

1 **Genome-wide study identifies association between HLA-B\*55:01 and penicillin**  
2 **allergy**

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54

55 **Abstract**

56 **Background**

57 Hypersensitivity reactions to drugs are often unpredictable and can be life-  
58 threatening, underscoring a need for understanding the underlying mechanisms and  
59 risk factors. The extent to which germline genetic variation influences the risk of  
60 commonly reported drug allergies such as penicillin allergy remains largely unknown.

61 **Methods**

62 We extracted data from the electronic health records of 52,000 Estonian and  
63 500,000 UK biobank participants to study the role of genetic variation in the  
64 occurrence of penicillin hypersensitivity reactions. We used imputed SNP to HLA  
65 typing data from up to 22,554 and 488,377 individuals from the Estonian and UK  
66 cohorts, respectively, to further fine-map the human leukocyte antigen (HLA)  
67 association and replicated our results in two additional cohorts involving a total of  
68 1.14 million individuals.

69 **Results**

70 Genome-wide meta-analysis of penicillin allergy revealed a significant association  
71 located in the HLA region on chromosome 6. The signal was further fine-mapped to  
72 the HLA-B\*55:01 allele (OR 1.47 95% CI 1.37-1.58, P-value  $4.63 \times 10^{-26}$ ) and  
73 confirmed by independent replication in two cohorts. The meta-analysis of all four  
74 cohorts in the study revealed a strong association of HLA-B\*55:01 allele with self-  
75 reported penicillin allergy (OR 1.33 95% CI 1.29-1.37, P-value  $2.23 \times 10^{-72}$ ). *In silico*  
76 follow-up suggests a potential effect on T lymphocytes at HLA-B\*55:01.

77 **Conclusion**

78 We present the first robust evidence for the role of an allele of the major  
79 histocompatibility complex (MHC) I gene HLA-B in the occurrence of penicillin  
80 allergy.

81

## 82 **MAIN**

83

84 Adverse drug reactions (ADRs) are common in clinical practice and are associated  
85 with high morbidity and mortality. A meta-analysis of prospective studies in the US  
86 revealed the incidence of serious ADRs to be 6.7% among hospitalized patients, and  
87 the cause of more than 100,000 deaths annually <sup>1</sup>. In Europe, ADRs are responsible  
88 for 3.5% of all hospital admissions, with 10.1% of patients experiencing ADRs during  
89 hospitalization and 197,000 fatal cases per year <sup>2,3</sup>. In the US, the cost of a single  
90 ADR event falls between 1,439 to 13,462 USD <sup>4</sup>.

91

92 ADRs are typically divided into two types of reactions. Type A reactions are more  
93 predictable and related to the pharmacological action of a drug, whereas type B  
94 reactions are idiosyncratic, less predictable, largely dose-independent, and typically  
95 driven by hypersensitivity reactions involving the immune system <sup>5</sup>. Although type B  
96 reactions are less frequent (<20%) than type A reactions, they tend to be more  
97 severe and more often lead to the withdrawal of a drug from the market <sup>6</sup>. Based on  
98 the timing of onset, drug allergy can be further divided into immediate or delayed  
99 effects <sup>7</sup>. One of the most common causes of type B reactions are antibiotics <sup>5</sup>,  
100 typically from the beta-lactam class, with the prevalence of penicillin allergy  
101 estimated to be as high as 25% in some settings <sup>8,9</sup>. Despite the relative frequency of  
102 such reactions, there are very few studies of the genetic determinants of penicillin

103 allergy<sup>10,11</sup>. This underscores the need for a better understanding of the  
104 mechanisms and risk factors, including the role of genetic variation, that contribute to  
105 hypersensitivity reactions.

106

107 The increasing availability of genetic and phenotypic data in large biobanks provides  
108 an opportune means for investigating the role of genetic variation in drug-induced  
109 hypersensitivity reactions. In the present study, we sought to identify genetic risk  
110 factors underlying penicillin-induced hypersensitivity reactions by harnessing data  
111 from the Estonian (EstBB) and UK Biobanks (UKBB), with further replication in large  
112 population-based cohorts.

113

## 114 **RESULTS**

### 115 **GENOME-WIDE ASSOCIATION ANALYSIS OF PENICILLIN HYPERSENSITIVITY**

116 To discover genetic factors that may predispose to penicillin allergy, we conducted a  
117 genome-wide association study (GWAS) of 19.1 million single-nucleotide  
118 polymorphisms (SNPs) and insertions/deletions in UKBB and EstBB (minor allele  
119 frequency filter in both cohorts MAF > 0.1%). Cases were defined as participants  
120 with a Z88.0 ICD10 code (“Allergy status to penicillin”) for a reported history of  
121 penicillin allergy. In total, we identified 15,690 unrelated individuals (4.2% of the total  
122 cohort size of 377,545) in UKBB with this diagnostic code. However, the  
123 corresponding number of cases in EstBB was only 7 (0.02% of the total cohort size  
124 of 32,608) suggesting heterogeneity in the use of the Z88.0 ICD10 code in different  
125 countries. We therefore also identified participants that had self-reported drug allergy  
126 at recruitment in EstBB and categorized the EstBB self-reported reactions by drug  
127 class J01C\* (beta-lactam antibacterials, penicillins) to match this to the respective

128 Z88.0 diagnostic code, resulting in 961 (2.9%) unrelated cases with penicillin allergy  
129 in EstBB. We validated the approach in EstBB by evaluating the association between  
130 the number of penicillin (using the Anatomical Therapeutic Chemical (ATC)  
131 Classification System code J01C\*) filled prescriptions per person and self-reported  
132 penicillin allergy. Using Poisson regression analysis, we identified a negative effect  
133 on the number of filled penicillin prescriptions among individuals with self-reported  
134 allergy in EstBB (P-value  $2.41 \times 10^{-15}$ , Estimate -0.18 i.e. prescription count is 16%  
135 lower for individuals with penicillin allergy).

136 We then meta-analyzed the results of the GWASes in these two cohorts separately,  
137 weighing effect size estimates using the inverse of the corresponding standard  
138 errors. We identified a strong genome-wide significant ( $p < 5 \times 10^{-8}$ ) signal for  
139 penicillin induced allergy (defined as ICD10 code Z88.0 or reported allergy to drugs  
140 in ATC J01C\* class) on chromosome 6 in the major histocompatibility complex  
141 (MHC) region (lead variant rs114892859, MAF(EstBB) = 0.7%, MAF(UKBB) = 2%, P  
142 =  $2.21 \times 10^{-28}$ , OR 1.02 95% CI 1.016-1.023) (**Figure 1 Table S1 in the**  
143 **Supplementary Appendix**).

144

#### 145 FINE-MAPPING THE PENICILLIN ALLERGY-ASSOCIATED HLA LOCUS

146 To further fine-map the causal variant of the identified association with penicillin  
147 allergy, we performed a functional annotation analysis with FUMA (Functional  
148 Mapping and Annotation of Genome-Wide Association Studies)<sup>12</sup>. We detected an  
149 independent intronic lead SNP for the penicillin allergy meta-analysis (GWAS top  
150 variant rs114892859, P-value  $2.21 \times 10^{-28}$ ) in the *MICA* gene (**Figure 1, B**). When  
151 testing the SNP for expression quantitative trait locus (eQTL) associations in blood  
152 based on data from the eQTLGen Consortium<sup>13</sup>, the variant appeared to be

153 associated with the expression levels of several nearby genes, with the most  
154 significant being *PSORS1C3* (P-value  $8.10 \times 10^{-62}$ ) and *MICA* (P-value  $1.21 \times 10^{-52}$ )  
155 (**Table S2 in the Supplementary Appendix**). We further performed an *in silico*  
156 investigation of the lead SNP rs114892859 and its best proxy (only proxy with  $r^2 > 0.9$   
157 in UKBB and EstBB; rs144626001) in HaploReg v4 to explore annotations and  
158 impact of the non-coding variant<sup>14</sup>. In particular rs114892859 had several  
159 annotations indicative of a regulatory function, including its location in both promoter  
160 and enhancer marks in T-cells and evidence of RNA polymerase II binding<sup>14,15</sup>.  
161 Interestingly, its proxy is more likely to be deleterious based on the scaled Combined  
162 Annotation Dependent Depletion (CADD) score (scaled score of 15.78 for  
163 rs144626001 (C/T) and 4.472 for rs114892859 (G/T))<sup>16,17</sup>.

164

165 Due to the high LD in the MHC region, we used imputed SNP to HLA typing data  
166 available at four-digit resolution<sup>18</sup> for up to 22,554 and 488,377 individuals from the  
167 Estonian and UK cohorts, respectively, to further fine-map the identified HLA  
168 association with penicillin allergy. In both cohorts a shared total of 103 alleles at four-  
169 digit level were present for all of the MHC class I genes (*HLA-A*, *HLA-B*, *HLA-C*) and  
170 59 alleles for three of the classical MHC class II genes (*HLA-DRB1*, *HLA-DQA1*,  
171 *HLA-DQB1*). To assess the variation in the frequencies of the HLA alleles in different  
172 populations, we compared the obtained allele frequencies in both cohorts (**Table S3**  
173 **in the Supplementary Appendix**) with the frequencies of HLA alleles in different  
174 European, Asian and African populations reported in the HLA frequency database  
175 (**Figure S2 and S3, Table S4 in the Supplementary Appendix**).

176

177 We then used an additive logistic regression model to test for associations between  
178 different four-digit HLA alleles and penicillin allergy in UKBB and EstBB. The results  
179 of both cohorts were meta-analyzed and P-values passing a Bonferroni correction  
180 ( $0.05/162 = 3.09 \times 10^{-4}$ , where 162 is the number of meta-analyzed HLA alleles) were  
181 considered significant (**Table S5 in the Supplementary Appendix**). One of the  
182 three results that surpassed the significance threshold had discordant effects in the  
183 two cohorts and one had a marginally significant association (P-value  $2.81 \times 10^{-4}$ ,  
184 **Table S5 in the Supplementary Appendix**). The strongest association we detected  
185 for penicillin allergy was the HLA-B\*55:01 allele (P-value  $4.63 \times 10^{-26}$ ; OR 1.47 95%  
186 CI 1.37-1.58).

187

#### 188 REPLICATION OF HLA-B\*55:01 ASSOCIATION WITH PENICILLIN ALLERGY

189 To further confirm association with penicillin allergy we analyzed the association of  
190 the HLA-B\*55:01 allele with self-reported penicillin allergy among 87,996 cases and  
191 1,031,087 controls from the 23andMe research cohort. We observed a strong  
192 association (P-value  $1.00 \times 10^{-47}$ ; OR 1.30 95% CI 1.25-1.34; **Figure 2**) with a similar  
193 effect size as seen for the HLA-B\*55:01 allele in the meta-analysis of the EstBB and  
194 UKBB. We obtained further confirmation for this association from the published  
195 dataset of Vanderbilt University's biobank BioVU, where the HLA-B\*55:01 allele was  
196 associated with allergy/adverse effect due to penicillin among 58 cases and 23,598  
197 controls (P-value  $1.79 \times 10^{-2}$ ; OR 2.15 95% CI 1.19-6.5; **Figure 2**)<sup>19</sup>. Meta-analysis of  
198 results from discovery and replication cohorts demonstrate a strong association of  
199 HLA-B\*55:01 allele with self-reported penicillin allergy (P-value  $2.23 \times 10^{-72}$ ; OR 1.33  
200 95% CI 1.29-1.37; **Figure 2**).

201

202 FURTHER ASSOCIATIONS AT HLA-B\*55:01

203 Finally, we used the Open Targets Genetics platform's UKBB PheWAS data<sup>20</sup> to  
204 further characterize the association of GWAS top variant rs114892859 that is also a  
205 strongly correlated tag-SNP ( $r^2 > 0.95$ ) of the HLA-B\*55:01 allele (**Table S6 in the**  
206 **Supplementary Appendix**) with other traits, and found strong associations with  
207 lower lymphocyte counts (P-value  $9.21 \times 10^{-14}$ , estimate -0.098 cells per nanoliter per  
208 allergy-increasing T allele) and lower white blood cell counts (P-value  $3.17 \times 10^{-9}$ ,  
209 estimate -0.078 cells per nanoliter per allergy-increasing T allele). To confirm this  
210 association, we extracted data on lymphocyte counts from the electronic health  
211 record (EHR) data of 4,567 EstBB participants, and observed the same inverse  
212 association of the HLA-B\*55:01 allele with lymphocyte counts (Estimate -0.148  
213 number of cells per nanoliter per T allele; P-value=0.047).

214

## 215 DISCUSSION

216

217 In the present study, we identify a strong genome-wide significant association of the  
218 HLA-B\*55:01 allele with penicillin allergy using data from four large cohorts: UKBB,  
219 EstBB, 23andMe and BioVu.

220

221 Hypersensitivity or allergic reactions to medications are type B adverse drug  
222 reactions that are known to be mediated by the immune system. One major driver of  
223 hypersensitivity reactions is thought to be the HLA system, which plays a role in  
224 inducing the immune response through T cell stimulation, and is encoded by the  
225 most polymorphic region in the human genome.<sup>21</sup> Genetic variation in the HLA  
226 region alters the shape of the peptide-binding pocket in HLA molecules, and enables



227 their binding to a vast number of different peptides – a crucial step in the adaptive  
228 immune response<sup>22</sup>. However, this ability of HLA molecules to bind a wide variety of  
229 peptides may also facilitate binding of exogenous molecules such as drugs,  
230 potentially leading to off-target drug effects and immune-mediated ADRs<sup>23</sup>. The  
231 precise mechanism of most HLA-drug interactions remains unknown, but it seems  
232 that T cell activation is necessary for the majority of HLA-mediated ADRs<sup>7,23,24</sup>.  
233 Despite the increasing evidence for a role of the HLA system in drug-induced  
234 hypersensitivity, much is still unclear, including how genetic variation in the HLA  
235 region predisposes to specific drug reactions.

236

237 Penicillin is the most common cause of drug allergy, with clinical manifestations  
238 ranging from relatively benign cutaneous reactions to life-threatening systemic  
239 syndromes<sup>8,9</sup>. There is a previous GWAS on the immediate type of penicillin allergy,  
240 where a borderline genome-wide significant protective association of an allele of the  
241 MHC class II gene *HLA-DRA* was detected and further replicated in a different cohort  
242<sup>25</sup>. Here we detect a robust association between penicillin allergy and an allele of the  
243 MHC class I gene *HLA-B*. The allele and its tag-SNP were also associated with  
244 lower lymphocyte levels and overlapped with T cell regulatory annotations, which  
245 suggests that the variant may predispose to a T-cell-mediated, delayed type of  
246 penicillin allergy. MHC I molecules are expressed by almost all cells and present  
247 peptides to cytotoxic CD8+ T cells, whereas MHC II molecules are expressed by  
248 antigen-presenting cells to present peptides to CD4+ T helper lymphocytes<sup>7,22</sup>.  
249 There are several examples of MHC I alleles associated with drug-induced  
250 hypersensitivity mediated by CD8+ T cells<sup>7,26,27</sup>. The involvement of T cells in  
251 delayed hypersensitivity reactions has been shown by isolating drug reactive T cell

252 clones<sup>28</sup>, and cytotoxic CD8+ T cells have been shown to be relevant especially in  
253 allergic skin reactions<sup>29–31</sup>. More than twenty years ago, CD8+ T cells reactive to  
254 penicillin were isolated from patients with delayed type of hypersensitivity to penicillin  
255<sup>32</sup>. The association with the HLA-B\*55:01 allele detected in our study might be a  
256 relevant factor in this previously established connection with CD8+ T cells. The HLA-  
257 B\*55:01 allele, together with other HLA-B alleles that share a common "E pocket  
258 sequence", has previously been associated with increased risk for eosinophilia and  
259 systemic symptoms, Stevens-Johnson Syndrome and toxic epidermal necrolysis  
260 (SJS/TEN) among patients treated with nevirapine<sup>33</sup>. The underlying mechanism in  
261 penicillin allergy remains a question and various models have been proposed for T-  
262 cell-mediated hypersensitivity<sup>26,31</sup>. For example, the hapten model suggests that  
263 drugs may alter proteins and thereby induce an immune response<sup>26,34</sup> – penicillins  
264 have been shown to bind proteins<sup>34,35</sup> to form hapten–carrier complexes, which may  
265 in turn elicit a T cell response<sup>36</sup>. Drugs may also bind with MHC molecules directly.  
266 For example, abacavir has been shown to bind non-covalently to the peptide-binding  
267 groove of HLA-B\*57:01, leading to a CD8+ T cell-mediated hypersensitivity response  
268<sup>37</sup>. Although we detect strong evidence for the involvement of HLA-B\*55:01 in  
269 penicillin allergy, and a marginally significant association in the MHC II gene DRB1,  
270 both need further functional investigation to explore their exact roles and  
271 mechanisms in the induced response.

272

273 The frequency of the HLA-B\*55:01 allele was slightly lower (0.7%) in EstBB than in  
274 UKBB (1.9%), however our comparison between European and Asian populations  
275 indicated a similar frequency (P-value 0.97) between these populations. It is

276 therefore possible that the HLA-B\*55:01 allele may be a common contributor to  
277 penicillin allergy among Asians as well, but this needs further investigation.  
278 It is being increasingly recognized that the involvement of HLA variation in  
279 hypersensitivity reactions goes beyond peptide specificity. Other factors, such as  
280 effects on HLA expression that influence the strength of the immune response have  
281 also been described<sup>38</sup>. The analysis of eQTLs based on the data of the eQTLGen  
282 Consortium<sup>13</sup> revealed that the T allele of the lead SNP rs114892859 identified in  
283 our GWAS of penicillin allergy appears to be associated with the expression of  
284 several nearby genes, including lower expression of both *HLA-B* and *HLA-C*, and an  
285 even stronger effect on RNA levels of *PSORS1C3* and *MICA* (**Table S2 in the**  
286 **Supplementary Appendix**). Interestingly, variants in the *PSORS1C3* gene have  
287 been associated with the risk of allopurinol, carbamazepine and phenytoin induced  
288 SJS/TEN hypersensitivity reactions<sup>39</sup>. *MICA* encodes the protein MHC class I  
289 polypeptide-related sequence A<sup>40</sup> which has been implicated in immune surveillance  
290<sup>41,42</sup>. Our findings therefore support the observation that variants associated with  
291 expression of HLA genes may contribute to the development of hypersensitivity  
292 reactions.

293

294 The main limitation of this study is the unverified nature of the phenotypes extracted  
295 from EHRs and self-reported data in the biobanks. Previous work has found that  
296 most individuals labeled as having beta-lactam hypersensitivity may not actually  
297 have true hypersensitivity<sup>8,43,9</sup>. Nevertheless, despite the possibility that some cases  
298 in our study may be misclassified, we detect a robust HLA association that was  
299 replicated in several independent cohorts against related phenotypes. The increased  
300 power arising from biobank-scale sample sizes therefore mitigates some of the

301 challenges associated with EHR data. The robustness of the genetic signal across  
302 cohorts with orthogonal phenotyping methods, ranging from EHR-sourced in UKBB  
303 to various forms of self-reported data in EstBB and 23andMe, also supports a true  
304 association. Finally, the modest effect size of the HLA-B\*55:01 allele (OR 1.33),  
305 particularly when compared to effect sizes of HLA alleles with established  
306 pharmacogenetic relevance<sup>44–46</sup>, suggests that this variant in isolation is unlikely to  
307 have clinically meaningful predictive value. Our work does provide the foundation for  
308 further studies to investigate the application of a polygenic risk score<sup>47</sup> (which  
309 combines the effects of many thousands of trait-associated variants into a single  
310 score), possibly in combination with phenotypic risk factors, in identifying individuals  
311 at elevated risk of penicillin allergy.

312 In summary, our results provide novel evidence of a robust genome-wide significant  
313 association of HLA and the HLA-B\*55:01 allele with penicillin allergy.

314

## 315 **METHODS**

316

### 317 **Phenotype definitions**

318 We studied individual-level genotypic and phenotypic data of 52,000 participants  
319 from the Estonian Biobank (EstBB) and 500,000 participants from UK Biobank  
320 (UKBB). Both are population-based cohorts, providing a rich variety of phenotypic  
321 and health-related information collected for each participant. All participants have  
322 signed a consent form to allow follow-up linkage of their electronic health records  
323 (EHR), thereby providing a longitudinal collection of phenotypic information. EstBB  
324 allows access to the records of the national Health Insurance Fund Treatment Bills  
325 (since 2004), Tartu University Hospital (since 2008), and North Estonia Medical

326 Center (since 2005). For every participant there is information on diagnoses in ICD-  
327 10 coding and drug dispensing data, including drug ATC codes, prescription status  
328 and purchase date (if available). We extracted information on penicillin allergy by  
329 searching the records of the participants for Z88.0 ICD10 code indicating patient-  
330 reported allergy status due to penicillin. Information on phenotypic features like age  
331 and gender were obtained from the biobank recruitment records. Since Z88.0 code  
332 seemed underreported in Estonia, we also used self-reported data on side-effects  
333 from penicillin for 1,015 (961 unrelated) participants who reported hypersensitivity  
334 due to J01C\* ATC drug group (Beta-Lactam Antibacterials, Penicillins) in their  
335 questionnaire when joining EstBB.

336

337 We also extracted likely penicillin allergies in the EstBB from the free text fields of  
338 the EHRs using a rule-based approach; the text had to contain any of the possible  
339 forms of the words 'allergy' or 'allergic' in Estonian as well as a potential variation of  
340 a penicillin name. As drug names are often misspelled, abbreviated or written using  
341 the English or Latin spelling instead of the standard Estonian one, we used a regular  
342 expression to capture as many variations of each penicillin name as possible. In  
343 addition, we applied rules regarding the distance between the words 'allergy' and the  
344 drug name as well as other words nearby to exclude negations of penicillin allergies  
345 in the definition.

346

347 To analyze the effect of self-reported allergy status on the number on penicillin  
348 prescriptions in EstBB we performed a Poisson regression among 37,825 unrelated  
349 individuals with J01C\* prescriptions considering age, gender and 10 principal  
350 components (PC) as covariates. Units were interpreted as follows: 1-

351  $\exp(\beta) * 100\% = 1 - \exp(-0.18) * 100\% = 16\%$ . The Poisson model was considered  
352 appropriate as there was no large overdispersion.

353

### 354 **Overview of genetic data**

355 The details on genotyping, quality control and imputation are fully described  
356 elsewhere for both EstBB<sup>48,49</sup> and UKBB<sup>50</sup>. In brief, of the included EstBB  
357 participants 33,277 have been genotyped using the Global Screening Array v1  
358 (GSA), 8,137 on the HumanOmniExpress beadchip (OMNI), 2,641 on the  
359 HumanCNV370-Duo BeadChips (370) and 7,832 on the Infinium CoreExome-24  
360 BeadChips from Illumina (CE). Furthermore, 2,056 individuals' whole genomes have  
361 been sequenced at the Genomics Platform of the Broad Institute. Sequenced reads  
362 were aligned against the GRCh37/hg19 version of the human genome reference  
363 using BWA-MEM1 v0.7.7. The genotype data was phased using Eagle2 (v. 2.3)<sup>51</sup>  
364 and imputed using BEAGLE (v. 4.1)<sup>52,53</sup>, software implementing a joint Estonian and  
365 Finnish reference panel (described in<sup>54</sup>). If one individual was genotyped with more  
366 than one microarray, duplicates were removed by prioritizing as follows: Whole  
367 genome > GSA > OMNI > 370 > CE. The total dataset comprises 32,608 unrelated  
368 participants that is based on the inclusion of individuals with PiHat < 0.2. When  
369 excluding relatives for a GWAS, we favored individuals who had self-reported ADRs  
370 due to drugs.

371 In UKBB, genotype data are available for 488,377 participants of which 49,950 are  
372 genotyped using the Applied Biosystems™ UK BiLEVE Axiom™ and the remaining  
373 438,427 individuals were genotyped using the Applied Biosystems™ UK Biobank  
374 Axiom™ Array by Affymetrix. The genotype data was phased using SHAPEIT3<sup>55</sup>,

375 and imputation was conducted using IMPUTE4<sup>53</sup> using a combined version of the  
376 Haplotype Reference Consortium (HRC) panel<sup>56</sup> and the UK10K panel<sup>57</sup>.  
377 We excluded individuals who have withdrawn their consent, have been labelled by  
378 UKBB to have poor heterozygosity or missingness, who have putative sex  
379 chromosome aneuploidy and who have >10 relatives in the dataset. We further  
380 removed all individuals with mismatching genetic and self-reported sex and ethnicity.  
381 GWAS was executed on unrelated individuals with confirmed white British ancestry.  
382 Only one individual from each pair of second- or higher-degree relatives (KING's  
383 kinship coefficient > 0.0884) were included, by favoring the carriers of Z88.0 ICD10  
384 code. After following these steps, we ended up with 377,545 unrelated individuals.  
385

### 386 **Genome-wide study and meta-analysis**

387 In the Estonian biobank, we conducted the penicillin GWAS among 31,760 unrelated  
388 individuals (PiHat < 0.2) of whom 961 were cases with self-reported allergy from  
389 J01C beta-lactam drugs and 30,799 undiagnosed controls. The controls were  
390 selected from a set of individuals with no self-reported ADRs or with ICD10  
391 diagnoses covered in a list of 79 ICD10 codes (described in<sup>58</sup>) with a possible drug-  
392 induced nature or diagnoses described as "due to drugs". The GWAS was run with  
393 the EPACTS software<sup>59</sup> using an additive genetic logistic model. To minimize the  
394 effects of population admixture and stratification, the analyses only included samples  
395 with European ancestry based on PC analysis (PCA) and were adjusted for the first  
396 ten PCs of the genotype matrix, as well as for age, sex and array.  
397

398 In the UKBB, GWAS on penicillin allergy (Z88.0) was performed among 15,690  
399 cases and 342,116 controls. Similarly as for EstBB, the controls were selected from

400 a set of individuals with no ICD10 diagnoses covered in a list of 79 ICD10 codes  
401 (described in <sup>58</sup>). GWAS of imputed genotype data was performed with the BOLT-  
402 LMM software tool <sup>60</sup> using a linear mixed model and considering the  
403 aforementioned covariates (10 PCs, age, sex). LD scores appropriate for the  
404 analysis of European-ancestry was used for calibration of the BOLT-LMM statistic  
405 reference.

406 We performed meta-analysis of 19,051,157 markers (MAF>0.1%) based on effect  
407 sizes and their standard errors using METAL <sup>61</sup>. Results were visualized with R  
408 software (3.3.2) <sup>62</sup>.

409

#### 410 **Post-GWAS annotation**

411

412 FUMA (Functional mapping and annotation of genetic associations) <sup>12</sup> is an  
413 integrative web-based platform using information from multiple biological resources,  
414 including e.g. information on eQTLs, chromatin interaction mappings, and LD  
415 structure to annotate GWASes. We applied FUMA to identify lead SNPs and  
416 genomic risk loci for results of the meta-analysis, using the European LD reference  
417 panel from 1000G <sup>63</sup>. Further eQTL associations were identified based on data from  
418 the the eQTLGen consortium, which is a meta-analysis of 37 datasets with blood  
419 gene expression data pertaining to 31,684 individuals <sup>13</sup>.

420

421 HaploReg <sup>14</sup> was used for exploring annotations, chromatin states, conservation, and  
422 regulatory motif alterations. To estimate the relative deleteriousness of the identified  
423 SNPs we use the Combined Annotation Dependent Depletion (CADD) framework <sup>16</sup>.

424



## 425 **HLA-typing**

426

427 HLA-typing of the EstBB genotype data was performed at the Broad Institute using  
428 the SNP2HLA tool <sup>64</sup>, which imputes HLA alleles from SNP genotype data.  
429 Single Nucleotide Variants (SNVs), small INsertions and DEletions (INDELs) and  
430 classical HLA variants were called using whole genome sequences of 2,244 study  
431 participants from the Estonian Biobank sequenced at 26.1x. We performed high-  
432 resolution (G-group) HLA calling of three class-I HLA genes (HLA-A, -B and -C) and  
433 three class-II HLA genes (HLA-DRB1, -DQA1 and -DQB1) using the HLA\*PRG  
434 algorithm <sup>65</sup>. SNVs and INDELs were called using GATK version 3.6 according to the  
435 best practices for variant discovery <sup>66</sup>. Classical HLA alleles, HLA amino acid  
436 residues and untyped SNPs were then imputed using SNP2HLA and the reference  
437 panel constructed using the 2,244 whole-genome sequenced Estonian samples. The  
438 imputation was done for genotype data generated on the GSA, and after quality  
439 control the four-digit HLA alleles of 22,554 individuals were used for analysis.

440

441 In UKBB we used four-digit imputed HLA data released by UKBB <sup>50</sup>. The imputation  
442 process, performed using HLA\*IMP:02 <sup>67</sup>, is described more fully elsewhere <sup>50,68</sup>. We  
443 applied posterior thresholding (at a threshold of 0.8) to the imputed data to create a  
444 marker representing the presence/absence of each HLA allele.

445

446 To compare obtained frequencies of HLA alleles with reported frequencies in  
447 European, Asian and African populations we used the database of Allele  
448 Frequencies of worldwide populations (<http://www.allelefrequencies.net/default.asp>).

449 We queried the frequencies of four-digit alleles choosing the following regions:

450 Europe, North-East Asia, South-Asia, South-East Asia, Western Asia, North Africa  
451 and Sub-Saharan Africa. Frequency comparisons were visualized with R software  
452 (3.3.2) <sup>62</sup>using ggplot2 package.

453

454 We performed separate additive logistic regression analysis with the called HLA  
455 alleles using R *glm* function in EstBB including age, sex and 10 PCs as covariates.  
456 In UKBB we performed association analysis of each four-digit allele with the Z88.0  
457 subcode using logistic regression function *glm* in R, adjusting for sex, age, age<sup>2</sup>,  
458 recruitment center, genotyping array, and the first 15 principal components (and  
459 excluding related [up to 2<sup>rd</sup> degree or closer] individuals and those of reported non-  
460 white ancestry). Meta-analysis of 162 HLA alleles was performed with the GWAMA  
461 software tool <sup>69</sup>. A Bonferroni-corrected P-value threshold of  $3.09 \times 10^{-4}$  was applied  
462 based on the number of tested alleles:  $0.05/162$ . Meta-analyzed results passing this  
463 threshold were considered significant.

464

#### 465 **HLA-B\*55:01 replication**

466 Replication analysis of the HLA-B\*55:01 allele was tested on 87,996 cases and  
467 1,031,087 controls of European ancestry (close relatives removed) from the  
468 23andMe research cohort. The self-reported phenotype of penicillin allergy was  
469 defined as an allergy test or allergic symptoms required for cases, with controls  
470 having no allergy. All individuals included in the analyses provided informed consent  
471 and participated in the research online, under a protocol approved by the external  
472 AAHRPP-accredited IRB, Ethical & Independent Review Services (E&I Review). A  
473 logistic regression assuming an additive model for allelic effects was used with  
474 adjusting for age, sex, indicator variables to represent the genotyping platforms and

475 the first five genotype principal components. In the 23andMe replication study, the  
476 HLA imputation was performed by using HIBAG<sup>70</sup> with the default settings. We  
477 imputed allelic dosage for HLA-A, B, C, DPB1, DQA1, DQB1 and DRB1 loci at four-  
478 digit resolution<sup>71</sup>.

479 Meta-analysis of the HLA-B\*55:01 association in four cohorts was performed with the  
480 GWAMA software tool<sup>69</sup> and results were visualized with R software (3.3.2)<sup>62</sup>.

481

## 482 **Phenome-wide study and HLA-B\*55:01 allele association with lymphocyte** 483 **levels**

484

485 To analyze other traits that are associated with the tag variant of the HLA-B\*55:01  
486 allele in the UK Biobank and GWAS Catalog summary statistics, we used the Open  
487 Targets Genetics platform<sup>20</sup>. To study the association between the HLA-B\*55:01  
488 allele and lymphocyte levels in EstBB, we extracted the information on measured  
489 lymphocyte levels (number of cells per nanoliter) from the free text fields of the  
490 medical history of 4,567 unrelated individuals with genotype data. After removing  
491 outliers based on the values of any data points which lie beyond the extremes of the  
492 whiskers (values > 3.58 and < 0.26), a linear regression was performed using R  
493 software and with age and sex as covariates.

494

## 495 **Acknowledgements**

496

497 This study has been supported by grants from the European Union's Horizon 2020  
498 research and innovation program under grant agreement number 692145; Estonian  
499 Research Council grant numbers PRG184, PRG687 and IUT24-6; and the Oak

500 Foundation. This work was carried out in part in the High Performance Computing  
501 Center of University of Tartu. We acknowledge the Finnish SISu Project and  
502 principal investigators Aarno Palotie, Jaana Suvisaari, Veikko Salomaa, and Priit  
503 Palta for sharing the Finnish imputation reference panel. This research has been  
504 conducted using the UK Biobank Resource under Application Number 11867. We  
505 thank the research participants of 23andMe for their contribution to this study and the  
506 23andMe Research Team. We further thank all the biobank participants in the  
507 Estonian, UK and Vanderbilt university biobanks for their contribution to this  
508 research.

509 J.B. is supported by funding from the Rhodes Trust, Clarendon Fund and the  
510 Medical Sciences Doctoral Training Centre, University of Oxford. J.C.C. is funded by  
511 the Oxford Medical Research Council Doctoral Training Partnership (Oxford MRC  
512 DTP) and the Nuffield Department of Clinical Medicine, University of Oxford. C.M.L.  
513 is supported by the Li Ka Shing Foundation; WT-SSI/John Fell funds; the NIHR  
514 Biomedical Research Centre, Oxford; Widenlife; and NIH (5P50HD028138-27).

515 M.V.H. works in a unit that receives funding from the MRC and is supported by a  
516 British Heart Foundation Intermediate Clinical Research Fellowship  
517 (FS/18/23/33512) and the National Institute for Health Research Oxford Biomedical  
518 Research Centre. Computation used the Oxford Biomedical Research Computing  
519 (BMRC) facility, a joint development between the Wellcome Centre for Human  
520 Genetics and the Big Data Institute supported by Health Data Research UK and the  
521 NIHR Oxford Biomedical Research Centre. Financial support was provided by the  
522 Wellcome Trust Core Award Grant Number 203141/Z/16/Z. The views expressed are  
523 those of the author(s) and not necessarily those of the NHS, the NIHR or the  
524 Department of Health.

525

526 **Author Contributions**

527

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529 supervised and generated genotype data or HLA typing data. D.S. and S.L.  
530 generated allergy data from free-text. K.K., J.B., M.L., T.J., J.C.C., J.F, W.W., A.A.,  
531 performed the data analysis. K.K., J.B., M.V.H. C.M.L., R.M., L.M., J.C.C. and J.F.  
532 conducted data interpretation. K.K. prepared the figures and tables. K.K, J.B., L.M.  
533 and J.F. drafted the manuscript. K.K., J.B., M.V.H. C.M.L., M.L., R.M., L.M., J.C.C.,  
534 W.W., A.A. and J.F. reviewed and edited the manuscript. All authors contributed to  
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545 Shringarpure, Chao Tian, Joyce Y. Tung, Robert J. Tunney, Vladimir Vacic, Xin  
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547

548 **Competing Interests statement**

549 C.M.L. has collaborated with Novo Nordisk and Bayer in research, and in  
550 accordance with a university agreement, did not accept any personal payment.  
551 W.W., A.A., and members of the 23andMe Research Team are employed by and  
552 hold stock or stock options in 23andMe, Inc.

553

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## 805 **Figure Legends**

806

807 **Figure 1. Manhattan plot (A) and HLA locus (B) of the genome-wide association study**  
808 **of allergy status to penicillin.**

809 The X-axes indicate chromosomal positions and Y-axes  $-\log_{10}$  of the P-values **(A)** Each dot  
810 represents a single nucleotide polymorphism (SNP). The dotted line indicates the genome-  
811 wide significance ( $P\text{-value} < 5.0 \times 10^{-8}$ ) P-value threshold. **(B)** SNPs are colored according to  
812 their linkage disequilibrium (LD; based on the 1000 Genome phase3 EUR reference panel)  
813 with the lead SNP. The SNP marked with a purple diamond is the top lead SNP  
814 rs114892859 identified depending on LD structure.

815

816 **Figure 2. HLA-B\*55:01 allele association with penicillin allergy-** The odds ratios (dots)  
817 and 95% confidence intervals (CI, horizontal lines) for HLA allele associated with penicillin  
818 allergy. The plot is annotated with P-values and case-control numbers. Color coding blue  
819 and black indicates the results for discovery cohorts Estonian UK biobank and replication  
820 results of the HLA\*B-55:01 allele in 23andMe research cohort (green) and Vanderbilt  
821 University's biobank BioVU (purple). Results of the meta-analysis of all four cohorts is  
822 indicated with a diamond (red).

823

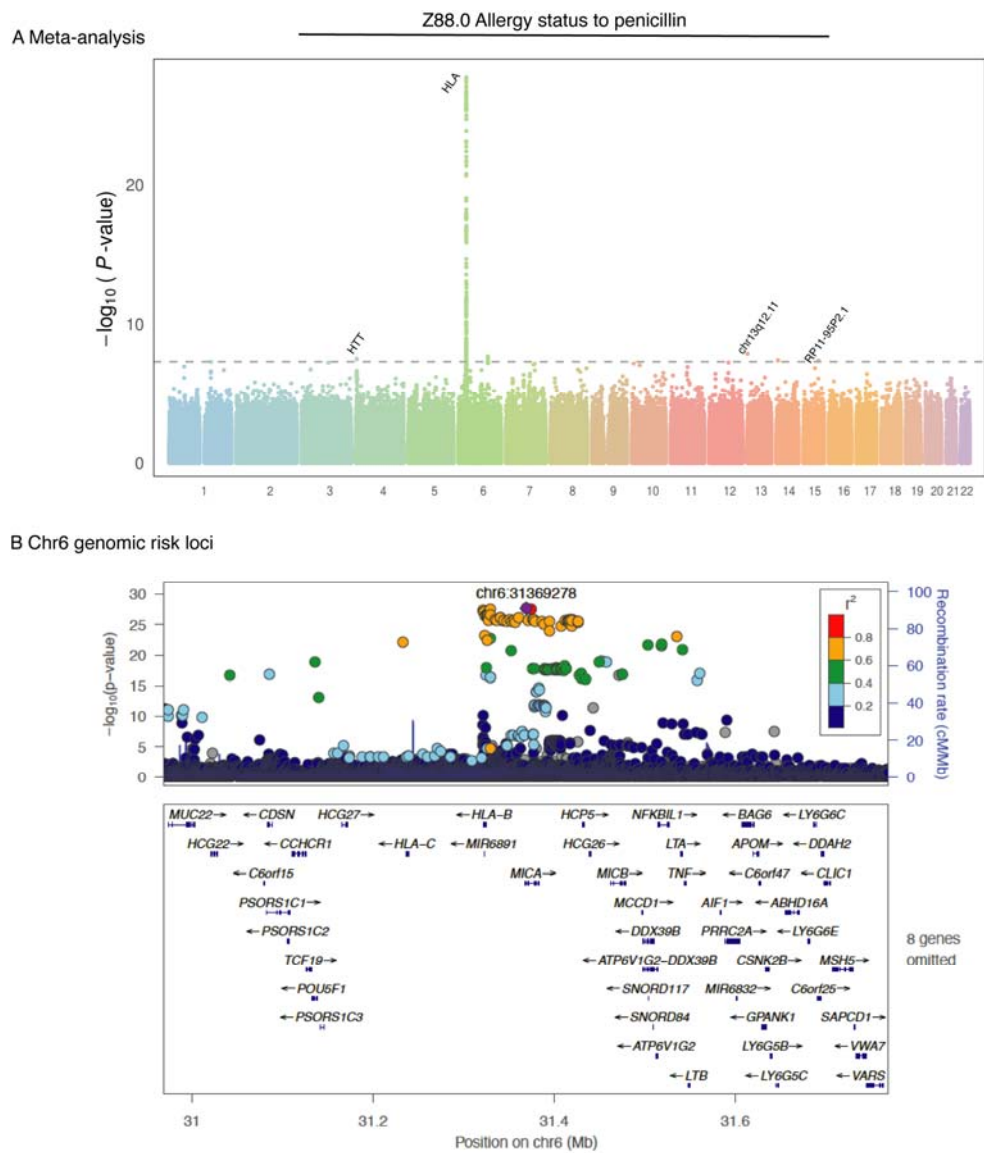
## 824 **Tables and Figures**

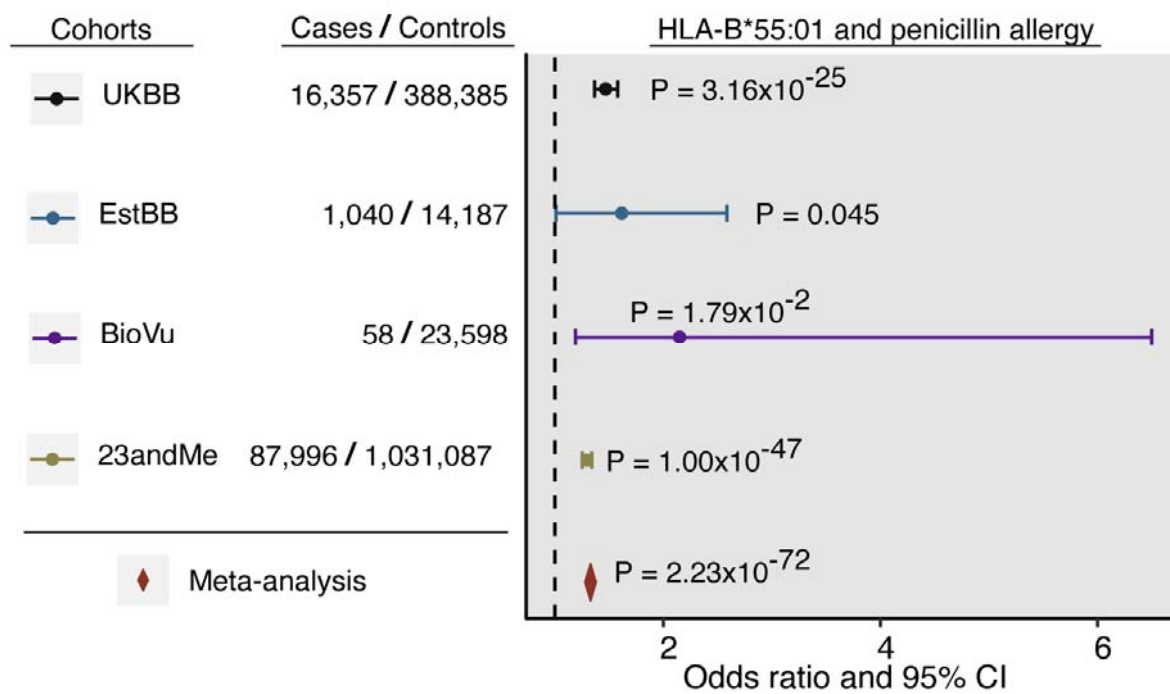
825



826 **Figure 1**

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