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Valid Belgian population frequency data on HLA A-B-DR haplotypes by means of family studies

by

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Abstract

In order to obtain reliable haplotype frequencies, 2251 Belgians belonging to the families of 468 haematological patients were typed for HLA A, B and DR. Three-locus haplotypes were determined by segregation analysis and a population genetic study was performed on the haplotypes of the 936 parents of the patients. No significant deviations from Hardy-Weinberg equilibrium were observed, showing the data to be representative for the population. Of the 518 observed haplotypes, 130 had a frequency of above 0.2% and represent 68.7% of the population. In forensic practice, the frequency of the other haplotypes can be set conservatively at 0.2%. In this way, with 99.32% of heterozygotes and a polymorphism information content of 99.05%, the HLA A-B-DR haplotype adds significant statistical weight in complex paternity cases. These data are also of interest in stem cell donor search, matching for organ transplantations and anthropological research.

Keywords: *HLA, haplotype, Belgian, population genetics*

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Introduction

Though at present most forensic casework is carried out using Short Tandem Repeat (STR) loci exclusively, in the field of paternity testing HLA typing is still performed, especially in laboratories that are part of a transfusion service [1-4]. These institutions have HLA typing facilities for transfusion, transplantation and disease association studies. The HLA system is the most polymorphic of the human genome, making it of possible great value to genetic relationship testing.

In Belgium too, we find three blood banks with an HLA laboratory involved in paternity testing. To calculate the inclusion probability in paternity casework, reliable population data on the genetic markers that are applied, are required. In order to obtain these data, we performed a population genetic study on HLA in Belgians. Due to the typical linkage desequilibrium between loci in the HLA region, it is mandatory to use haplotype frequencies in inclusion calculations and not simply apply the product rule across all typed loci. We therefore determined HLA A-B-DR haplotype frequencies in the Belgian population by direct counting among 468 families.

Material and Methods

Family samples

We studied 468 indigenous Belgian families, who were HLA typed in view of possible bone marrow transplantation from *of* to a family member. Thus, 2251 individuals were tested, including 468 patients, 747 siblings and their 936 parents.

HLA typing

HLA A, B and DR were typed on T- and B-cell enriched suspensions by complement dependent lymphocytotoxicity [5] and/or on extracted DNA by PCR and reverse dot blot hybridisation with specific oligonucleotide probes (InnoLIPA, Innogenetics, Zwijnaarde, Belgium) [6]. Nomenclature for factors of the HLA system 2001 [7] was used for assigning HLA-A, B and DR antigens. Three locus haplotypes were deduced from Mendelian inheritance in the families.

Statistical analysis

Based on the HLA typing results of family members, we determined the genotypes and haplotypes of 936 unrelated parents. Extended hap-

lotype frequencies were calculated by direct counting among the parents. Hardy-Weinberg equilibrium (HWE) was tested through the exact test of Guo and Thompson [8] using Arlequin version 2.000 software [9]. The power of exclusion (PEX) and mean paternity index (MPI) were calculated according to Brenner and Morris [10], the polymorphism information content (PIC) according to Botstein [11].
PEX represents the probability of excluding paternity of a falsely accused man. MPI can be used to express that in an inclusion case, the tested man is on average MPI times more likely the father than not. PIC is a measure of the forensically useful degree of polymorphism of a genetic marker. It depends on the number of alleles and their distribution.

Results

Results of serological as well as high and low resolution DNA typing were pooled into the following categories of "broad" antigens: HLA A1, A2, A3, A9, A10, A11, A19, A28; HLA B5, B7, B8, B12, B13, B14, B15, B16, B17, B18, B21, B22, B27, B35, B37, B40, B41, B47, B53, B70, B73; HLA DR1, DR2, DR3, DR4, DR5, DR6, DR7, DR8, DR9, DR10.

In the study population of 936 Belgians, we observed 518 different haplotypes. However, only haplotypes with a count of 4 or more (frequency > 0.2%) are presented (Table 1). We thus show 130 haplotypes with frequencies between 0.0604 (HLA A1-B8-DR3) and 0.0021 (HLA A9-B5-DR6). They represent 68.7% of observed haplotypes. Having counts of 3 or lower, the 388 other haplotypes are not shown and frequencies not calculated. However, they are available from the authors on simple request. As a whole, they account for 31.3% of the study population. The figure of 518 haplotypes observed among 936 unrelated individuals is well below the number of 1680 theoretical haplotypes possible with 8 A, 21 B and 10 DR alleles. This clearly illustrates linkage desequilibrium, well known for the HLA region on chromosome 6.

HWE was tested on the loci HLA A, HLA B and HLA DR separately, revealing no significant deviation from HWE (Table 2). The exact test of Guo & Thompson tests whether the difference between the observed genotype frequencies and the genotype frequencies expected according to the law of Hardy and Weinberg, is statistically significant. P gives the probability of finding this difference (observed – expected) when the data are in accordance with HW. It is arbitrarily but generally accepted in population genetics to consider the data in accordance with HW when the

TABLE 1
HLA A-B-DR haplotype frequencies in a Belgian population sample (n = 936). Only haplotypes with frequency >0.002 are presented.

haplotype	counts	frequency	SE	haplotype	counts	frequency	SE
A1 B8 DR3	113	0.0604	0.0055	A19 B14 DR1	7	0.0037	0.0014
A3 B7 DR2	51	0.0272	0.0038	A19 B40 DR5	7	0.0037	0.0014
A2 B7 DR2	48	0.0256	0.0037	A2 B21 DR7	7	0.0037	0.0014
A3 B35 DR1	39	0.0208	0.0033	A9 B12 DR6	7	0.0037	0.0014
A19 B12 DR7	38	0.0203	0.0033	A2 B7 DR3	7	0.0037	0.0014
A2 B15 DR4	36	0.0192	0.0032	A3 B7 DR1	7	0.0037	0.0014
A2 B12 DR4	31	0.0166	0.0029	A19 B35 DR5	7	0.0037	0.0014
A2 B12 DR5	23	0.0123	0.0025	A11 B35 DR1	7	0.0037	0.0014
A2 B18 DR5	19	0.0101	0.0023	A2 B37 DR10	7	0.0037	0.0014
A2 B5 DR5	17	0.0091	0.0022	A9 B18 DR7	7	0.0037	0.0014
A9 B7 DR2	17	0.0091	0.0022	A1 B8 DR7	7	0.0037	0.0014
A2 B17 DR7	16	0.0085	0.0021	A9 B17 DR4	7	0.0037	0.0014
A2 B12 DR7	15	0.0080	0.0021	A2 B12 DR6	6	0.0032	0.0013
A3 B7 DR4	15	0.0080	0.0021	A19 B12 DR5	6	0.0032	0.0013
A1 B17 DR7	14	0.0075	0.0020	A19 B8 DR3	6	0.0032	0.0013
A3 B35 DR4	14	0.0075	0.0020	A2 B35 DR1	6	0.0032	0.0013
A1 B8 DR6	14	0.0075	0.0020	A2 B7 DR5	6	0.0032	0.0013
A9 B17 DR7	14	0.0075	0.0020	A9 B15 DR5	6	0.0032	0.0013
A3 B7 DR5	14	0.0075	0.0020	A2 B13 DR7	6	0.0032	0.0013
A9 B35 DR1	13	0.0069	0.0019	A1 B12 DR4	6	0.0032	0.0013
A3 B18 DR3	13	0.0069	0.0019	A11 B35 DR5	6	0.0032	0.0013
A19 B40 DR6	12	0.0064	0.0018	A2 B15 DR1	6	0.0032	0.0013
A11 B35 DR6	12	0.0064	0.0018	A9 B12 DR4	6	0.0032	0.0013
A19 B5 DR5	12	0.0064	0.0018	A9 B7 DR6	6	0.0032	0.0013
A19 B18 DR5	12	0.0064	0.0018	A19 B5 DR6	6	0.0032	0.0013
A2 B5 DR1	12	0.0064	0.0018	A3 B7 DR3	6	0.0032	0.0013
A9 B15 DR6	12	0.0064	0.0018	A19 B15 DR6	6	0.0032	0.0013
A2 B40 DR6	12	0.0064	0.0018	A28 B53 DR6	6	0.0032	0.0013
A2 B16 DR6	11	0.0059	0.0018	A3 B35 DR2	6	0.0032	0.0013
A2 B15 DR2	11	0.0059	0.0018	A2 B40 DR5	6	0.0032	0.0013
A3 B7 DR6	11	0.0059	0.0018	A3 B7 DR7	6	0.0032	0.0013
A1 B7 DR6	11	0.0059	0.0018	A3 B40 DR4	6	0.0032	0.0013
A19 B18 DR3	11	0.0059	0.0018	A3 B35 DR6	5	0.0027	0.0012
A2 B7 DR4	11	0.0059	0.0018	A2 B5 DR2	5	0.0027	0.0012
A19 B40 DR4	10	0.0053	0.0017	A19 B27 DR4	5	0.0027	0.0012
A2 B27 DR5	10	0.0053	0.0017	A1 B5 DR2	5	0.0027	0.0012
A10 B18 DR2	10	0.0053	0.0017	A11 B22 DR6	5	0.0027	0.0012
A2 B5 DR6	9	0.0048	0.0016	A19 B7 DR2	5	0.0027	0.0012
A3 B14 DR1	9	0.0048	0.0016	A2 B18 DR2	5	0.0027	0.0012
A10 B16 DR6	9	0.0048	0.0016	A1 B27 DR4	5	0.0027	0.0012
A1 B7 DR2	9	0.0048	0.0016	A9 B12 DR7	5	0.0027	0.0012
A2 B15 DR6	9	0.0048	0.0016	A2 B15 DR5	5	0.0027	0.0012
A9 B22 DR6	9	0.0048	0.0016	A1 B8 DR2	5	0.0027	0.0012
A19 B12 DR6	8	0.0043	0.0015	A3 B18 DR5	5	0.0027	0.0012
A2 B40 DR4	8	0.0043	0.0015	A1 B35 DR1	5	0.0027	0.0012
A9 B35 DR5	8	0.0043	0.0015	A3 B8 DR3	5	0.0027	0.0012
A9 B8 DR3	8	0.0043	0.0015	A1 B8 DR4	5	0.0027	0.0012
A2 B40 DR2	8	0.0043	0.0015	A2 B27 DR6	5	0.0027	0.0012

haplotype	counts	frequency	SE	haplotype	counts	frequency	SE
A1 B37 DR5	5	0.0027	0.0012	A2 B27 DR4	5	0.0027	0.0012
A3 B12 DR6	5	0.0027	0.0012	A11 B27 DR2	4	0.0021	0.0011
A3 B15 DR4	5	0.0027	0.0012	A19 B22 DR6	4	0.0021	0.0011
A3 B5 DR1	5	0.0027	0.0012	A9 B15 DR7	4	0.0021	0.0011
A9 B22 DR5	5	0.0027	0.0012	A19 B12 DR1	4	0.0021	0.0011
A3 B7 DR8	5	0.0027	0.0012	A19 B40 DR2	4	0.0021	0.0011
A9 B12 DR5	5	0.0027	0.0012	A9 B7 DR4	4	0.0021	0.0011
A2 B17 DR6	5	0.0027	0.0012	A9 B5 DR5	4	0.0021	0.0011
A10 B40 DR6	5	0.0027	0.0012	A2 B14 DR7	4	0.0021	0.0011
A28 B12 DR6	5	0.0027	0.0012	A2 B35 DR7	4	0.0021	0.0011
A2 B22 DR5	5	0.0027	0.0012	A9 B35 DR6	4	0.0021	0.0011
A10 B12 DR5	5	0.0027	0.0012	A19 B16 DR6	4	0.0021	0.0011
A9 B21 DR5	5	0.0027	0.0012	A3 B16 DR6	4	0.0021	0.0011
A11 B5 DR1	5	0.0027	0.0012	A11 B35 DR2	4	0.0021	0.0011
A19 B13 DR7	5	0.0027	0.0012	A2 B37 DR5	4	0.0021	0.0011
A11 B35 DR4	5	0.0027	0.0012	A9 B16 DR5	4	0.0021	0.0011
A11 B8 DR3	5	0.0027	0.0012	A9 B5 DR6	4	0.0021	0.0011

TABLE 2
Exact test for Hardy-Weinberg equilibrium for 3 HLA loci

Locus	Observed heterozygosity	Expected heterozygosity	P	SD
HLA A	0.8312	0.834	0.426	0.0011
HLA B	0.9380	0.918	0.141	0.0005
HLA DR	0.8860	0.867	0.386	0.0110

p value is >0.05. This means that the chance to obtain the experimental data, when the tested population is in HWE, is >0.05. On the other hand, a p value <0.05 means the probability of observing the actual results when the sample meets HW, is below 0.05. Then, the population is said not to be in HWE.

The statistical parameters concerning forensic interest of HLA A-B-DR haplotypes can be found in Table 3. For the sake of comparison,

TABLE 3
Statistical parameters showing forensic usefulness of system

Parameter	HLA A-B-DR	TH01	D12S1090
Power of exclusion	0.9812	0.5553	0.8164
Mean paternity index	73.5	2.23	5.55
Polymorphism information content	0.9905	0.7532	0.9243

this table also lists the aforementioned parameters for TH01 [12], the most widely applied genetic marker in paternity casework, and for D12S1090 [13], one of the most polymorphic STR loci. The characteristics of HLA A-B-DR show off favourably.

Discussion

In the past, HLA typing was more regularly applied in paternity testing [14] than nowadays. Still, only HLA A-B typing was performed due to the lack of molecular typing techniques and the often poor quality of HLA-DR typing sera [5,15]. With the advent of DNA-typing techniques however, HLA-DR typing became virtually error free.

The use of 3-locus haplotypes as described here offers two major advantages. Firstly, the deduction of the haplotype is more straightforward when 3 loci are considered. Secondly, a supplementary locus with 10 alleles and 87% heterozygotes significantly increases the statistical power of the system.

With three polymorphic loci typed, large numbers of different haplotypes are produced and even the most common are found at low frequencies that are difficult to estimate with a high degree of precision. In order to avoid these difficulties HLA alleles and serological “split” antigens were pooled into the corresponding “broad” specificities. This level of typing resolution is readily attained by all available molecular HLA typing assays [16], whether based on PCR with sequence specific primers or followed by specific oligonucleotide hybridisation. The quantity of DNA required in the former method is eventually higher than in the latter, possibly precluding its use in criminalistic casework. The latter method however was shown to be sufficiently sensitive for the use in forensic investigations, even on severely degraded samples [17].

Haplotypes can be determined by segregation analysis in families and by maximum likelihood estimates [18] performed on phenotypes of random individuals. Theoretically, the first method is superior since haplotypes are directly identified and not statistically estimated. This is stated in the Standards for Histocompatibility Testing of the European Federation of Immunogenetics [19]: “determination of haplotypes and genotypes can only be done by family studies”.

To obtain a reliable measurement of haplotype frequencies in a highly polymorphic system, consistent numbers of individuals must be included. This is not always realistic in family studies [20, 21] and therefore large

databases of phenotypes, such as registries of blood or bone marrow donors have been used [22, 23]. Due to their sheer size, the frequencies calculated from national registries of bone marrow donors seem very accurate [24]. For the registry of Belgium for example, 37,484 individuals are used in the analysis, yielding an accuracy of the estimated HLA A-B-DR haplotype frequencies of 0.000027 [25]. The quality of those data for population genetic analysis may however be questionable and needs validation [26]. In an evaluation of 29 national registries, only 3 were found in HWE for the three loci HLA A, B and DR [27]. Several reasons are causing these problems. Firstly, in many registries, donors are on entry typed only for HLA A and B. When selected for a patient because of HLA A-B match, HLA DR is typed. Thus a selection bias is introduced, causing overrepresentation of the more frequent HLA A-B phenotypes in the HLA DR-typed subset. Secondly, bone marrow registries have no information on ethnicity and can be considered genetic "melting pots". Phenotypes of mixtures of ethnic groups do not have a good fit for HWE. Finally, the quality of the typing result itself in a family study of haematological patients is inevitably superior to that in a bone marrow registry. In the former situation, patient typing is repeated, often with a second technique, on a control sample. Results can be further validated through the family study and segregation of the haplotypes themselves. In the unrelated donor registry on the other hand, masses of donors are typed at the lowest cost possible and the result is only verified in the rare cases when a match is found in a patient. The Belgian bone marrow donor registry combines all of these problems and can, according to Schipper [27], not be used for population genetic studies.

Our study combines a substantial sample size with the advantages of family analysis.

The "non-randomness" of the test might raise questions on the validity of the results for the Belgian population as a whole. Indeed, the group of parents are related to patients with a distinct disease and may therefore seem a selective group in the population.

The major argument to conclude that the data can be extrapolated is the finding that the sample is in HWE. According to the HW theorem, the population size that generates the sample is very large – infinite in fact. It furthermore implies that any selection is excluded.

We also compared our frequencies with the Belgian registry of bone marrow donors [25]. This large population can be considered a control group for the sample we examined. Though actual frequencies differ,

both groups show the same ranking of the “top five” frequent haplotypes.

If a haplotype that is not presented in Table 1 is found in a paternity case, its frequency can be cautiously estimated as 0.002. Application of HLA A-B-DR haplotyping is especially useful as supplementary testing in complex paternity situations, such as mutation cases or incomplete cases. Due to the extensive polymorphism, inclusion probabilities will significantly increase, leading to reliable conclusions.

These data are eventually also of interest in stem cell donor search, matching for organ transplantation and anthropological research.

Samenvatting

Om betrouwbare haplotype frequenties te bekomen, werd typering voor HLA A, B en DR uitgevoerd bij 2251 Belgen, familieleden van 486 hematologische patiënten. Via segregatie-analyse kon het drie-locus haplotype afgeleid worden en werd een populatie-genetische studie verricht op de 936 ouders van de patiënten. Er was geen significante afwijking van Hardy-Weinberg evenwicht, wat aantoont dat de data representatief zijn voor de populatie. Van de 518 verschillende geobserveerde haplotypes, waren er 130 met een frequentie boven 0,2%. Zij vertegenwoordigen 68,7% van de populatie. In de forensische praktijk kan de frequentie van de overige haplotypes als 0,2% beschouwd worden. Met 99,32% aan heterozygoten en een graad van polymorfisme van 99,05% verhoogt het HLA A-B-DR haplotype de statistische bewijskracht bij complexe gevallen van paterniteitsonderzoek. Deze data hebben ook hun belang bij het zoeken van een compatibele donor van hematopoietische stamcellen, bij matching voor orgaantransplantatie en bij antropologisch onderzoek.

Résumé

Afin d’obtenir des fréquences haplotypiques valides, 2251 Belges, membres de famille de 486 patients hématologiques, ont été typés pour HLA A, B et DR. A moyen d’analyse de ségrégation, le trois-locus haplotype a été déduit et une étude de génétique de population a été conduite sur les 936 parents des malades. Aucune déviation significative de l’équilibre Hardy-Weinberg n’a été observée, ce qui démontre que ces données sont représentatives pour la population. Parmi les 518 haplotypes différents observés, il y en avait 130 avec une fréquence supérieure à 0,2%. Ceux-ci représentent 68,7% de la population. En pratique, il est permis de considérer la fréquence des autres haplotypes comme étant 0,2%. Avec 99,32% d’hétérozygotes et un degré de polymorphisme de 99,05%, la puissance statistique est augmentée dans les cas complexes d’examen de paternité. Ces données sont aussi intéressantes dans les recherches de donneur de cellules souche hématopoiétiques, dans le matching pour la transplantation d’organes et dans la recherche anthropologique.

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