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DNA BANK QUALITY ANALYSIS FROM ITS INCEPTION IN 1989 UP TILL NOW

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The DNA bank of Histocompatibility Laboratory "Jean Dausset" in Saint Louis Hospital is one of the oldest and important of HLA Labs in France. The first DNA samples were stored in 1989 and the total DNA samples today are >111000. Most of the samples were extracted manually and from 2011 by automatic methods (Fujifilm, Qiagen). For historical and practical reasons all samples are stored at 4°C. Eight or sixteen DNA samples, depending on the year of extraction, were chosen at random - a total of 136 samples. We tested the integrity and the quality of DNA samples by a combination of several criteria: Monitoring of DNA degradation by electrophoresis on agarose gel, DNA quality control – by spectrophotometric analysis and pH control of storage buffer Tris–EDTA (TE). We found deterioration in 22.8% of DNA samples studied with a very significant degradation in 1992, 2009 and 2010. No degradation was observed for years 2000, 2002, 2004, 2006, 2012. Excluding the above mentioned years the results show 9% DNA degradation between 1989 and 2012 and only 6% in 1989. We found contamination with RNA and protein respectively in 18% and 3% and the pH < 7 in TE buffer in 76% of the studied samples. No obvious correlation was found between DNA purity, the pH and the DNA degradation. Our results suggest that genomic DNA samples can be stored for a long time at 4 degrees C with good yields and quality. The impact of manual extraction and "homemade" buffers may reveal unpleasant surprises. That is why the standardization of DNA extraction with the automats and the use of commercial kits become essential. The storage of DNA samples at 4 °C is an acceptable and cost-effective alternative to expensive frozen storage and reduces the costs of biomedical community.

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ESTIMATION OF THE 6-DIGIT LEVEL ALLELE FREQUENCIES OF HLA-DR IN KOREANS USING AMBIGUITY-SOLVING DNA TYPING

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Since Korean society is fast becoming multi-ethnic, the determination of ambiguous HLA types using HLA allele frequencies is losing its usefulness. We thus tried to develop new methods that would directly resolve the ambiguities in HLA-DR genotyping using blood from 150 unrelated healthy Koreans. All alleles of HLA-DR were first amplified by 3 multi-group specific amplification (MGSA) tubes and then sequenced. Tube 1 was designed for *DRB1*01*, 15 and 16 allele groups, tube 2 for 03, 08, 10, 11, 12, 13 and 14, and tube 3 for 04, 07 and 09. Codon 86 GTG PCR tube (tube 4) was added to solve phase ambiguity. Exon 2 of the HLA-DRB1 locus was primarily analyzed. In cases of incomplete sequence ambiguity, additional exon 1, exon 3 and exon 3-4 were amplified. In case of phase ambiguity, Heterozygous Ambiguity Resolving Primer (HARP) was utilized. One case needed re-sequencing after re-amplification using Group Specific Amplification (GSA) and Sequence Specific Primer (SSP). Our MGSA method reduced the ambiguity to 9.3% while 42.7% of ambiguity occurs in conventional SBT method. All of the phase ambiguities were resolved by HARP, GSA, SSP or additional exon analyses. In this study, 29 alleles and 14 serologic phenotypes were identified. None of same 4 digit allele had different 6 digit allele in the test samples. However, 21.4% of alleles previously reported as *DRB1*12:01* was identified as *DRB1*12:10*. In addition, all of previously reported *DRB1*14:01* was identified as *DRB1*14:54:01*. By using the algorithm proposed in this study, we could completely solve the typing ambiguities of 150 Koreans. The algorithm which uses MGSA tube from the beginning is somewhat expensive, but is useful regarding timely and accurate reporting. The MGSA method is applicable to various populations and thus could be shared by most HLA laboratories. However, since HARP and SSP should be specific for a population, efforts to solve HLA ambiguity should continue as a society is becoming multi-ethnic.

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ANXA11 ASSOCIATION WITH SARCOIDOSIS SUSCEPTIBILITY: A META-ANALYSIS OF NON-FAMILY-BASED STUDIES

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Sarcoidosis is a multisystemic disorder of unknown etiology, characterized by the formation of noncaseating granulomas, predominantly in the lungs and lymph nodes. The sarcoidosis association with the ANXA11 rs1049550 SNP has been previously reported in case–control studies. We carried out this meta-analysis in order to collect all the relevant studies to further clarify the association of ANXA11 SNP rs1049550 C/T (R230C variant) polymorphism with Sarcoidosis susceptibility. Relevant published data were retrieved through Medline, PubMed and Web of Science pertaining to Sarcoidosis and ANXA polymorphisms. Odds ratios (OR) with 95 % confidence intervals (CI) were used to assess the strength of the association. Z test was used to determine the significance of the pooled OR. Statistical heterogeneity was measured using the Q statistic. The effect of heterogeneity was quantified using the I2-statistic. Visual inspection of asymmetry in funnel plots was conducted. Begg's rank correlation method and Egger weighted regression method were also used to statistically

assess the publication bias. Statistical analyses were performed with STATA12.0 software. A total of 6 studies, including 3297 sarcoidosis cases and 3346 healthy controls, were collected in this meta-analysis. For T vs. C, no heterogeneity ($Q = 4.79$, $p = 0.44$, $I2 = 0.0\%$) was observed among individual estimates, and original data were combined using the fixed-effects model. For the total population, we found that ANXA11 T allele was less common in the Sarcoidosis group than in the control group and we obtained an effect summary OR = 0.69, with a 95 % CI = 0.64-0.74, and $p < 0.001$, which shows a protective association of SNP rs1049550 T allele to sarcoidosis. Our comprehensive meta-analysis indicated that there is sufficient evidence to demonstrate a conclusive association between the ANXA11 SNP rs1049550 and sarcoidosis susceptibility.

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GROUP-SPECIFIC AMPLIFICATION OF HLA-DQA1 REVEALED A NUMBER OF GENOMIC FULL LENGTH SEQUENCES INCLUDING THE NOVEL HLA ALLELES *DQA1*01:10* AND *DQA1*01:11*

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In this paper we describe a subgroup-specific amplification assay for HLA−DQA1 that encompasses the whole coding region and allows us to sequence full length HLA-DQA1 genes. We would like to introduce the novel alleles *HLA-DQA1*01:10* and *HLA-DQA1*01:11*. Moreover, we were able to confirm the full length genomic sequence data of the alleles *HLA-DQA1*01:07, HLA-DQA1*03:01:01, HLA-DQA1*03:02, HLA-DQA1*04:01:02, HLA-DQA1*04:02, HLA-DQA1*05:03, HLA-DQA1*05:05:01:02* and *HLA-DQA1*06:01:01*. A complete genomic overview of all six HLA-DQA1 allele groups is now available from the submission of our data to the IMGT/HLA database. Since our approach facilitates the analysis of all HLA-DQA1 allele sequences, HLA-DQA1 may become the first HLA locus from which all subgroup members will be known in detail in the near future.

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THE NOVEL *HLA-A** ALLELE *A*32:53* IDENTIFIED BY SEQUENCE BASED TYPING

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Routine human leukocyte antigen (HLA) typing for class I (*A*,B*,C**) and class II (*DRB1*, DQB1**) by sequence based typing of a male patient suffering from myelodysplastic syndrome was performed using commercial kits (Atria Genetics Inc., South San Francisco, CA) following the manufacturer's protocol. Allele assignment of HLA A exon 2, 3 and 4 with SBTengine (Genome Diagnostics B.V, Utrecht, Netherlands) gave no matched result with the currently used IMGT/HLA Database suggesting the presence of a novel allele whereas additional SSP typing (Olerup SSP AB, Stockholm, Sweden) showed no unclear result. To separate both alleles, amplification and subsequent sequence based typing with primers located in Intron 1 (5′-GTCGGGCGGGTCTCAGCC - 3′) and allele-specific primers located in intron 4 (5′- CAGAGAGGCTCCTGCTTTCCG - 3′ and 5′-CAGAGAGGCTCCTGCTTTCCC - 3′) was done. Sequence analyses of the segregated alleles identified the common *A*02:01* allele and the new *A*32* allele identical to *A*32:01* except one nucleotide exchange in exon 4 at position 649 (G>A) causing an amino acid exchange (Ala > Thr). The sequence was submitted to EMBL Nucleotide Sequence Database and IMGT/HLA Sequence Database under the accession number HE971711 and was officially named *A*32:53* by the World Health Organization (WHO) Nomenclature Committee.

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DISCREPANCIES BETWEEN THE HLA REGISTRY TYPINGS AND THE VERIFICATION TYPING RESULTS: CZECH NATIONAL MARROW DONORS REGISTRY 2013 ANNUAL REPORT

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The HLA typing plays a crucial role in the selection of the unrelated donors for the hematopoietic stem cell transplantation. The accuracy of the HLA registry typings of the donors is important for timely search of the HLA matched donor for a specific patient. The goal of this study is to analyse HLA discrepancies detected in the verification typings performed during the search of the unrelated Czech donors in 2013. The primary registry HLA data were originated in our laboratory as well as in our 7 cooperating donor centres. The most volunteer donors were serologically typed for HLA-A and HLA-B and DNA typed for DRB1 at the time of recruitment. Newly all recruited donors have been DNA typed also for HLA-A and HLA-B since 2009. HLA verification typings were performed by DNA methods at a level of 2 or 4 digits. Verification typing results were collected separately for HLA-A/B (serology), HLA-A/B (DNA typing) and DRB1 (DNA typing). We analysed 498 verification typings in total (144 for HLA-A/B serology, 73 for HLA-A/B DNA typing and 281 for DRB1 DNA typing). We detected 8 errors (5.6%) in the primary serological HLA data (4 errors at the locus A and 4 errors at the locus B). Discrepancies were: homozygous serological typing vs. heterozygous DNA typing (i.e. serologically missed antigen, 4 errors), serological antigen mistyping (3 errors) and serologically over-assigned antigen (1 error). We detected 2 errors (0.7%) in DRB1 DNA registry typings. The second antigen was not identified in these both DRB1 DNA typings (i.e. false homozygosity). The allelic drop-out in SSO