Bioinformatics Article

Construction and benchmarking of a multi-ethnic reference panel for the imputation of HLA class I and II alleles

Human Molecular Genetics, 2018, Vol. 00, No.00

Human Molecular Genetics, 2018, Vol. 00, No.00

Frauke Degenhardt[1,#], Mareike Wendorff[1,#], Michael Wittig[1], Eva Ellinghaus[2], Lisa W Datta[3], John Schembri[4], Siew C Ng[5], Elisa Rosati[1], Matthias Hübenthal[1], David Ellinghaus[1], Eun S Jung[1,6], Wolfgang Lieb[7], Shifteh Abedian[8,9], Reza Malekzadeh[9], Jae H Cheon[6], Pierre Ellul[4], Ajit Sood[10], Vandana Midha[10], Thelma Bk[11], Sunny H Wong[5], Stefan Schreiber[1,12], Keiko Yamazaki[13,14], Michiaki Kubo[15], Gabrielle Boucher[16], John Rioux[16], Tobias L Lenz[17], Steven R Brant[3,18,19], Andre Franke[1,*]

[1]Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Kiel, Germany,

[2]K.G. Jebsen Inflammation Research Centre, Institute of Clinical Medicine, University of Oslo, Oslo University Hospital, Rikshospitalet, Oslo, Norway,

[3]Department of Medicine, Meyerhoff Inflammatory Bowel Disease Center, Johns Hopkins University School of Medicine, Baltimore, Maryland, U.S.A.,

[4]Department of Gastroenterology, Mater Dei Hospital, Msida, Malta,

[5]Department of Medicine and Therapeutics, Institute of Digestive Disease, LKS Institute of Health Science, State Key Laboratory of Digestive Disease, The Chinese University of Hong Kong, Hong Kong, China,

[6]Department of Internal Medicine and Institute of Gastroenterology, Yonsei University College of Medicine, Seoul, Republic of Korea,

[7]Biobank PopGen and Institute of Epidemiology, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany,

[8]Department of Epidemiology, University Medical Center Groningen, Groningen, The Netherlands,

[9]Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran, Iran,

[10]Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana, Punjab, India,

[11]Department of Genetics, University of Delhi South Campus, New Delhi, India,

[12]Department of Medicine, Christian-Albrechts-University of Kiel, Kiel, Germany,

[13]Laboratory for Genotyping Development, Center for Integrative Medical Sciences, RIKEN Yokohama Institute, Yokohama, Japan,

[14] Division of Genomic Epidemiology and Clinical Trials, Clinical Trials Research Center, Nihon University School of Medicine, Tokyo, Japan,

[15] RIKEN Center for Integrative Medical Sciences, Yokohama, Japan,

[16]Université de Montréal and the Montreal Heart Institute, Research Center, Montréal, Québec, Canada,

[17]Research Group for Evolutionary Immunogenomics, Max Planck Institute for Evolutionary Biology, Plön, Germany,

[18]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, U.S.A. and

[19]Department of Medicine, Rutgers Robert Wood Johnson School of Medicine and Department of Genetics, Rutgers University, New Brunswick and Piscataway, New Jersey, U.S.A

[*]Corresponding Author: Prof. Andre Franke, Institute of Clinical Molecular Biology Christian-Albrechts-University of Kiel Rosalind-Franklin-Street 12, D-24105 Kiel E-mail: a.franke@mucosa.de Telephone: +49 (0) 431 / 500 - 15 109 FAX: +49 (0) 431 / 500 - 15 168

[#]The authors wish it to be known that, in their opinion, the first 2 authors should be regarded as joint First Authors.

Abstract

Genotype imputation of the human leukocyte antigen region (HLA) is a cost-effective means to infer classical HLA alleles from inexpensive and dense SNP array data. In the research setting, imputation helps avoid costs for wet lab-based HLA typing and thus renders association analyses of the HLA in large cohorts feasible. Yet, most HLA imputation reference panels target Caucasian ethnicities and

multi-ethnic panels are scarce. We compiled a high-quality multi-ethnic reference panel based on genotypes measured with lllumina's Immunochip genotyping array and HLA types established using a high-resolution next generation sequencing approach. Our reference panel includes more than 1,300 samples from Germany, Malta, China, India, Iran, Japan, Korea and samples of African American ancestry for all classical HLA class I and II alleles including *HLA-DRB3/4/5*. Applying extensive cross validation, we benchmarked the imputation using the HLA imputation tool HIBAG, our multi-ethnic reference and an independent, previously published dataset compiled of subpopulations of the 1000 Genomes project. We achieved average imputation accuracies higher than 0.924 for the commonly studied *HLA-A, -B, -C, -DQB1* and *-DRB1* genes across all ethnicities. We investigated allele specific imputation challenges in regard to geographic origin of the samples using sensitivity and specificity measurements as well as allele frequencies and identified HLA alleles that are challenging to impute for each of the populations separately. In conclusion, our new multi-ethnic reference dataset allows for high resolution HLA imputation of genotypes at all classical HLA class I and class II genes including the *HLA-DRB3/4/5* loci based on diverse ancestry populations.

Introduction

The major histocompatibility complex (MHC), in humans also named human leukocyte antigen (HLA), is a highly variable gene cassette with major functions in the immune system. The HLA region spans approximately 5 Mb on chromosome 6p21 with genomic positions ranging from 29 Mb to 34 Mb. Genes in this region code for proteins that are involved in many complex functions of the adaptive and innate immune system, like the presentation of peptides to the host immune system and also code for proteins that aid peptide presentation or antigen recognition. Results from over ten years of genome-wide association studies (GWAS) support the HLA as one of the most important disease susceptibility loci for almost every immune-mediated and autoimmune disease. In many cases, the strongest association signals are found within the highly polymorphic classical HLA genes in the class I and II regions, a finding made long before the GWAS era for many of these diseases (1). Therefore, pinpointing the exact genetic variants in the HLA region, which are associated with these diseases is of utmost importance to disentangle the underlying genetic pathophysiology (2). This is complicated by the highly polymorphic nature of the region, resulting in the need for large disease cohorts to increase statistical power in the detection of genetic association. The costs per sample for Sanger- and next generation sequencing-based (NGS) HLA typing is still at least double that of a genome-wide single nucleotide polymorphism (SNP) array analysis with the new chip platforms. Therefore, imputation methods and reference panels have been developed to provide geneticists with a tool to infer HLA alleles at the classical loci *in silico* using inexpensive and dense SNP array data. These have led to significant advances in fine-mapping of disease relevant genetic variants for many inflammatory and autoimmune diseases (3-5). Published and established HLA imputation tools are amongst others SNP2HLA, HIBAG, and HLA*IMP (6-8). Imputation of the HLA requires reference panels with high coverage of alleles and genotypes in the region of interest as well as a broad spectrum of samples in order to capture as many different alleles as possible. Additionally, the ancestral background of the reference panel used to impute a dataset of interest must be as close as possible to the study population as shown for instance by Jia *et al.* 2013 (7). Most HLA imputation reference panels target Caucasian ethnicities and although there has been progress in the development of ancestrally diverse HLA reference panels studies in which multi-ethnic analyses are performed are still scarce and limited in size (e.g. for chronic inflammatory diseases, (9)). Several imputation references have been published in the past using various genotyping chips and at different resolutions. All reference panels have significantly

advanced HLA imputation and analysis conducted with the produced data. However, to date, no full context 4-digit multi-ethnic HLA imputation reference panel exists for fine mapping of the HLA region across the totality of the mentioned loci.

With this study, we aimed to create a comprehensive high-quality multi-ethnic HLA reference dataset, including *HLA-DPA1, -DPB1* and *-DRB3/4/5,* using populations of African American, East Asian (Japan, South Korea, China), European (Germany, Malta) and Middle Eastern (India and Iran) descent.

We generated HLA allele calls from next generation sequencing reads (NGS) for ulcerative colitis and control individuals of each population, using HLAssign (Wittig *et al.*, (10)) and genotype information using the Illumina Immunochip SNP array (Figure 1). Using multidimensional scaling (MDS) analysis we analysed population structure based on HLA allele frequencies. The combination of called HLA alleles and SNP array genotypes served as training datasets for our new multi-ethnic reference using the HLA imputation tool HIBAG (Zheng *et al.*, (6)). We benchmarked the imputation, applying extensive cross validation on our multi-ethnic reference panel (Supplementary Figure 1). The performance of our final model was additionally assessed using the previously published HLA calls of the 1000 Genomes project. We also conducted a literature search into the genetic architecture of *HLA-DRB3/4/5* in relation to *HLA-DRB1*, as the presence of the *HLA-DRB3/4/5* are highly dependent on which *HLA-DRB1* allele is carried by an individual. These loci are of particular interest, since they represent a functional variation that has not been considered in many of the previously published reference datasets and hence have been largely excluded in association studies.

Results

MDS-based clustering of reference samples on HLA allele frequencies

Using multidimensional scaling (MDS) analysis on relative frequencies of single HLA G grouped alleles across each cohort, we observed distinct clusters for individuals with East Asian, African and European backgrounds (Figure 2), except for *HLA-DRB3/4/5* and *HLA-DQB1*. The different subpopulations of our multi-ethnic study population cluster well with respective ethnicities of the 1000 Genomes population. For the 1000 Genomes population exons 2 and 3 (class I) or exon 2 (class II) were typed only for loci *HLA-A, -B, -C, -DQB1* and *-DRB1* but not for *HLA-DPA1*, *-DPB1* and *-DRB3/4/5*. However, to the best of our knowledge no custom G groups were defined; Gourraud *et al.*, (11). Samples did not show population specific clustering for *HLA-DQB1*, because frequencies of the HLA alleles in European individuals were similar to those in the Yoruban, African American and European individuals of the 1000 Genomes population. We did not detect consistent clusters for the *HLA-DRB3/4/5* genes, possibly because there was not enough variability to allow good clustering results. In our multi-ethnic dataset we only observe four, three, and six different 4-digit alleles for the *HLA-DRB3/4/5* genes, respectively. In addition, these genes also included a high percentage of null alleles (*HLA-DRB3*: 48.45-81.28%, *HLA-DRB4*: 65.78-84.52%, *HLA-DRB5*: 71.28-85.66%, Table 1), that dominate the frequency spectrum and thus the MDS analysis. With “null allele” we here refer to the absence of a locus in a given individual. These null alleles are named DRB3*00:00, DRB4*00:00 and DRB5*00:00 throughout this paper. In summary, the MDS analysis reveals significant population heterogeneity for the classical HLA genes and thus, imputation tools should be able to account for this heterogeneity by using population-matched and diverse reference panels.

Imputation Benchmark

We performed HLA imputation of the HLA class I loci *HLA-A, -B, -C* and class II loci *HLA-DQA1, -DQB1, -DPA1, -DPB1, -DRB1 and -DRB3/4/5* using HIBAG and three different constellations: (i) our multi-ethnic reference panel in full 4-digit context (Figure 3 and next paragraph), (ii) our multi-ethnic reference panel combined with the 1000 Genomes dataset on G group level (Supplementary Figure 2 and Supplementary Table 1), and (iii) our multi-ethnic reference panel on G group level as a comparison (Supplementary Figure 3 and Supplementary Table 2). We also used the 1000 Genomes panel to test the performance of our data (Table 2) with special focus on the imputation for the non-European population panels, as one of the main innovations of this work.

Using a cross validation approach (Supplementary Figure 1), we divided the data of each specific population into five random subsamples irrespective of case-control status. For each of the subsets, using the remaining 80% of the population, as well as the HLA allele and genotype information of all other populations, we trained a HIBAG model. The HLA alleles were predicted for the 20% of data from the analysed population that were not used for training. We calculated accuracies for each of the five subsamples of our population of interest and imputation accuracies for unrelated individuals of the 1000 Genomes population. The results of the cross validation are depicted in Figure 3 and Table 3. Overall accuracies were high with average accuracies ranging from 0.924 in Chinese to 0.967 in Maltese (Table 3; Supplementary Table 3). More specifically, high overall accuracies were achieved for the *HLA-C*, *HLA-DP* and *HLA-DQ* loci whereas the *HLA-A, -B* and *-DRB1* loci were more challenging to impute across all ethnicities with accuracies as low as 0.862 for *HLA-DRB1* in the Iranian panel. This is also reflected in the posterior probability curves depicted in Figure 3b. Posterior probabilities in HIBAG are used as an additional measure to control prediction accuracies and are generated as an average over all classifiers. Low overall posterior probabilities for a locus indicate that the majority of the alleles were challenging to impute. Note, that correct calls, e.g. for rare alleles, also tend to have smaller posterior probabilities, while incorrect calls can have a high posterior probability when haplotypes of two alleles are similar across many classifiers. Therefore, we decided to additionally use other measures such as sensitivity and specificity, and allele specific accuracy to evaluate allele specific results in the following analyses. With 29-55 alleles per population, and 75% (Malta) to 82% (Japan) of the alleles having frequencies of < 1% (Supplementary Table 4 and Supplementary Table 5), *HLA-B* presented a particular challenge for imputation. Similarly challenging were *HLA-A* and *-DRB1*, which

are discussed further below. The remaining loci were not as variable or had a smaller and more even frequency spectrum (Supplementary Table 5), such that posterior probabilities were higher. *HLA-DPA1* and *-DPB1* had the most "on target" SNPs (30 and 51 SNPs, respectively) (Supplementary Table 6), reflecting the fact, that these loci are least variable and therefore better suited to be captured on a SNP genotyping array. Overall, between 682 (*HLA-DPB1*) and 1,794 (*HLA-A*) SNPs were located within the different gene loci including flanking regions of 500 kb upstream and downstream of each gene. A median of 41.5 (*HLA-DRB5*) to 81 (*HLA-A*) SNPs were used by the single classifiers of HIBAG.

In the following, we show the results of the imputation with our own reference dataset divided by ethnic background and also compare our data to previously reported HLA imputation accuracies on published datasets from Dilthey *et al.* (8), Jia *et al.* (7), Okada *et al.* (12), Kim *et al.* (13) and Zheng *et al*. (6) (Table 4). It is of importance to note, that high accuracies for a reference panel using a specific benchmarking panel are best achieved when the benchmarking panel follows the same allele nomenclature and grouping as the panel used for imputation. We could not determine to which extent this was considered in each of the above studies, but we estimate that the effect should not be detrimental if differences only occur between slightly different custom allele groupings (i.e. we assume that the allele that a grouping is based on is also the most frequent allele) and not between different levels of grouping (i.e. full context versus G groups). A summary of these datasets is described in Table 4. The following results are specific to the imputation of HLA alleles into the respective populations using our multi-ethnic 4-digit full context reference panel. If not stated otherwise, mean accuracies were compared for 4-digit allele imputations of *HLA-A*, *-B*, *-C*, *-DQB1* and *-DRB1*. These are the loci that are present for all imputation references (Table 4). Within the cross-validation framework, accuracies for a gene were calculated as an average across the different cross-validation runs it as has been done previously (12, 13) and enables better comparison of these values between studies. We also report median, minimum and maximum values in Supplementary Table 3. We report accuracies across all imputed alleles in Table 3, Supplementary Table 1 and Supplementary Table 2. A few alleles were especially challenging to impute, both within our as well as in previously published reference panels. These alleles usually have comparably lower sensitivity scores or specificity scores and similar haplotype structures within the same 2-digit allele groups (Supplementary Tables 7 and 8, Supplementary Tables 5-8 of Zheng *et al.*, (6)). This is especially important in the context of association analyses where the greatest impact from these issues is seen with higher frequency variants (AF >1%) and thus needs to be considered carefully. Note, that this also depends on the ethnicity of the samples evaluated. We describe A*02:01/A*02:03, DRB1*11:01/DRB1*11:04 and

DRB1*04:03/DRB1*04:04 below for illustration purposes.

African American panel

The imputation of HLA alleles into our own African American dataset achieved an average imputation accuracy on full context 4-digit level of 0.951 across all analysed loci and of 0.937 on average for loci *HLA-A, -B, -C, -DQB1* and *-DRB1* only (Table 3). Employing our multi-ethnic reference dataset on G group level (ii), we were able to impute alleles of the genes *HLA-A, -B, -C, -DQB1* and *-DRB1* of the 1000 Genomes African ancestry data with a mean accuracy of 0.904 and highest accuracies for the Luhya Kenyan samples alone (0.880 to 0.980; mean of 0.913; Table 2). In comparison, Zheng *et al.* (6) imputed HLA alleles of random subsets of their African American HLARES data combined with the Yoruba Nigerians (YRI) HapMap samples with a reported mean accuracy of 0.818 using their tool HIBAG (Table 4b). Jia *et al.* (7), imputed the HLA alleles of Yoruba Nigerian HapMap samples using their Caucasian T1DGC reference panel with accuracies between 0.203 (*HLA-DRB1*) and 0.984 (*HLA-C*) across all loci and an overall mean accuracy of 0.750 (Table 4a).

East Asian panel

Employing our multi-ethnic reference dataset (i) to impute HLA alleles into our Chinese samples, we achieved accuracies of 0.868 (*HLA-B*) to 1.000 (*HLA-DRB3/4*) and of 0.924 on average for *HLA-A*, *-B*, *-C*, *-DQB1* and *-DRB1*. We imputed HLA alleles into our Japanese samples with accuracies of 0.936 (*HLA-A*) to 1.000 (*HLA-DRB3/5*) and 0.958 on average for *HLA-A*, *-B*, *-C*, *-DQB1* and *-DRB1*. For our Korean samples imputation accuracies of 0.918 (*HLA-DRB1*) to 1.000 (*HLA-DRB4*) were reached, with an average accuracy of 0.947 (Table 3). Additionally, we imputed the HLA alleles of the East Asian 1000 Genomes data on G group level (ii) with mean accuracies higher than 0.953 (Table 2).

In comparison, Okada *et al.* (12), Jia *et al.* (7), Kim *et al.* (13) and Zheng *et al*. (6) reported mean accuracies between 0.77 to 0.922 for *HLA-A*, *-B*, *-C*, *-DQB1* and *-DRB1* (Table 4) for East Asian populations using their respective HLA imputation panels. *HLA-DPA1* or *HLA-DRB3/4/5* are not considered in any of the publications for East Asian ethnicities. For single loci the reported imputation accuracies vary between 0.656 (*HLA-B* with T1DGC reference for Han Chinese in Bejing (CHB) and Japanese samples (JPT), Jia *et al.* (7)) and 0.984 (HLA-C with a Korean reference panel and the same test population, Kim *et al*. (13)).

In the cross-validation benchmark the accuracy of locus HLA-A in the Chinese population (Figure 3a) was decreased due to a misclassification of A*02:03 to A*02:01 in 32% of 37 samples in which this allele occurred. This misclassification is due to the high similarity between these alleles (Supplementary Text). When excluding A*02:03 from accuracy calculations for *HLA-A*, accuracies improved for the Chinese subpopulation from 0.900 to 0.954 (Table 3).

Iranian and Indian panels

Overall imputation accuracies for our Indian and Iranian panels over all loci were 0.944 and 0.935, respectively. The accuracies were high for all loci except *HLA-B* (0.875 and 0.885, respectively) and -*DRB1* (0.924 and 0.862, respectively) (Table 3).

The accuracy of the Iranian samples in the cross-validation benchmark (Figure 3a) at *HLA-DRB1* was low due to a misclassification of DRB1*11:04 to DRB1*11:01 in 39% of the 36 Iranian samples in which this allele occurs (Supplementary Text). When excluding the DRB1*11:04 as well as the DRB1*04:04 and DRB1*04:03 alleles (see below) from accuracy calculations for *HLA-DRB1*, the accuracies improved from 0.862 to 0.956 (Table 3). Mean sensitivity values for DRB1*11:04 for the cross-validation runs were 0.307 for the Iranian population and 0.208 for the Indian population (Supplementary Table 8). The frequency of this allele was 2.82% and 13.85%, respectively (Supplementary Table 5). The improvement of the overall accuracy by excluding these alleles in the Indian samples (0.924 to 0.952) was not as big as in the Iranian samples because of the lower allele frequency. Previously reported sensitivity values for the DRB1*11 alleles (Supplementary Tables 5-8 of Zheng *et al.*, (6)) range from 0.627 (DRB1*11:04) to 0.993 (DRB1*11:01) in the European population. In this previous study, misclassifications occurred for DRB1*11:04, too, which was called as DRB1*11:01 in 93% of cases when a misclassification occurred in European samples (6). This is in line with our own results.

Imputation for non-reference populations

The Latin American admixed populations of the 1000 Genomes dataset (containing Amerindian and European, for Puerto Rico also West African ancestral admixture, here grouped into Mexican, Columbian and Puerto Rican populations) were imputed with mean accuracies ranging from 0.821 for the Mexican, 0.855 for the Columbian to 0.913 for the Puerto Rican population (Table 2). In particular, *HLA-B* and *-DRB1* showed low imputation accuracies (0.688 to 0.857 and 0.598 to 0.821, respectively) while all remaining loci had accuracies higher than 0.857 (Table 2). Overall, the Puerto Rican dataset showed highest accuracies and only 40 out of 134 total measured alleles had sensitivity values of lower than 1.000 (Supplementary Table 9). Out of these 40 alleles, 22 have an AF <0.1% in the Puerto Rican panel. Accuracies for loci imputed within the Puerto Rican dataset ranged from 0.821 (*HLA-DRB1*) to 0.979 (*HLA-DQB1*) (Table 2).

HLA-DRB3/4/5 haplotypes

Many imputation tools allow the imputation of *HLA-A, -B, -C, -DQB1* and *-DRB1* but only a few studies have reported on the imputation of the *HLA-DRB3/4/5*, such as Dilthey *et al*. (8), who analysed *HLA-DRB3/4/5* imputation in Caucasian datasets (Table 4c). These genes can be present or absent in an individual depending on the *HLA-DRB1* genotype. For the evaluation of the imputation of these genes and to elucidate which DRB3/4/5 loci are known to be located on the same haplotype as a specific *HLA-DRB1*, we conducted an extensive literature review and present the results below. We mainly focus on the information reported by Holdsworth *et al.* (14), Robbins *et al.* (15) and Bontrop *et al.* (16). According to literature, alleles of the *HLA-DRB3, -DRB4 and -DRB5* (*HLA-DRB3/4/5*) loci occur within a specific *HLA-DRB1* context, being present in some haplotypes and absent in others. The results of this review are summarized in Figure 4. Haplotypes with *HLA-DRB1* always carry the pseudogene *HLA-DRB9,* which is located downstream of *HLA-DRB1* and that consists of two exons (17). DRB1*01, DRB1*08 and DRB1*10 do not present with any *HLA-DRB3/4/5* allele. Haplotypes with DRB1*03, *11, *12, *13 and *14 are found with *HLA-DRB2* and *-DRB3*. DRB1*04, *07, *09 are found with *HLA-DRB4* as well as *-DRB7* and *-DRB8*. Finally, DRB1*15 and *16 are reported to be located on the same haplotype as *HLA-DRB5*. Exceptions to his rule have been described for DRB1*15 and *16, where especially in African Americans *HLA-DRB5/6* can be missing. DRB1*07 has been reported to occur with a non-expressed form of DRB4*04:01 (15) and DRB1*08 has also been previously identified together with DRB3*03:01 (15).

We investigated our herein-described multi-ethnic dataset of *HLA-DRB1* and *-DRB3/4/5* for congruence with these previous findings. In short, we determined the *HLA-DRB1* alleles for every sample and checked whether we could also find the expected *HLA-DRB3/4/5* alleles or the absence of these in the same sample. All but four samples followed the haplotype structures depicted in Figure 4. After re-analysis of the remaining four samples we concluded that these samples must have been contaminated, since three or more alleles could plausibly be called for all analysed loci, with one allele having a smaller number of reads that aligned to it. In further six samples we found one of the exceptions described in the literature. One Maltese sample did not have *HLA-DRB4* while DRB1*07:01 was present and five African American samples did not have *HLA-DRB5* while DRB1*15:03 or DRB1*16:02 was present.

Frequencies of *HLA-DRB3/4/5* are shown in Table 1. Overall, *HLA-DRB3* is the most variable of those genes according to its frequency spectrum, with DRB3*02:02 being the most common non-null allele

with an allele frequency ranging from 8.82% in our Japanese panel to 37.98% in our Iranian panel. For *HLA-DRB4,* DRB4*01:03 is the most common non-null allele with frequencies ranging from 8.71% in the African American to 32.09% in the Japanese panel. DRB5*01:01 is the most common non-null allele in all but the Iranian and Japanese panels with frequencies of 5.43% in the Iranian to 21.53% in the Chinese panel, while DRB5*01:02 has a frequency of 20.59% in the Japanese panel and a frequency of 6.98% in the Iranian panel. Our data suggest, that DRB1*15:01 is located on the same haplotype as DRB5*01:01, while DRB1*15:02 (which is very common in Japanese samples) is located on the same haplotype as DRB5*01:02 (Supplementary Table 10). Accuracies of the *HLA-DRB3/4/5* imputations are high (>0.971; Table 3 and Figure 3a). Sensitivity measures for the *HLA-DRB3/4/5* are generally high, however, for low frequency variants (e.g. DRB3*02:24 in the Iranian, Maltese and German panels at frequencies of < 0.62%) values as low as 0 were measured. DRB4*01:02 in the Japanese panel, DRB3*01:01 and DRB4*01:01 in the African American panel are common alleles (AF > 1%) classified with mean sensitivity values of lower than 0.800 (0.375, 0.739, 0.690, respectively). We also observed, using the tool Disentangler (18), that the phasing of *HLA-DRB3/4/5* alleles might present a challenge, with many of the null alleles occurring on haplotypes with *HLA-DRB1*, when the respective *HLA-DRB3/4/5* allele is present (Supplementary Figure 4, *HLA-DRB3/4/5* are excluded here). The analysis of this particular topic, however, is beyond the scope of this paper.

Discussion

We compiled three different imputation panels as pre-trained HIBAG models that can be used for HLA imputation in different ethnicities: (i) a multi-ethnic reference with 4-digit full context HLA alleles and (ii) a multi-ethnic reference with 4-digit HLA alleles as G groups. Both panels include *HLA-A, -B, -C, -DQA1, -DQB1, -DPA1, -DPB1, -DRB1,* and *-DRB3/4/5*, and (iii) a multi-ethnic reference panel combined with the 1000 Genomes data (including data from *HLA-A, -B, -C, -DQB1, -DRB1, -DPA1, -DPB1* at a 4-digit G group resolution). Our reference panels have high accuracy values across different ethnicities and subsets of the data and also achieve high accuracies in non-reference ethnicities (Tables 2 and 3). The accuracies in non-reference ethnicities are high but lower than for our reference datasets, as even though our reference is highly diverse the worldwide diversity of the HLA is still not sufficiently captured. Average accuracies of our multi-ethnic reference are larger than 0.924. Tabulated results describing the accuracy measures of panels (ii) and (iii) are presented in Supplementary Table 1 and Supplementary Table 2. Using our reference data, few alleles remain challenging to impute. This affects alleles of the *HLA-DRB1* locus, like the DRB1*11 and DRB1*04 group, which has already been described as problematic in previous benchmarks of other imputation reference panels (Jia *et al.* (7), Zheng *et al. (6)*, Dilthey *et al. (8)*) as well as alleles of the highly diverse *HLA-A* and *-C* genes. We therefore recommend using a 2-digit resolution for these alleles and to consider the imputation difficulties in the interpretation of association results for these alleles. We further suggest that the interpretation of specificity and sensitivity measures should be done separately by ethnic background, since measures can vary between ancestries, i.e. haplotypes for an allele that are highly predictive in one ethnicity may not be highly predictive in another ethnicity. We also verified that SNPs missing in the dataset for which HLA alleles are imputed – and that exist in the reference – can negatively affect the imputation accuracy. This was the case for DRB1*04:03 and DRB1*04:04, where exclusion of 4.4% of the SNPs used by the HIBAG had a major impact on the imputation accuracy for these alleles (Supplementary Text). We therefore suggest, as a general rule, to cautiously investigate the coverage of SNPs used by any imputation reference panel prior to imputation with the respective panel into a dataset. Posterior probabilities are often used to improve the quality of the dataset. Indeed, we also observe that the accuracies improve when using a posterior probability threshold. However, for some alleles similar haplotype structures can cause incorrect calls despite high posterior probabilities. Especially for rare alleles, correct calls are possible at a very low posterior probability. We therefore

suggest using the sensitivity and specificity tables we provide in Supplementary Table 8 to perform data filtering as well as checking the posterior probability.

In summary, imputing HLA alleles into multi-ethnic genome-wide association datasets with our reference panels provides accurate results and can aid HLA fine mapping studies especially in non-Caucasian populations in the future. It allows for HLA imputation using the most recent HLA allele nomenclature at a full context 4-digit resolution and a high diversity of different populations.

Nevertheless, larger sample sizes and even more diverse reference panels are needed to adequately cover the existing global HLA polymorphism and frequency spectrum particularly for the ethnicities not included in our panel and also to impute especially rare HLA alleles with high accuracy. DRB1*01:03, for instance is an allele that has a higher frequency in North American Caucasians (0.9-1.9%) than European Caucasians (~0.6%) (19). As over a million of samples will have been genotyped and whole-genome sequenced in the near future, it is just a matter of warranting global coverage, thus to include representatives from every ethnicity for these efforts. Still, most genetic research focuses on Caucasian ancestry cohorts and neglects large segments of human populations. Decreasing costs of high-resolution NGS-based HLA typing approaches – including phased data sets from long-read technologies – will further fuel the development of more comprehensive and even more accurate imputation reference panels.

Materials And Methods

Resolution of imputation reference panels

Several imputation references have been published in the past using various genotyping chips allowing for an imputation of different HLA genes at different resolutions, i.e. full context 4-digit (2-field), G group and P group resolution (as defined by the IMGT/HLA database) or custom groups (mostly before 2010). Full context 4-digit levels provide information on the gene name, their allele group and the protein sequence of the HLA molecule (i.e. A*01:02 – Gene: A; allele group: 01; protein: 02). Alleles that are within the same G group have identical nucleotide sequences for exons 2 and 3 (HLA class I) or exon 2 only (HLA class II) and may differ in sequence in the other exons. Alleles that are within the same P group encode for identical amino acid sequences in exons 2 and 3 or exon 2 only. P and G group annotations were introduced in 2010 and a major update in allele naming was conducted (ftp://ftp.ebi.ac.uk/pub/databases/ipd/imgt/hla/Nomenclature_2009.txt), amongst others the separator ":" was introduced and alleles were renamed especially alleles of the *HLA-A, -B, -C* and *-DPB1* genes. Notably, HLA allele calling conducted before this time with alleles typed only at exons 2 and 3 or exon 2 may not follow the known G group and P group conventions published by the IMGT/HLA, i.e. HLA alleles might be grouped in custom groups and some of the alleles will carry outdated allele names. This issue should be considered when merging reference panels, such that all included alleles should map to the same allele groups and also in benchmarking studies using external data. G grouping published by the IMGT/HLA database is based on the highest resolution that is recorded for an allele (i.e. 8-digits or lower). Note, that the post-calling G grouping based on 4-digit alleles is problematic for some alleles listed in Supplementary Table 11.

Cohorts & Data Preparation

Multi-ethnic dataset

DNA of 96 healthy individuals and 96 ulcerative colitis (UC) patients were collected from different studies of Chinese, German, Indian, Iranian, Japanese, Korean and Maltese populations that have been published and described elsewhere (20, 21). In short, Chinese samples were collected in and around Hong Kong (Chinese University of Hong Kong), Korean samples in South Korea (Yonsei University College of Medicine and Asan Medical Centre, Seoul), Japanese samples in Tokyo (Institute of Medical Science, University of Tokyo, RIKEN Yokohama Institute and Japan Biobank), Iranian samples were collected in Tehran (Tehran University of Medical Science), Indian samples in North India (Dayanand Medical College and Hospital, Ludhiana), all self-reported North Indian which was consistent with their genetically determined background. German samples in North Germany and Maltese samples in Malta (Department of Gastroenterology, Mater Dei Hospital, Msida, Malta). In addition to the data from the published UC studies, DNA samples were obtained from 192 healthy controls and 192 UC patients, all self-reported as African American (AA) which was consistent with their genetically determined background as each had an admixture of West African and European ancestry (22). These subjects were recruited in the United States of America and Canada by the Johns Hopkins Multicenter African American IBD Study as well as other Genetics Research Centers of the NIDDK IBD Genetics Consortium. We also received 192 (96 healthy, 96 UC) pre-analysed Japanese samples directly from RIKEN Yokohama Institute.

High density SNP-array data interrogating a wide proportion of the extended HLA region were produced for these samples using the Illumina Immunochip (all but Malta) with 196,524 markers addressing immune relevant genes or the Illumina Infimum ImmunoArray 24 (Malta only) with 253,702 markers and subjected to strict quality control criteria as described in the **Supplementary Methods.** DNA was isolated and processed as described previously (10) in preparation for sequencing. Sequencing was performed on an Illumina HiSeq2500 (http://systems.illumina.com) with 100 bp or 125 paired-end runs on a panel of both case and control data in a pool of 96 libraries per lane. 192 Japanese samples were provided by the RIKEN Yokohama Institute and sequenced using 125 bp paired-end runs on the HiSeq2500 with pools of 94 libraries per lane. 4-digit HLA Alleles for all classical HLA I and HLA II genes *HLA-A, -B, -C, -DQA1, -DQB1, -DPA1, -DPB1, -DRB1* as well as – *DRB3/4/5* were manually curated and called using HLAssign (10). In short, only reads mapping exactly to a reference based on HLA sequences published with the IMGT/HLA database version 3.27.0 (23) were used for calling, taking into consideration evenness of read mapping, read equality and specific

read mapping as described by Wittig *et al.* (10). We also cautiously looked at cross-mapping events (reads mapping to multiple HLA loci) and SNP patterns to identify e.g. alleles originating from concatenation of true alleles. In total 1,360 samples were used in this study, having been sequenced and called successfully based on their DNA quality and internal HLAssign measures, i.e. sufficiently large read coverage and also having passed our stringent criteria for the quality control of the Illumina Immunochip array data (**Supplementary Methods**). The *HLA-DRB3/4/5* calls were additionally evaluated for plausibility with respect to the called *HLA-DRB1* genotype. *HLA-DRB3/4/5* alleles, according to reported studies (14-16), occur on certain haplotypes in tight linkage with specific *HLA-DRB1* variants and can either be present or not present at all (i.e. null allele, described as DRB3*00:00, DRB4*00:00 and DRB5*00:00 in the following) or as one functional *HLA-DRB3/4/5* allele in combination with two of the *HLA-DRB3/*4/5 null alleles. For a detailed overview we compiled Figure 4. A total of 312 African American (158 Controls, 154 UC cases), 162 German (78 Controls, 84 Cases), 140 Chinese (68 Controls, 72 Cases), 143 Indian (78 Controls, 65 Cases), 132 Iranian (63 Controls, 69 Cases), 189 Japanese (96 Controls and 93 Cases), 122 South Korean (81 Controls and 41 Cases) and 160 Maltese (75 Controls and 85 Cases) samples were available for construction of HLA imputation models with HIBAG.

1000 Genomes dataset

Using the Phase 3 [version from 20130502] 1000 Genomes reference dataset (24) and Vcftools (version 0.1.12b), we extracted 174,538 phased SNPs that are present in both the Phase 3 dataset and on the Illumina Immunochip used for the main part of our trans-ethnic data. We then performed quality control as described in the **Supplementary Methods** leaving out batch and population stratification analyses. HLA data were downloaded from ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/working/20140725_hla_genotypes/ and are described here: http://www.internationalgenome.org/category/population/. Publicly available data from the 1000 Genomes dataset do not include *HLA-DPA1*, *-DPB1*, *-DQA1* and *DRB3/4/5* allele calls. In Total 162 samples of African Ancestry, 193 of South American Ancestry, 260 sample of East Asian ancestry and 322 samples of European ancestry were available for construction of HLA imputation models with HIBAG. The HapMap data used in other studies (Table 4) are a part of the 1000 Genomes dataset.

Calling of *HLA-DRB3/4/5 alleles*

Data were analysed visually using HLAssign (10). HLAssign does not calculate phases of the HLA alleles and thus does not make hemizygous calls (i.e. recognize null alleles) such that *HLA-DRB3/4/5* genotypes were edited with respect to the *HLA-DRB1* allele post calling. For consistency with the *HLA-DRB3/4/5* with literature (Figure 3), we introduced null alleles, DRB3*00:00, DRB4*00:00 or DRB5*00:00 when the *HLA-DRB1* locus was called as DRB1*01, DRB1*08 or DRB1*10 respectively. DRB3*00:00 was assigned if no *HLA-DRB3* was present in the corresponding *HLA-DRB1* haplotype. Equally, DRB4*00:00 and DRB5*00:00 were assigned if haplotypes corresponding to the absence of *HLA-DRB4* or *-DRB5* were called. Samples with inconclusive *HLA-DRB3/4/5* detected during HLAssign analysis were reanalysed using HLAReporter (25). HLAReporter performs *de novo* assembly on the NGS reads within the investigated HLA locus using the alignment tool TASR (26) and compares these to either G groups or full context alleles known in the IMGT/HLA database with the parameters (-m 50, -o 5, -r 0.7, -u 0, -i 1, -t 0, -e 33, -c 0) for on target reads. Contigs for samples with equal G group predictions were aligned against each other to generate longer overlapping regions using contigs with a coverage higher than 15 and then realigned to the known IMGT/HLA reference alleles.

MDS analysis

Relative allele frequencies were calculated for each allele across the entire multi-ethnic and 1000 Genomes HLA data within the *HLA-A, -B, -C, -DQ* and *-DR* loci. Multidimensional scaling analysis (MDS) was then performed on alleles for which all data subpopulations had an allele frequency (AF) of at least 1% to excluded to produce a clustering that is not biased by similarity in low frequency variants. The MDS analysis was performed using R and the stats-Package (cmdscale) with a Euclidean distance measure. For the MDS analysis across all loci we used HLA loci *HLA-A, -B, -C, -DQB1* and -*DRB1*.

Training of the reference panel

We performed HLA imputation using the published imputation tool HIBAG (HLA Imputation using attribute BAGging) (6). This is a machine learning tool implemented in R that employs ensemble classifiers built on bootstrap samples that has been shown to perform with high accuracy in HLA imputation across multi-ethnic datasets (6). In short, a training set with both HLA alleles and SNPs typed in the extended HLA region on chromosome 6, between 29 and 34 Mb (xHLA), is used to build several classifiers based on bootstrap samples and a subset of SNPs, similarly to random forest as proposed by Breiman *et al.* (27) which minimize the out-of-bag (OOB) errors. Once a model is trained, it can be used as reference to predict HLA alleles from unknown samples using their respective SNP genotype information, utilizing the posterior probability as measure of confidence. For the benchmark we performed a 5x cross validation using HIBAG (6) and HLA and SNP genotype data from two sources: Our multi-ethnic cohort described above and the publicly available 1000 Genomes dataset (24). The 1000 Genomes dataset was typed for *HLA-A, -B, -C, -DPB1* and *-DRB1*, while the multi-ethnic dataset contained all classical HLA Class I and Class II loci and additionally *HLA-DRB3/4/5*. For the 1000 Genomes dataset, typed HLA data were available for samples of the following ethnicities: African, South American Ancestry, East Asian and European. We grouped our data into three different datasets: (i) our multi-ethnic reference containing eight different cohorts described above, (ii) the same reference as in (i) with HLA alleles transformed into their respective G groups (G groups combine alleles with identical exon 2 and 3 [HLA Class I] or exon 2 [HLA Class II] nucleotide sequence) using hla_nom_g.txt downloaded from hlaalleles.org date: 2017-07-10, IPD-IMGT/HLA version 3.29.0); and (iii) our multi-ethnic panel and the 1000 Genomes dataset combined. In total we used 1,360 samples and 8,803 SNPs within the xHLA region for the multi-ethnic reference, as well as 937 samples from the 1000 Genomes data and 8,889 SNPs within the xHLA region from the 1000 Genomes dataset, with 2,267 samples and 8,417 SNPs for the combined dataset as well as their respective HLA calls. For the 1000 Genomes panel, we checked for nomenclature issues, making sure that all of the HLA alleles used in the 1000 Genomes panel mapped to the nomenclature for HLA alleles used since April, 2010 (ftp://ftp.ebi.ac.uk/pub/databases/ipd/imgt/hla/Nomenclature_2009.txt). For alleles with unambiguous G groups (Supplementary Table 11), we assigned the lower number allele for reference panels (ii) and (iii). Genotype data were prepared as described in Supplementary Methods. Samples with typed HLA information were extracted from each quality-controlled, genotyped dataset. The different cohorts were merged and those SNPs with a consistent minor allele frequency (MAF) of <1% (across all cohorts typed for the particular SNP) were excluded. The data were randomly split into 5 equal parts per cohort

with respect to case-control status, thus ensuring that a training set would include both case and control data. Using HIBAG (version.1.8.3), we trained our models using the reference containing the merged subpopulations, excluding 20% of the population of interest and 100 classifiers, as suggested by the authors of the tool (Supplementary Figure 1).

Validation of the reference panel

The quality-controlled genotype data for each cohort was imputed using Beagle version 4.1 with the cohort itself serving as an internal reference to fill in any remaining missing data. Pretrained HIBAG HLA models (see above) were provided with the respective 20% of the remaining data of each analysed population (Supplementary Figure 1), using the genomic position as the identifier. HLA calls were calculated and stored with their respective posterior probabilities. Accuracies and the number of samples to be excluded were calculated for different posterior probability threshold and compared between the different populations.

Calculation of Accuracies

Imputation accuracies were calculated on best-guess alleles compared to the known alleles of the typed data. Accuracies for best-guess alleles were calculated by counting the number of alleles imputed correctly per locus and dividing by the number of samples multiplied by two. Per locus and per allele accuracies were evaluated. We also calculated single allele specificity and sensitivity values if possible. For this we evaluated each allele separately, counting the number of times an allele was predicted correctly as present (True Positive; TP) or absent (True Negative; TN) and the number of times an allele was incorrectly predicted as present (False Positive; FP) or absent (False Negative; FN). We then used the standard-definitions to calculate sensitivity and specificity from these values.

$$\text{Sensitivity} = TP/(TP+FN)$$

$$\text{Specificity} = TN/(TN+FP)$$

$$\text{Accuracy} = (TP+TN)/(TP+TN+FP+FN)$$

For the calculation of the accuracy, specificity and sensitivity values within the cross validation, the mean values across the different runs were calculated for each locus or allele, as well as median, minimum and maximum values for comparison. To establish which alleles might have low sensitivity and specificity values in a general setting for (i), we calculated these measures using a model based on the entire population (i).

Imputation reference panels for comparison

A Caucasian reference panel based on genotypes retrieved from the Type 1 Diabetes Genome Consortium (T1DGC) (29), as well as a Pan Asian dataset (30) using three different Asian populations, were published along with SNP2HLA (7) and are available on request from the SNP2HLA authors. Here, loci *HLA-A, -B, -C, -DQA1, -DQB1, -DPB1 and -DRB1* were typed (Table 4a). Two additional Asian reference panels based on SNP2HLA were published at 4-digit resolution. First, a Korean reference panel was published in 2014 (13) for the imputation of amino acids and HLA alleles into East Asian populations for *HLA-A, -B, -C, -DQB1, -DPB1* and *-DRB1* and second, a Japanese reference dataset was published in 2015 by Okada *et al.* (12) with an evaluation of loci *HLA-A, -B, -C, -DQB1* and *-DRB1*. For these two last reference panels we assume that they were typed at full context 4-digit resolution, which has not been explicitly mentioned in the respective publications (12, 13) but we find that the typed alleles best fit to the full 4-digit context based on which alleles are present. Pre-trained multi-ethnic HLA models with European, Asian, Hispanic and African ancestry (based on a total of 3,738 samples) are provided with the HLA imputation tool HIBAG (6). The samples used for these models were obtained from HLARES (samples GlaxoSmithKline clinical trials) (6) and HapMap, a part of the 1000 Genomes dataset. Together with the tool different imputation references for application on several SNP arrays are available. Loci *HLA-A, -B, -C, -DQA1, -DQB1, -DPB1 and -DRB1* were evaluated at 4-digit resolution (Table 4b). The remaining considered reference panels based on HLA*IMP:02 (8) were typed before the allele nomenclature update in 2010 and may be typed based on customary groupings (possibly G groups) (Table 4c).

Availability of resources

The herein-described reference data sets are available on request from the authors (email contact: f.degenhardt@ikmb.uni-kiel.de) as pretrained HIBAG models and are mapped to IMGT/HLA database version 3.27.0 with G group definitions derived from IMGT/HLA database version 3.29.0. Note that allele names at 4-digit levels did not change between these two releases. The training of these models was performed as described above without exclusion of any samples. A script that will estimate the haplotype similarity between alleles based on the genotype positions available in a dataset, is also available upon request.

Supplementary Materials

Supplementary Materials are available at *HMG* online.

Acknowledgements

We acknowledge the efforts of individuals from Johns Hopkins University and the other MAAIS recruitment centers who contributed to recruitment of the African American samples used in the study as per reference (22). Additional African American samples used were also provided by Judy H. Cho (Icahn School of Medicine at Mount Sinai, New York, NIH grant [DK062422]), Richard H. Duerr (University of Pittsburgh, NIH grant [DK062420]), and Mark Silverberg (University of Toronto, NIH grant [DK062423]). All subjects gave informed consent for their samples to be used for genetics research studies related to inflammatory bowel disease. We also want to acknowledge Garima Juyay (Department of Genetics, University of Delhi South Campus, New Delhi, India), Yuta Fuyuno (Laboratory for Genotyping Development, Center for Integrative Medical Sciences, RIKEN Yokohama Institute, Yokohama, Japan and Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan), Atsushi Takahashi (Laboratory for Statistical Analysis, Center for Integrative Medical Sciences, RIKEN Yokohama Institute, Yokohama, Japan) and Behrooz Alizadeh (University of Groningen, University Medical Centre Groningen, Department Epidemiology, Groningen, the Netherland) for their involvement in this project. We thank Marie Dowds (Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Kiel, Germany) and Philip Stuart (Department of Dermatology, University of Michigan Medical School, Ann Arbor, Michigan, U.S.A.) for helpful discussions.

Mareike Wendorff is supported by the German Research Foundation (DFG) through the Research Training Group 1743, "Genes, Environment and Inflammation". This project received infrastructure support from the DFG Excellence Cluster No. 306 "Inflammation at Interfaces". Eva Ellinghaus has received funding from the European Union Seventh Framework Programme (FP7-PEOPLE-2013-COFUND) under grant agreement No. 609020 (Scientia Fellows). Funding for the Multicenter African American IBD Study (MAAIS) samples was provided by the U.S.A. National Institutes of Health (NIH) [DK062431] (S.R.B.). Shifteh Abedian is supported by joint funding from the University Medical Center Groningen, Groningen, The Netherlands, and Institute for Digestive System Disease, Tehran University of Medical Sciences, Tehran, Iran. The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7-PEOPLE-2013-COFUND) under grant agreement [609020] - Scientia Fellows. This work was supported by a grant from the BioBank Japan Project and, in part, by a Grant-in-Aid for Scientific Research (B) (26293180) funded by the Ministry of Education, Culture, Sports, Science, and Technology, Japan. This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI18C0094). Funding for the Indian samples was provided by the Centre of Excellence in Genome Sciences and Predictive Medicine (Grant # BT/01/COE/07/UDSC/2008) from the Department of Biotechnology, Government of India). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosures Of Interest.

The authors have no conflict of interest to declare.

References

1 Rose, N.R. (1978) HLA and disease. *Arch. Intern. Med.*, **138**, 527-528.

2 Dendrou, C.A., Petersen, J., Rossjohn, J. and Fugger, L. (2018) HLA variation and disease. *Nat. Rev. Immunol.*, **18**, 325-339.

3 Okada, Y., Yamazaki, K., Umeno, J., Takahashi, A., Kumasaka, N., Ashikawa, K., Aoi, T., Takazoe, M., Matsui, T., Hirano, A. *et al.* (2011) HLA-Cw*1202-B*5201-DRB1*1502 haplotype increases risk for ulcerative colitis but reduces risk for Crohn's disease. *Gastroenterology*, **141**, 864-871 e861-865.

4 Goyette, P., Boucher, G., Mallon, D., Ellinghaus, E., Jostins, L., Huang, H., Ripke, S., Gusareva, E.S., Annese, V., Hauser, S.L. *et al.* (2015) High-density mapping of the MHC identifies a shared role

for HLA-DRB1*01:03 in inflammatory bowel diseases and heterozygous advantage in ulcerative colitis. *Nat. Genet.*, **47**, 172-179.

5 Patsopoulos, N.A., Barcellos, L.F., Hintzen, R.Q., Schaefer, C., van Duijn, C.M., Noble, J.A., Raj, T., IMSGC, ANZgene, Gourraud, P.A. *et al.* (2013) Fine-mapping the genetic association of the major histocompatibility complex in multiple sclerosis: HLA and non-HLA effects. *PLoS Genet.*, **9**, e1003926.

6 Zheng, X., Shen, J., Cox, C., Wakefield, J.C., Ehm, M.G., Nelson, M.R. and Weir, B.S. (2014) HIBAG-HLA genotype imputation with attribute bagging. *Pharmacogenomics J.*, **14**, 192-200.

7 Jia, X., Han, B., Onengut-Gumuscu, S., Chen, W.M., Concannon, P.J., Rich, S.S., Raychaudhuri, S. and de Bakker, P.I. (2013) Imputing amino acid polymorphisms in human leukocyte antigens. *PLoS One*, **8**, e64683.

8 Dilthey, A., Leslie, S., Moutsianas, L., Shen, J., Cox, C., Nelson, M.R. and McVean, G. (2013) Multi-population classical HLA type imputation. *PLoS Comput. Biol.*, **9**, e1002877.

9 Franke, A. (2017) Inflammatory Bowel Disease: A Global Disease That Needs a Broader Ensemble of Populations. *Gastroenterology*, **152**, 14-16.

10 Wittig, M., Anmarkrud, J.A., Kassens, J.C., Koch, S., Forster, M., Ellinghaus, E., Hov, J.R., Sauer, S., Schimmler, M., Ziemann, M. *et al.* (2015) Development of a high-resolution NGS-based HLA-typing and analysis pipeline. *Nucleic Acids Res.*, **43**, e70.

11 Gourraud, P.A., Khankhanian, P., Cereb, N., Yang, S.Y., Feolo, M., Maiers, M., Rioux, J.D., Hauser, S. and Oksenberg, J. (2014) HLA diversity in the 1000 genomes dataset. *PLoS One*, **9**, e97282.

12 Okada, Y., Momozawa, Y., Ashikawa, K., Kanai, M., Matsuda, K., Kamatani, Y., Takahashi, A. and Kubo, M. (2015) Construction of a population-specific HLA imputation reference panel and its application to Graves' disease risk in Japanese. *Nat. Genet.*, **47**, 798-802.

13 Kim, K., Bang, S.Y., Lee, H.S. and Bae, S.C. (2014) Construction and application of a Korean reference panel for imputing classical alleles and amino acids of human leukocyte antigen genes. *PLoS One*, **9**, e112546.

14 Holdsworth, R., Hurley, C. K., Marsh, S. G., Lau, M., Noreen, H. J., Kempenich, J. H., Setterholm, M. ,Maiers, M. (2009) The HLA dictionary 2008: a summary of HLA-A, -B, -C, -DRB1/3/4/5, and -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR, and -DQ antigens. *Tissue Antigens*, **73**, 95-170.

15 Robbins, F., Hurley, C.K., Tang, T., Yao, H., Lin, Y.S., Wade, J., Goeken, N. and Hartzman, R.J. (1997) Diversity associated with the second expressed HLA-DRB locus in the human population.

Immunogenetics, **46**, 104-110.

16 Bontrop, R.E., Otting, N., de Groot, N.G. and Doxiadis, G.G. (1999) Major histocompatibility complex class II polymorphisms in primates. *Immunol. Rev.*, **167**, 339-350.

17 Gongora, R., Figueroa, F. and Klein, J. (1996) The HLA-DRB9 gene and the origin of HLA-DR haplotypes. *Hum. Immunol.*, **51**, 23-31.

18 Kumasaka, N., Okada, Y., Takahashi, A., Kubo, M., Nakamura, Y. and Kamatani, N. (2011), In *12th International Congress of Human Genetics/61st Annual Meeting of The American Society of Human Genetics*, Montreal, Canada, Abstract / Program #708F.

19 Gonzalez-Galarza, F.F., Takeshita, L.Y., Santos, E.J., Kempson, F., Maia, M.H., da Silva, A.L., Teles e Silva, A.L., Ghattaoraya, G.S., Alfirevic, A., Jones, A.R. *et al.* (2015) Allele frequency net 2015 update: new features for HLA epitopes, KIR and disease and HLA adverse drug reaction associations. *Nucleic Acids Res*, **43**, D784-788.

20 Liu, J.Z., van Sommeren, S., Huang, H., Ng, S.C., Alberts, R., Takahashi, A., Ripke, S., Lee, J.C., Jostins, L., Shah, T. *et al.* (2015) Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat. Genet.*, **47**, 979-986.

21 Brant, S.R., Okou, D.T., Simpson, C.L., Cutler, D.J., Haritunians, T., Bradfield, J.P., Chopra, P., Prince, J., Begum, F., Kumar, A. *et al.* (2017) Genome-Wide Association Study Identifies African-Specific Susceptibility Loci in African Americans With Inflammatory Bowel Disease. *Gastroenterology*, **152**, 206-217 e202.

22 Huang, C., Haritunians, T., Okou, D.T., Cutler, D.J., Zwick, M.E., Taylor, K.D., Datta, L.W., Maranville, J.C., Liu, Z., Ellis, S. *et al.* (2015) Characterization of genetic loci that affect susceptibility to inflammatory bowel diseases in African Americans. *Gastroenterology*, **149**, 1575-1586.

23 Robinson, J., Halliwell, J.A., Hayhurst, J.D., Flicek, P., Parham, P. and Marsh, S.G. (2015) The IPD and IMGT/HLA database: allele variant databases. *Nucleic Acids Res.*, **43**, D423-431.

24 Auton, A., Brooks, L.D., Durbin, R.M., Garrison, E.P., Kang, H.M., Korbel, J.O., Marchini, J.L., McCarthy, S., McVean, G.A. and Abecasis, G.R. (2015) A global reference for human genetic variation. *Nature*, **526**, 68-74.

25 Huang, Y., Yang, J., Ying, D., Zhang, Y., Shotelersuk, V., Hirankarn, N., Sham, P.C., Lau, Y.L. and Yang, W. (2015) HLAreporter: a tool for HLA typing from next generation sequencing data. *Genome Med.*, **7**, 25.

26 Warren, R.L. and Holt, R.A. (2011) Targeted assembly of short sequence reads. *PLoS One*, **6**, e19816.

27 Breiman, L. (2001) Random Forests. *Mach. Learn.*, **45**.

28 Browning, B.L. and Browning, S.R. (2016) Genotype Imputation with Millions of Reference Samples. *Am J. Hum. Genet.*, **98**, 116-126.

29 Mychaleckyj, J.C., Noble, J.A., Moonsamy, P.V., Carlson, J.A., Varney, M.D., Post, J., Helmberg, W., Pierce, J.J., Bonella, P., Fear, A.L. *et al.* (2010) HLA genotyping in the international Type 1 Diabetes Genetics Consortium. *Clin. Trials*, **7**, 75-87.

30 Pillai, N.E., Okada, Y., Saw, W.Y., Ong, R.T., Wang, X., Tantoso, E., Xu, W., Peterson, T.A., Bielawny, T., Ali, M. *et al.* (2014) Predicting HLA alleles from high-resolution SNP data in three Southeast Asian populations. *Hum. Mol. Genet.*, **23**, 4443-4451.

Figure 1 – Flowchart of steps taken in preparation and benchmarking of our multi-ethnic reference panel. HLA allele calls were made based on next-generation sequencing (NGS) reads. Genotype information was measured using the Illumina Immunochip. These data were combined to train a HIBAG imputation model. Benchmarking was performed using a 5x cross validation and the independent, previously published, 1000 Genomes dataset.

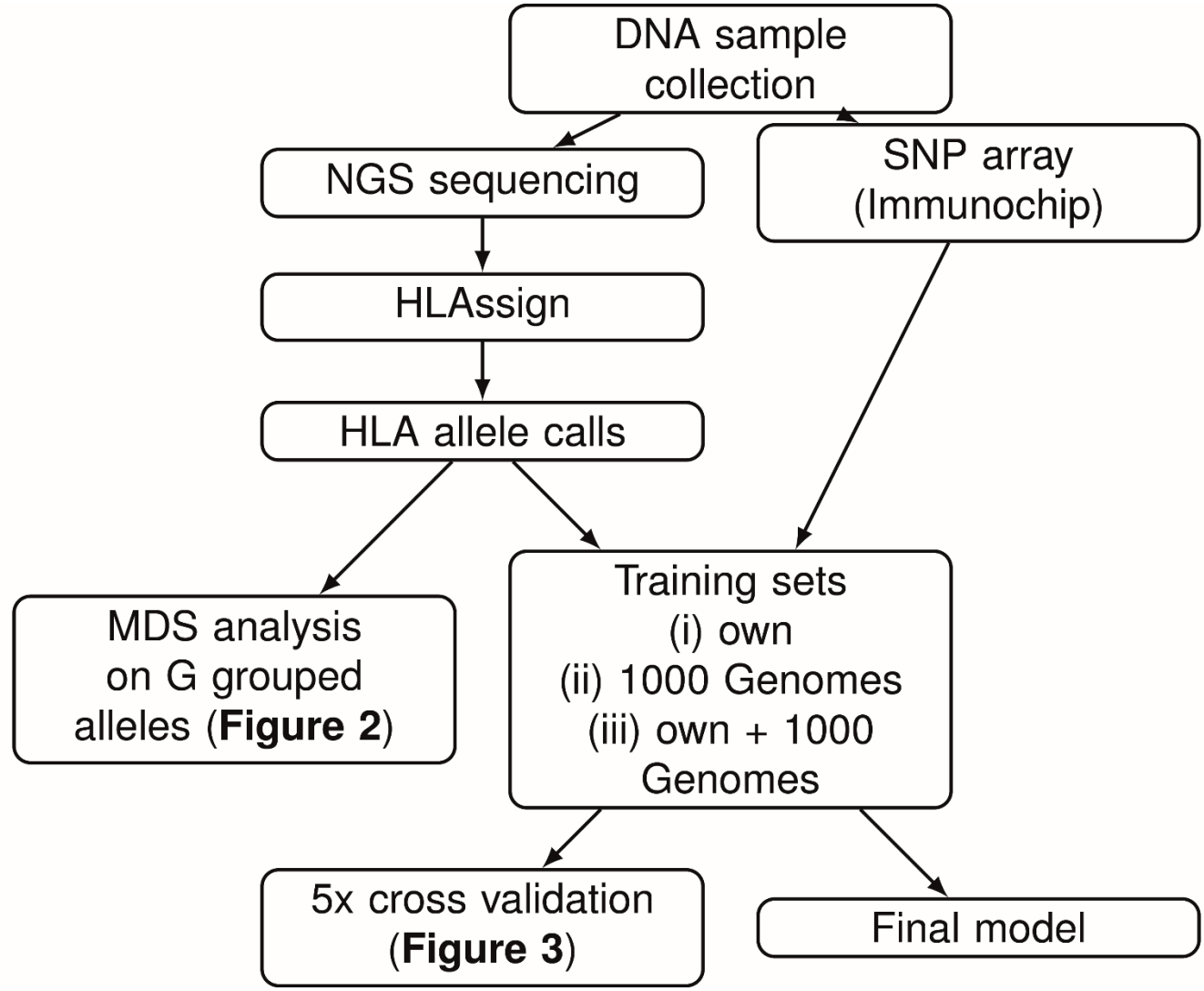


Figure 2 – MDS analysis of HLA typed allele data: The MDS analysis was performed using a Euclidean distance measure. Alleles with a frequency <1% were excluded to produce a clustering that is not biased by similarity in low frequency variants. Colours show the origin of the cohort. Red: African American (AA) and African background; Green: European and Middle Eastern background: German (GER), Indian (IND), Iranian (IRN), Maltese (MLT); Blue: Asian background, Hong-Kong Chinese (CHN), South Korean (KOR) and Japanese (JPN); Purple: Non-reference admixed American individuals. Capital acronyms in the panels depict the 1000 Genomes populations as described in Auton *et al.*, (24). The 1000 Genomes populations include Americans of African Ancestry in the Southwest USA (ASW), Africans from Kenya (LWK), Nigeria (YRI), Columbian (CLM), Mexican (MXL) and Puerto Rican (PUR), Han Chinese in Beijing (CHB), Southern Han Chinese (CHS), Japanese in Tokyo (JPT), Finnish (FIN), British (GBR), Tuscan (TSI) and samples with Western European Ancestry collected in the CEPH diversity panel (CEU). For *HLA-DPA1*, *-DPB1*, *-DQA1* and the *-DRB3/4/5* loci no data was available in those panels. For the MDS analysis across all loci (HLA CLASS I_II) we included *HLA-A, -B, -C, -DQB1* and *-DRB1*. Samples of our own cohorts cluster well with the corresponding 1000 Genomes population.

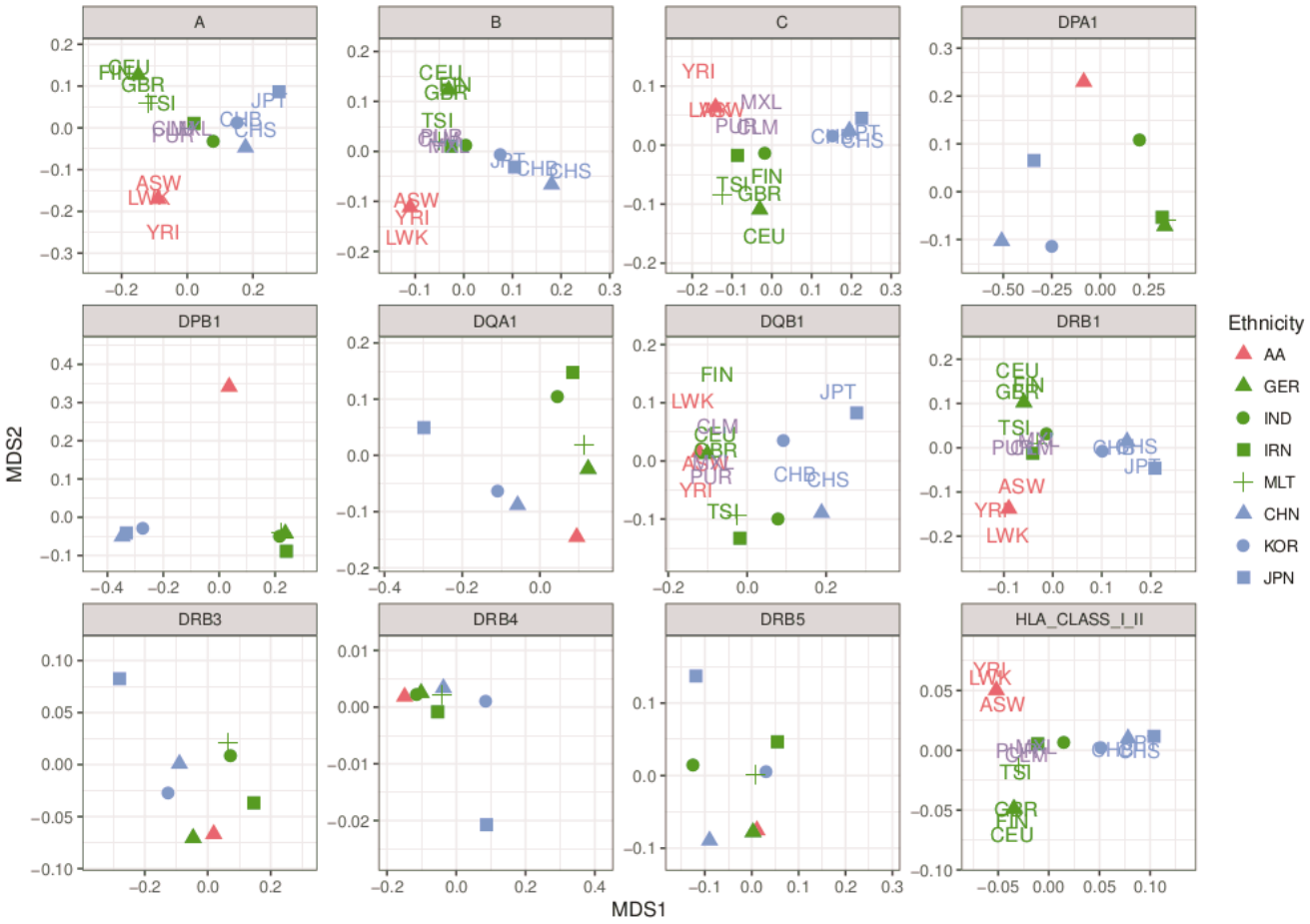
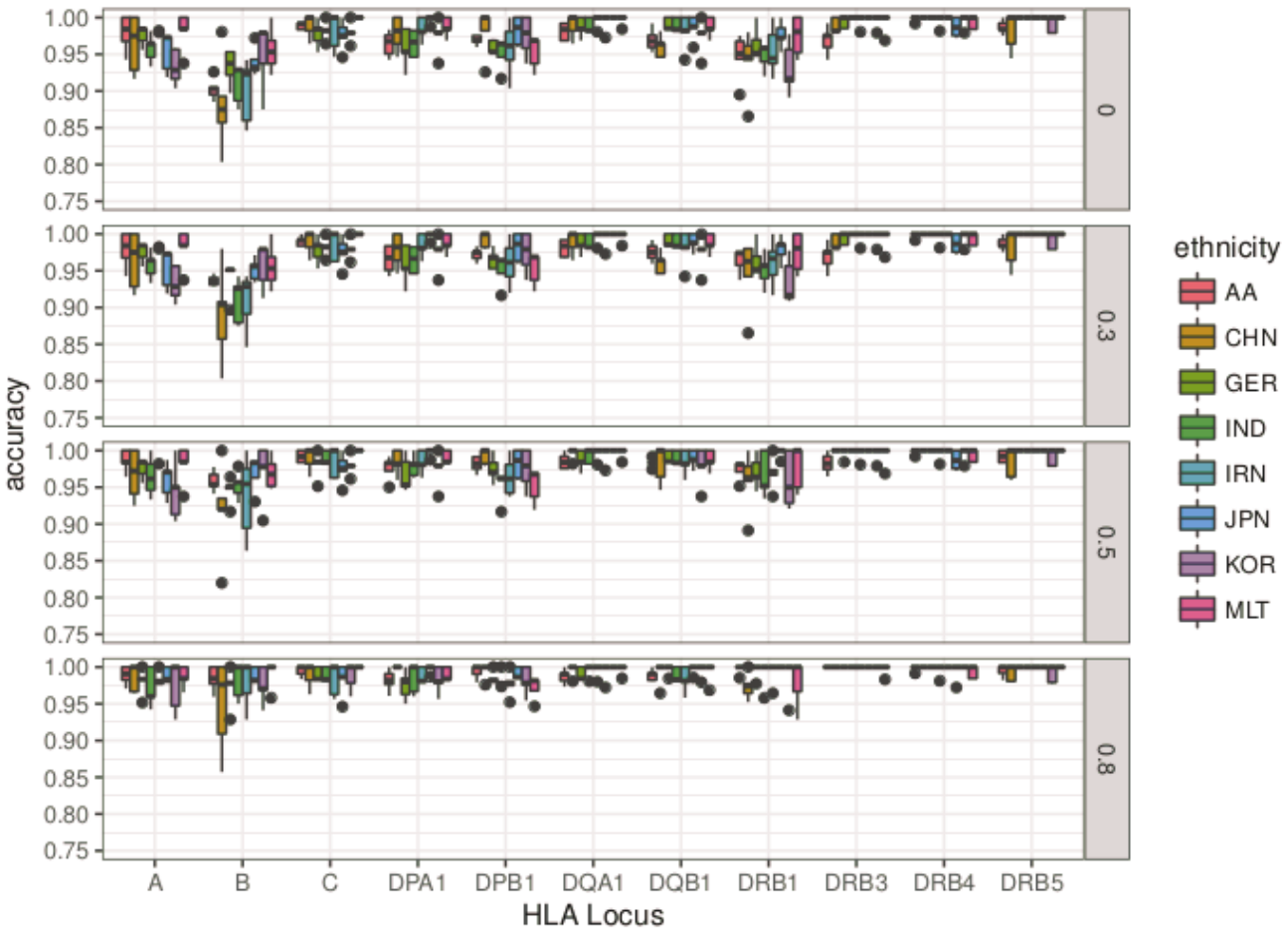


Figure 3 – Imputation accuracies employing the multi-ethnic reference panel: Accuracies and post-imputation probabilities of HLA imputation with HIBAG using a 5-fold cross-validation scheme and the multi-ethnic dataset with full 4-digit allele information. 20% of the data with a specific ethnic background were used as the validation set after training a model that used 80% of the remaining data and all data from other ethnic backgrounds. We included 1,360 African American (AA), Hong-Kong Chinese (CHN), German (GER), Indian (IND), Iranian (IRN), Japanese (JPN), South Korean (KOR) and Maltese (MLT) samples in total. **(a)** Accuracies are depicted according to post-imputation probabilities with cut-off thresholds at 0 (no confidence filtering), 0.3, 0.5, 0.8 (only high confidence genotypes). Loci are shown according to alphabetical order. Imputation accuracies are especially high for *HLA-C*, *-DPA1*, *-DPB1*, *-DQB1* and the *-DRB3/4/5*. *HLA-DRB1* accuracies are especially lowered by misclassifications of DRB1*04:03, DRB1*04:04 and DRB1*11:04. **(b)** Posterior probabilities are depicted as proportion of the number of samples with a posterior probability smaller than a threshold (x-axis).



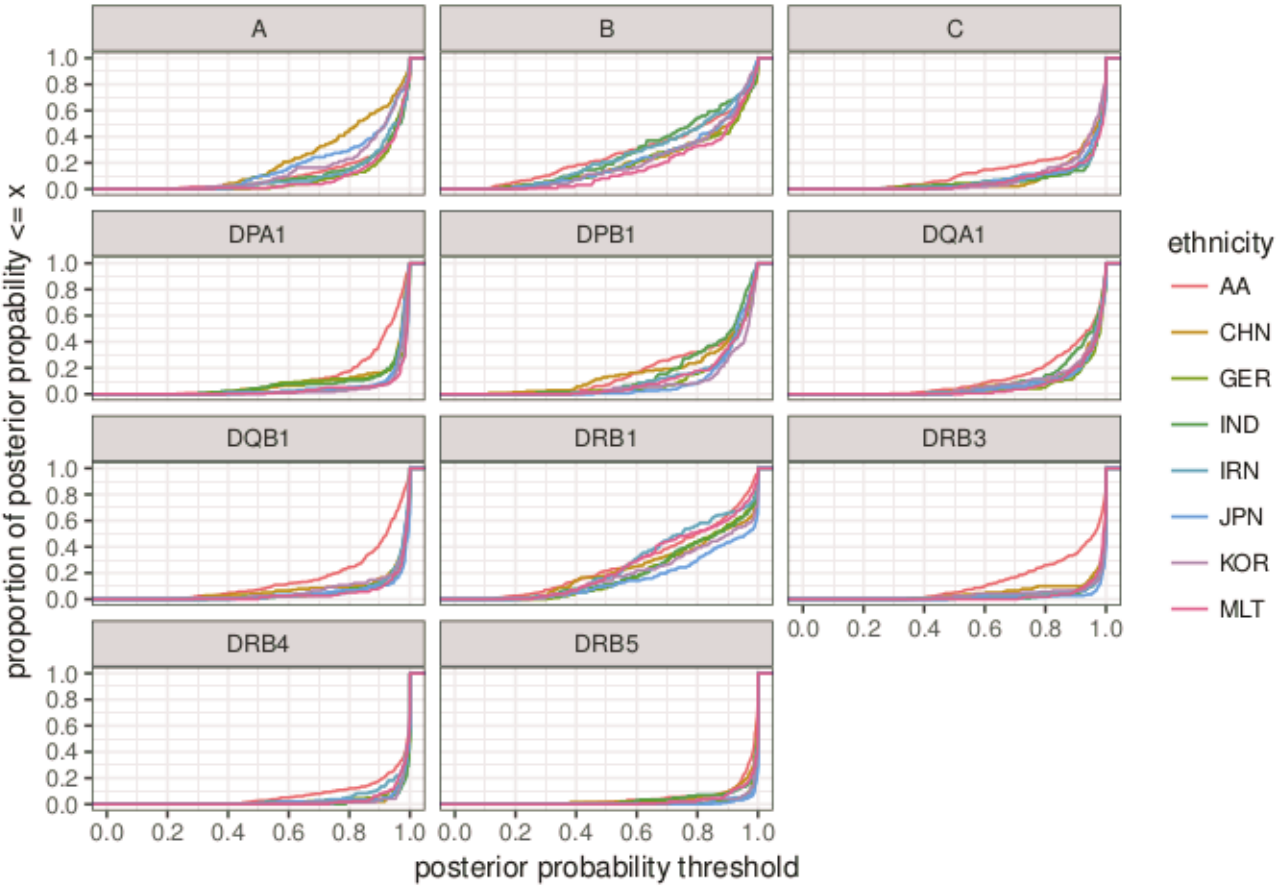


Figure 4 – Known architecture of *HLA-DRB3/4/5*: HLA haplotypes that usually contain a specific *HLA-DRB1* allele (*HLA-DRB1* column) are shown. Two-digit alleles are denoted. All loci are depicted in order of their genomic location. *HLA-DRA*, *HLA-DRB1* and *HLA-DRB9* coincide with all haplotypes. The remaining loci are present or absent depending on the haplotype. The most prevalent haplotypes with the known exceptions are shown in the rows below. Exceptions are sometimes seen for *DRB1**08, DRB1*07, DRB1*15 and DRB1*16. DRB1*08 can occur with *HLA-DRB3*, DRB1*07 can occur without an expressed form of *HLA-DRB4* and DRB1*15 and DRB1*16 can occur without *HLA-DRB5/6*. Loci that usually occur together are joined by a line. The name of the corresponding serotype is shown on the left and haplotypes are ordered by serotype name. Information for this figure was retrieved from Bontrop *et al.*, Holdsworth *et al.* and Robbins *et al.* (14-16).

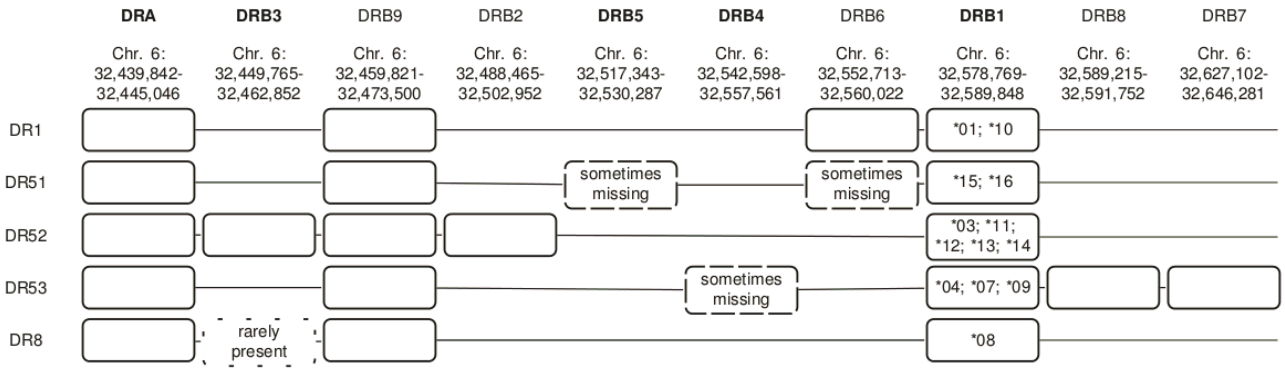


Table 1 – Frequencies of DRB3/4/5 in our multi-ethnic reference panel: Frequencies of *HLA-DRB3/4/5* in the typed HLA data for African American (AA), Hong-Kong Chinese (CHN), German (GER), Indian (IND), Iranian (IRN), Japanese (JPN), South Korean (KOR) and Maltese (MLT) populations at full 4-digit context. Null alleles have the highest frequencies. For *HLA-DRB4* mainly one other allele, DRB4*01:03 exists. DRB5*01:01 is second most abundant of the *HLA-DRB5* alleles in all but the Japanese and Iranian panels, where DRB5*01:02 is seen more often.

	AA	**CHN**	**GER**	**IND**	**IRN**	**JPN**	**KOR**	**MLT**
DRB3*00:00	51.61	64.60	59.88	56.74	48.45	81.28	64.34	55.00
DRB3*01:01	11.13	2.55	14.51	5.32	8.53	4.55	11.07	4.69
DRB3*02:02	27.74	19.34	22.53	32.98	37.98	8.82	16.39	33.75
DRB3*02:24	0.00	0.00	0.62	0.00	0.39	0.00	0.00	0.31
DRB3*03:01	9.52	13.50	2.47	4.96	4.65	5.35	8.20	6.25
DRB4*00:00	84.52	75.91	80.25	80.85	75.97	65.78	68.44	75.63
DRB4*01:01	6.77	0.00	2.47	0.35	1.55	0.00	0.00	3.75
DRB4*01:02	0.00	0.00	0.00	0.00	0.39	2.14	0.41	0.00
DRB4*01:03	8.71	24.09	17.28	18.79	22.09	32.09	31.15	20.31
DRB4*03:01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.31
DRB5*00:00	81.94	72.63	80.56	71.28	85.66	71.66	82.38	81.56
DRB5*01:01	15.97	21.53	16.67	15.96	5.43	6.42	11.07	10.00
DRB5*01:02	0.32	1.82	0.62	12.77	6.98	20.59	4.51	3.75
DRB5*01:03	0.00	0.73	0.00	0.00	0.00	0.00	0.00	0.00
DRB5*01:08	0.32	2.19	0.00	0.00	0.00	0.27	0.41	0.00
DRB5*02:02	0.97	0.36	2.16	0.00	1.94	1.07	1.64	4.69
DRB5*02:03	0.00	0.73	0.00	0.00	0.00	0.00	0.00	0.00
DRB5*02:13	0.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2 – Imputation accuracies for 1000 Genomes populations: Population groups are depicted in **bold** and the subpopulations in *italic* type. African (AFR) samples are divided into Americans of African Ancestry in the Southwest USA (ASW), Africans from Kenya (LWK) and Nigeria (YRI). Admixed American (AMR) samples are split into samples with Columbian (CLM), Mexican (MXL) and Puerto Rican (PUR) ancestry. East Asians (EAS) were collected as Han Chinese in Beijing (CHB), Southern Han Chinese (CHS) and Japanese in Tokyo (JPT). Samples with European Ancestry (EUR) are Finnish (FIN), British (GBR), Tuscan (TSI) and samples with Western European Ancestry collected in the CEPH diversity panel (CEU). Accuracies of *HLA-DRB1** are *HLA-DRB1* measured without DRB1*04:03, DRB1*04:04 and DRB1*11:04, which improved accuracies for all ethnicities. HLA-A* are accuracies measured without A*02:03, which improved accuracies for the Chinese samples. Overall accuracies were highest for EUR samples and lowest for the non-AMR, for which no samples with similar backgrounds are included in our novel imputation reference.

	#samples	***A***	***B***	***C***	***DQB1***	***DRB1***	**mean**	*A**	*DRB1**
AFR	**162**	0.920	0.833	0.932	0.951	0.886	0.904	0.920	**0.906**
ASW	*41*	0.939	0.805	0.915	0.939	0.902	0.900	0.939	**0.923**
LWK	*75*	0.880	0.853	0.960	0.980	0.893	0.913	0.880	**0.899**
YRI	*46*	0.967	0.826	0.902	0.913	0.859	0.893	0.967	**0.902**
AMR	**193**	0.909	0.756	0.972	0.984	0.710	0.866	0.909	**0.766**
CLM	*67*	0.925	0.709	0.970	0.985	0.687	0.855	0.925	**0.711**
MXL	*56*	0.857	0.688	0.973	0.991	0.598	0.821	0.857	**0.674**
PUR	*70*	0.936	0.857	0.971	0.979	0.821	0.913	0.936	**0.888**
EAS	**260**	0.929	0.931	0.975	0.992	0.940	0.953	**0.941**	**0.951**
CHB	*82*	0.939	0.921	0.988	0.994	0.939	0.956	**0.948**	**0.967**
CHS	*92*	0.935	0.924	0.967	0.995	0.935	0.951	**0.963**	**0.944**
JPT	*86*	0.913	0.948	0.971	0.988	0.948	0.953	0.913	0.943
EUR	**322**	0.983	0.944	0.994	0.989	0.890	0.960	0.983	**0.968**
CEU	*52*	0.981	0.922	0.971	1.000	0.865	0.948	0.981	**0.987**
FIN	*95*	0.984	0.974	1.000	0.989	0.926	0.975	0.984	**0.959**
GBR	*86*	0.977	0.959	1.000	0.983	0.884	0.960	0.977	**0.993**
TSI	*89*	0.989	0.910	0.994	0.989	0.871	0.951	0.989	**0.944**

Table 3 – Imputation accuracies of the imputation with the multi-ethnic reference panel: 20% of the data with a specific ethnic background were used as validation set after training a model with 80% of the remaining data and all data from other ethnic backgrounds. We included 1,360 African American (AA), Hong-Kong Chinese (CHN), German (GER), Indian (IND), Iranian (IRN), Japanese (JPN), South Korean (KOR) and Maltese (MLT) samples in total in the imputation reference. Shown are mean accuracies of the HLA imputation with HIBAG using a 5-fold cross-validation scheme and the multi-ethnic dataset with full 4-digit allele information. The given mean considers only the loci highlighted in **bold**, as these are loci also analysed in all previous publications. Accuracies of *HLA-DRB1** are *HLA-DRB1* measured without DRB1*04:03, DRB1*04:04 and DRB1*11:04, which improves accuracies for all ethnicities. *HLA-A** are accuracies measured without A*02:03, which improves accuracies for the Chinese samples. Overall, *HLA-B* is most challenging to impute. Mean accuracies are higher than 0.925 across all cross validation runs. Best results are achieved for the GER, JPN and MLT populations.

	AA	**CHN**	**GER**	**IND**	**IRN**	**JPN**	**KOR**	**MLT**
#samples	**312**	**140**	**162**	**143**	**132**	**189**	**122**	**160**
A	0.969	0.900	0.976	0.955	0.973	0.936	0.939	0.984
B	0.877	0.868	0.917	0.875	0.885	0.938	0.934	0.947
C	0.953	0.986	0.975	0.979	0.974	0.973	0.968	0.988
DPA1	0.969	0.979	0.960	0.968	0.985	0.995	0.975	0.988
DPB1	0.925	0.949	0.960	0.944	0.954	0.979	0.963	0.956
DQA1	0.942	0.975	0.975	0.965	0.962	0.968	0.959	0.978
DQB1	0.962	0.964	0.988	0.990	0.981	0.984	0.975	0.984
DRB1	0.925	0.903	0.948	0.924	0.862	0.960	0.918	0.931
DRB3	0.971	1.000	1.000	1.000	1.000	1.000	0.996	0.994
DRB4	0.977	1.000	0.991	0.996	0.996	0.990	1.000	0.988
DRB5	0.987	0.982	1.000	1.000	1.000	1.000	0.992	1.000
mean	0.937	0.924	0.961	0.944	0.935	0.958	0.947	0.967
*A**	0.969	0.954	0.976	0.954	0.973	0.935	0.937	0.984
*DRB1**	0.930	0.904	0.954	0.952	0.956	0.968	0.926	0.971

Table 4 – Previously reported imputation accuracies**:** Accuracies measured for HLA reference panels, which are mainly based on Caucasian and Asian data, with origin of the publications and cohorts used for training and validation as well as a comparison to accuracies achieved with our own multi-ethnic reference panel (i) in the cross-validation experiment on our own data (see also **Table 3**) and on the 1000 Genomes cohorts (see also **Table 2**). Accuracies of the cross-validation (own) framework and of the imputation into the 1000 Genomes population are shown. Mean accuracies are calculated across *HLA-A, -B, -C, -DPB1* and *-DRB1* (loci highlighted in **bold**). Mean accuracies of the listed reference panels are lower compared to our own reference panel in the majority of the cases, especially in the non-European population. **(a)** Accuracies published with SNP2HLA. The international Type 1 Diabetes Genome Consortium (T1DGC) reference panel (7) published along with SNP2HLA was used to gain the accuracies on the 1948 British Birth Cohort and the HapMap-CEPH Cohort, two European ancestry panels. The T1DGC panel was further used for imputing the Yoruban Nigerian (YRI), the East Asian Han Chinese from Beijing (CHB) and the Japanese from Tokyo (JPT) samples of the 1000 Genomes datasets. For the East Asian 1000 Genomes panels accuracies reached by later published ethnic-specific references (12, 13) are also listed. **(b)** Accuracies published with HIBAG using the HLARES data from GlaxoSmithKline (GSK) clinical trials of specific ethnic background combined with 1000 Genomes datasets (6). **(c)** Accuracies published with HLA*IMP:02 using different combinations of the Gold Standard (GS = 1948 Birth Cohort/ HapMap CEU and CEPH CEU+) and the HLARES data as references (8).

(a) SNP2HLA

Source	**Jia *et al.*, 2013**				**Okada *e***
					2015
imputation reference	**T1DGC**				**Japanese**
# training samples	5,225				916
test population	**1948 British Birth Cohort**	**CEPH**	**YRI**	**CHB & JPT**	**JPT**
# test samples	918	90	not specified	not specified	44
A	0.981	0.991	0.699	0.981	0.908
B	0.968	0.968	0.905	0.656	0.943
C	0.969	0.991	0.984	0.688	0.989
DPA1	/	/	/	/	/

DPB1	/		/		/		/		/
DQA1	/		0.985		0.649		0.963		/
DQB1	0.983		0.991		0.961		0.964		0.894
DRB1	0.933		0.969		0.203		0.923		0.843
DRB3	/		/		/		/		/
DRB4	/		/		/		/		/
DRB5	/		/		/		/		/
mean	0.967		0.983		0.729		0.864		0.915
mean *A-C, DQB1, DRB1*	0.967		0.982		0.75		0.842		0.915
mean *A-C,*	**own**								
DQB1, DRB1	GER	0.961	GER	0.961	AA	0.937	CHN	0.924	CHN
	MLT	0.967	MLT	0.967			JPN	0.958	JPN
							KOR	0.947	KOR
	1000 Genomes								
	EUR	0.96	EUR	0.96	ASW	0.9	CHB	0.956	CHB
					LWK	0.913	CHS	0.951	CHS
					YRI	0.893	JPT	0.953	JPT

(b) HIBAG

Source	**Zheng *et al.*, 2014**			
imputation reference	**HLARES data of Asian ancestry & CHB & JPT**	**HLARES data of Hispanic ancestry**	**African American HLARES data & 60 African YRI**	**HLARES data of European ancestry**
# training samples	720 + 90 (minus test)	439 (minus test)	173 + 60 (minus test)	2668 (minus test)
test population	**random subset**	**random subset**	**random subset**	**random subset**
# test samples	subset	subset	subset	subset
A	0.921	0.934	0.924	0.982
B	0.875	0.75	0.768	0.966
C	0.966	0.962	0.885	0.988
DPA1	/	/	/	/
DPB1	0.898	0.931	0.8	0.947

DQA1	0.868		0.938		0.794		0.964	
DQB1	0.96		0.957		0.742		0.992	
DRB1	0.887		0.82		0.771		0.921	
DRB3	/		/		/		/	
DRB4	/		/		/		/	
DRB5	/		/		/		/	
mean	0.911		0.899		0.812		0.966	
mean *A-C, DQB1, DRB1*	0.922		0.885		0.818		0.97	
mean *A-C,*	**own**							
DQB1, DRB1	CHN	0.924			AA	0.937	GER	0.961
	JPN	0.958					MLT	0.967
	KOR	0.947						
	1000 Genomes							
	CHB	0.956	PUR	0.913	ASW	0.9	EUR	0.96
	CHS	0.951			LWK	0.913		
	JPT	0.953			YRI	0.893		

(c) HLA*IMP:02

Source	**Dilthey *et al.*, 2013**				
imputation reference	**GS**	**HLARES _EU**	**GS & HLARES_ALL**		
# training samples	1,585	1,758	2,055		
test population	**HLARES _EU**	**random subset**	**African Americans of random subset**	**Asians of random subset**	**Europe of rand**
# test samples	1,060	872	1,008 (all populations)		
A	0.96	0.97	0.73	0.79	0.96
B	0.9	0.95	0.73	0.68	0.95
C	0.96	0.96	0.97	0.82	0.97
DPA1	/	/	/	/	/
DPB1	/	0.90 (2-digit)	/	/	/
DQA1	0.87	0.97	1	0.73	0.96

DQB1	0.98		0.98		0.87		0.83		0.97
DRB1	0.88		0.91		0.71		0.72		0.9
DRB3	/		0.94 (2 digit)		/		/		/
DRB4	/		0.98 (2 digit)		/		/		/
DRB5	/		0.99 (2 digit)		/		/		/
mean	0.93		0.95		0.84		0.76		0.95
mean *A-C, DQB1, DRB1*	0.94		0.95		0.8		0.77		0.95
mean *A-C,*	**own**								
DQB1, DRB1	GER	0.961	GER	0.961	AA	0.937	CHN	0.924	GER
	MLT	0.967	MLT	0.967			JPN	0.958	MLT
							KOR	0.947	
	1000 Genomes								
	EUR	0.96	EUR	0.96	ASW	0.9	CHB	0.956	EUR
					LWK	0.913	CHS	0.951	
					YRI	0.893	JPT	0.953	

ABBREVIATIONS

AA	African American
AF	Allele frequency
AFR	African
AMR	Ad Mixed American
ASW	Americans of African Ancestry in SW USA
bp	base pair
CEU	Utah Residents (CEPH) with Northern and Western European Ancestry
CHB	Han Chinese in Beijing, China
CHN	Chinese
Chr.	Chromosome
CHS	Southern Han Chinese
CLM	Colombians from Medellin, Colombia

DNA	Deoxyribonucleic acid
EAS	East Asian
EUR	European
FIN	Finnish in Finland
FN	False Negative
FP	False Positive
G group	HLA group on genome level
GBR	British in England and Scotland
GER	German
GWAS	Genome wide association study
HLA	Human Leukocyte Antigen
IRN	Iranian
IND	Indian
JPT	Japanese in Tokyo, Japan
JPN	Japanese
KOR	South Korean
LWK	Luhya in Webuye, Kenya
MAF	Minor Allele Frequency
max	Maximum
Mb	Mega base
MDS	Multidimensional scaling
MHC	Major Histocompatibility Complex
min	Minimum
MLT	Maltese
MXL	Mexican Ancestry from Los Angeles USA
NGS	Next generation sequencing
P group	HLA group on protein level
PUR	Puerto Ricans from Puerto Rico

SNP	Single nucleotide polymorphism
TN	True Negative
TP	True Positive
TSI	Toscani in Italia
UC	Ulcerative Colitis
xHLA	Extended Human Leukocyte Antigen
YRI	Yoruba in Ibadan, Nigeria

Supplementary Figure 1 – Cross validation scheme for the HLA benchmarking: Number of samples used for training of the respective reference using the example of a cross-validation model applied to the African American data panel.

Supplementary Figure 2 – Imputation accuracies employing the multi-ethnic reference combined with the 1000 Genomes dataset: Accuracies and post-imputation probabilities of HLA imputation with HIBAG (1) using a 5x cross validation scheme and the trans-ethnic and 1000 Genomes dataset (4) with 4-digit G group allele information. 20% of the data with a specific ethnical background were used as the validation set after training a model with 80% of the remaining data and all data with other ethnical backgrounds. We used 1,360 African American (AA), Hong-Kong Chinese (CHN), Caucasian (GER), Indian (IND), Iranian (IRN), Japanese (JPN), South Korean (KOR) and Maltese (MLT) samples and 937 samples from the 1000 Genomes dataset in total. **(a)** Accuracies are depicted according to post-imputation probabilities with cutoff thresholds at 0, 0.3, 0.5 and 0.8. Loci are shown according to alphabetical order. **(b)** Posterior probabilities are depicted as proportion of the number of samples with a posterior probability smaller than a threshold (x-axis).

Supplementary Figure 3 – Imputation accuracies employing the multi-ethnic reference panel in G group context: Accuracies and post-imputation probabilities of HLA imputation with HIBAG using a 5x cross validation scheme and the multi-ethnic dataset with 4-digit G group allele information. 20% of the data with a specific ethnical background were used as the validation set after training a model with 80% of the remaining data and all data with other ethnical backgrounds. We used 1,360 African American (AA), Hong-Kong Chinese (CHN), Caucasian (GER), Indian (IND), Iranian (IRN), Japanese (JPN), South Korean (KOR) and Maltese (MLT) samples in total. **(a)** Accuracies are depicted according to post-imputation probabilities with cutoff thresholds at 0, 0.3, 0.5 and 0.8. Loci are shown according to alphabetical order. **(b)** Posterior probabilities are depicted as proportion of the number of samples with a posterior probability smaller than a threshold (x-axis).

Supplementary Figure 4 – Disentenglar plots: Disentenglar (5) plot of alleles with a MAF >1% for **(a)** African

American (AA), **(b)** Chinese (CHN), **(c)** European (EUR), **(d)** Indian (IND), **(e)** Iranian (IRN), **(f)** South Korean (KOR), **(g)** Japanese (JPN) and **(h)** Maltese (MLT) data. Typing was performed on a 4-digit level using HLAssign (6). Plot shows frequencies as height of the bar and haplotype connections as grey lines. HLA loci are ordered by genomic location.