposed. We evaluated different concentrations of a readily available alkylating drug, melphalan, in the flow cytometric diagnosis of FA. Nineteen controls, 13 patients diagnosed as or suspected of having FA (Table 1), 15 FA parents and 27 patients with various non-FA cytopenias were studied after having given informed consent. Peripheral blood mononuclear cells (PBMCs) were incubated (10<sup>6</sup>/mL) for 72 h in IMDM plus 10% FCS and 1% phytohemagglutinin (PHA) in the presence or not of melphalan (Alkeran, GlaxoWellcome) 0.01, 0.05, 0.1, 0.5, 1, 2 µg/mL (added 24 h after the initiation of culture). PBMCs were then treated with an automated DNA staining kit (DNA-prep, Coulter). The PI fluorescence of individual nuclei was measured by a flow cytometer Epics-XL2 (Coulter). The results were analyzed by Multicycle software (Phoenix Flow Systems). Percentages of cells in the G2 phase were compared by Wilcoxon's test. Specificity and sensitivity were calculated using the MiniStat program.<sup>6</sup>

In ten patients with a positive DEB test and various expressions of cytopenia and physical abnormalities

**Table 1. Clinical and laboratory data of 10 FA patients. Cell cycle is evaluated after exposure to melphalan 0.1 and 0.5 µg/mL. Three cases (a, b, c), suspected to be FA (DEB test negative or border-line, G2 block), are shown at the bottom.**

Age (yrs)	Hematologic features	Physical abnormalities	DEB test	%G2		
				0.1 µg/mL	0.5 µg/mL	
1	6	thrombocytopenia	facies, kidney ectopia, vesicoureteral reflux, skin hyperpigmentation	pos	27	31
2	6	pancytopenia	facies, short stature, left kidney hypoplasia, right kidney ectopia and dysplasia with vesicoureteral reflux, atrial septal defect, hip dysplasia, deafness	pos	40	38
3	8	neutropenia	microcephaly, short stature, thumb adduction, dental hypoplasia	pos	29	54
4	6	normal	facies, microcephaly, short stature, hypertelorism	pos	46	52
5	6	normal	facies, short stature, vesicoureteral reflux, left kidney hypoplasia, café-au-lait spots	pos	30	68
6	8	pancytopenia	facies, short stature, right kidney hypoplasia	pos	—	50
7	12	pancytopenia	—	pos	—	60
8	10	pancytopenia	facies, café-au-lait spots, hexadactyly	pos	41	54
9	7	thrombocytopenia	horseshoe kidney, duodenal stenosis, hip dysplasia	pos	37	31
10	16	pancytopenia	facies	pos	64	62
a	5	thrombocytopenia	growth retardation, kidney ectopia, cryptorchidism	neg	26	39
b	11	pancytopenia	thumb defect	border-line	61	39
c	1	normal	atrial septal defect, kidney ectopia with vesicoureteral reflux, double right thumb	border-line	39	60

DEB test: pos = cells with chromosome breaks >45%; border-line = cells with chromosome breaks >11<45%; neg = cells with chromosome breaks <11%