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## KCND3 is a novel susceptibility locus for early repolarization

2 Alexander Teumer<sup>1,2\*</sup>, Teresa Trenkwalder<sup>3\*</sup>, Thorsten Kessler<sup>3</sup>, Yalda Jamshidi<sup>4</sup>, Marten E. van den  
3 Berg<sup>5</sup>, Bernhard Kaess<sup>6</sup>, Christopher P. Nelson<sup>7,8</sup>, Rachel Bastiaenen<sup>9</sup>, Marzia De Bortoli<sup>10</sup>,  
4 Alessandra Rossini<sup>10</sup>, Isabel Deisenhofer<sup>3</sup>, Klaus Stark<sup>11</sup>, Solmaz Assa<sup>12</sup>, Peter S. Braund<sup>7,8</sup>, Claudia  
5 Cabrera<sup>13,14,15</sup>, Anna F. Dominiczak<sup>16</sup>, Martin Gögele<sup>10</sup>, Leanne M. Hall<sup>7,8</sup>, M. Arfan Ikram<sup>5</sup>, Maryam  
6 Kavousi<sup>5</sup>, Karl J. Lackner<sup>17,18</sup>, Lifelines Cohort Study<sup>19</sup>, Christian Müller<sup>20</sup>, Thomas Münzel<sup>18,21</sup>,  
7 Matthias Nauck<sup>2,22</sup>, Sandosh Padmanabhan<sup>16</sup>, Norbert Pfeiffer<sup>23</sup>, Tim D. Spector<sup>24</sup>, Andre G.  
8 Uitterlinden<sup>5</sup>, Niek Verweij<sup>12</sup>, Uwe Völker<sup>2,25</sup>, Helen R. Warren<sup>13,14</sup>, Mobeen Zafar<sup>12</sup>, Stephan B.  
9 Felix<sup>2,26</sup>, Jan A. Kors<sup>27</sup>, Harold Snieder<sup>28</sup>, Patricia B. Munroe<sup>13,14</sup>, Cristian Pattaro<sup>10</sup>, Christian  
10 Fuchsberger<sup>10</sup>, Georg Schmidt<sup>29,30</sup>, Ilja M. Nolte<sup>28</sup>, Heribert Schunkert<sup>3,30</sup>, Peter Pramstaller<sup>10</sup>, Philipp  
11 S. Wild<sup>31</sup>, Pim van der Harst<sup>12</sup>, Bruno H. Stricker<sup>5</sup>, Renate B. Schnabel<sup>20</sup>, Nilesh J. Samani<sup>7,8</sup>,  
12 Christian Hengstenberg<sup>32</sup>, Marcus Dörner<sup>2,26</sup>, Elijah R. Behr<sup>33</sup>, Wibke Reinhard<sup>3</sup>

13

14

15

1 Institute for Community Medicine, University Medicine Greifswald, Germany

16

2 DZHK (German Center for Cardiovascular Research), partner site Greifswald, Greifswald, Germany

17

3 Klinik für Herz- und Kreislauferkrankungen, Deutsches Herzzentrum München, School of Medicine,  
Technical University of Munich, Munich, Germany

18

19

4 Genetics Research Centre, Institute of Molecular and Clinical Sciences, St George's University of

20

London, United Kingdom

21

5 Department of Epidemiology, Erasmus MC - University Medical Center Rotterdam, The Netherlands

22

6 Medizinische Klinik I, St. Josefs-Hospital, Wiesbaden, Germany

23

7 Department of Cardiovascular Sciences, Cardiovascular Research Centre, Leicester, Leicester,

24

United Kingdom

25

8 NIHR Leicester Biomedical Research Centre University of Leicester, Leicester, United Kingdom

26

9 Cardiology Clinical Academic Group, Institute of Molecular and Clinical Sciences, St George's,

27

University of London, United Kingdom

28

10 Institute for Biomedicine, Eurac Research, affiliated with the University of Lübeck, Bolzano, Italy

29

11 Department of Genetic Epidemiology, University Regensburg, Germany

30

12 Department of Cardiology, University of Groningen, University Medical Center Groningen, The

31

Netherlands

32

13 Clinical Pharmacology, William Harvey Research Institute, Barts and The London, Queen Mary

33

University of London, London, UK

34

14 NIHR Barts Cardiovascular Biomedical Research Centre, Barts and The London School of

35

Medicine and Dentistry, Queen Mary University of London, London, UK

36

15 Centre for Translational Bioinformatics, William Harvey Research Institute, Barts and the London

37

School of Medicine and Dentistry, Charterhouse Square, London, UK

38

16 Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences,

39

University of Glasgow, Glasgow, Scotland, UK

40

17 Institute of Clinical Chemistry and Laboratory Medicine, University Medical Center of the Johannes

41

Gutenberg-University Mainz, Mainz, Germany

42

18 German Center for Cardiovascular Research (DZHK), partner site RhineMain, Mainz, Germany

43

19 The list of group authors is provided in the study acknowledgments

44

20 Department of General and Interventional Cardiology, University Heart Center Hamburg-

45

Eppendorf, Germany, DZHK (German Center for Cardiovascular Research), partner site

46

Hamburg/Kiel/Lübeck, Germany

47

21 Center for Cardiology - Cardiology I, University Medical Center of the Johannes Gutenberg-

48

University Mainz, Mainz, Germany

49

22 Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald,

50

Greifswald, Germany

51

23 Department of Ophthalmology, University Medical Center of the Johannes Gutenberg-University

52

Mainz, Mainz, Germany

53

24 Department of Twin Research and Genetic Epidemiology, King's College London, London, UK

54

25 Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald,

55

Greifswald, Germany

56

26 Department of Internal Medicine B, University Medicine Greifswald, Greifswald, Germany

57

27 Department of Medical Informatics, Erasmus MC - University Medical Center Rotterdam, The

58

Netherlands

59 28 Department of Epidemiology, University of Groningen, University Medical Center Groningen,  
60 Groningen, The Netherlands  
61 29 Innere Medizin I, Klinikum rechts der Isar, Technical University Munich, Munich, Germany  
62 30 Deutsches Zentrum für Herz- und Kreislauf-Forschung (DZHK) e.V. (German Center for  
63 Cardiovascular Research), Partner Site Munich Heart Alliance, Munich, Germany  
64 31 Preventive Cardiology and Preventive Medicine, Center for Cardiology, University Medical Center  
65 of the Johannes Gutenberg-University Mainz, Mainz, Germany; Center for Thrombosis and  
66 Hemostasis, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz,  
67 Germany; DZHK (German Center for Cardiovascular Research), partner site Rhine-Main, Mainz,  
68 Germany  
69 32 Division of Cardiology, Department of Internal Medicine II, Medical University of Vienna, Vienna,  
70 Austria  
71 33 Cardiology Clinical Academic Group, Institute of Molecular and Clinical Sciences, St George's,  
72 University of London, United Kingdom AND St George's University Hospitals NHS Foundation Trust,  
73 London, United Kingdom  
74  
75 \* These authors contributed equally to this work.  
76

77 Corresponding Author:  
78 PD Dr. med. Wibke Reinhard  
79 Klinik für Herz- und Kreislauferkrankungen  
80 Deutsches Herzzentrum München, Technische Universität München  
81 Lazarettstrasse 36  
82 80636 München  
83 Germany  
84 +49 89 1218 4005  
85  
86

87 **Abstract**

88 The presence of an early repolarization pattern (ERP) on the surface electrocardiogram (ECG) is  
89 associated with risk of ventricular fibrillation and sudden cardiac death. Family studies have shown  
90 that ERP is a highly heritable trait but molecular genetic determinants are unknown. We assessed the  
91 ERP in 12-lead ECGs of 39,456 individuals and conducted a two-stage meta-analysis of genome-wide  
92 association studies (GWAS). In the discovery phase, we included 2,181 cases and 23,641 controls  
93 from eight European ancestry studies and identified 19 genome-wide significant ( $p < 5E-8$ ) variants in  
94 the *KCND3* (potassium voltage gated channel subfamily D member 3) gene with a p-value of  $4.6E-10$ .  
95 Replication of two loci in four additional studies including 1,124 cases and 12,510 controls confirmed  
96 the association at the *KCND3* gene locus with a pooled odds ratio of 0.82,  $p = 7.7E-12$  (rs1545300  
97 minor allele T). A subsequent GWAS meta-analysis combining all samples did not reveal additional  
98 loci. The lead SNP of the discovery stage (rs12090194) was in strong linkage disequilibrium with  
99 rs1545300 ( $r^2 = 0.96$ ,  $D' = 1$ ). Summary statistics based conditional analysis did not reveal any  
100 secondary signals. Co-localization analyses indicate causal effects of *KCND3* gene expression levels  
101 on ERP in both the left ventricle of the heart and in tibial artery.  
102 In this study we identified for the first time a genome-wide significant association of a genetic variant  
103 with ERP. Our findings of a locus in the *KCND3* gene not only provide insights into the genetic  
104 determinants but also into the pathophysiological mechanism of ERP, revealing a promising candidate  
105 for functional studies.  
106

## 107 **Introduction**

108 The early repolarization pattern (ERP) is a common ECG finding characterized by an elevation at the  
109 QRS-ST junction (J-point) of at least 0.1 mV in two adjacent ECG leads. The prevalence of ERP in the  
110 general population ranges from 2 to 13% being more common in young athletic men(1–5). The  
111 classical notion of ERP being a benign ECG phenotype was challenged in 2008 by the landmark study  
112 of Haissaguerre and colleagues showing an association of ERP with increased risk of ventricular  
113 fibrillation and sudden cardiac death(6): the Early Repolarization Syndrome (ERS)(7). Since then  
114 several studies demonstrated an elevated risk of cardiovascular and all-cause mortality in individuals  
115 with ERP underscoring its arrhythmogenic potential(2, 8, 9). Although the mechanistic basis for  
116 malignant arrhythmias in ERS is unclear, it has been suggested that they occur as a result of an  
117 augmented transmural electrical dispersion of repolarization. Ex vivo studies point towards a central  
118 role of the cardiac transient outward potassium current ( $I_{to}$ ) in the development of both, ERP and  
119 ERS(10). Furthermore, case descriptions of ERS identified genetic variations in genes encoding  
120 proteins for cardiac ion channels(11–13). Studies among relatives of sudden arrhythmic death  
121 syndrome show that ERP is more prevalent in the relatives than in controls indicating that ERP is an  
122 important potentially inheritable pro-arrhythmic trait(14, 15). Moreover, in family studies the heritability  
123 estimate for the presence of ERP was  $h^2=0.49$  (16). However, estimates for common SNP heritability  
124 from unrelated individuals are lower(17). This may explain why the only available genome-wide  
125 association study (GWAS) on ERP failed to identify genetic variants reaching genome-wide  
126 significance(18), and indicates the need for GWAS with more power by including a larger number of  
127 ERP cases.

128 In order to identify genetic variations that convey susceptibility to ERP we performed a GWAS and  
129 meta-analysis in European ancestry individuals, using a combined two-stage GWAS approach with a  
130 discovery phase of 2,181 ERP cases and 23,641 controls from eight cohorts, and replicated the  
131 results in 1,124 cases and 12,510 controls from four additional cohorts.

## 132 **Results**

133 Clinical characteristics of the study cohorts are depicted in **Table 1**. The proportion of ERP based on  
134 the definition by Haisaguerre and Macfarlane(6, 19) ranged from 6% to 14% which is in line with  
135 previously reported prevalence in the general population(2–4).

136 *Novel variants associated with ERP*

137 We performed a GWAS meta-analysis in up to 2,181 cases and 23,641 controls from eight discovery  
138 cohorts. In total, 6,976,246 SNPs passed quality control (see Methods). We identified 19 variants  
139 spanning 49 kb in *KCND3* (Potassium Voltage-Gated Channel Subfamily D Member 3) as well as  
140 rs139772527 (effect allele frequency [EAF] 1.4%, OR=2.57,  $p=2.0E-8$ ) near *HBZ* (Hemoglobin Subunit  
141 Zeta) to be genome-wide significantly associated ( $p<5E-8$ ) with ERP. The SNP with the lowest p-value  
142 of the region (lead SNP) at *KCND3* was the intronic rs12090194 (EAF 32.5%, OR=0.80,  $p=4.6E-10$ ),  
143 and was replicated in an independent sample of 1,124 cases and 12,510 controls from four additional  
144 cohorts ( $p_{\text{replication}}=2.5E-3$ ,  $p_{\text{combined}}=9.3E-12$ , **Table 2**). The SNP rs139772527 near *HBZ* did not fulfil  
145 the criteria for replication ( $p_{\text{replication}}=0.28$ ,  $p_{\text{combined}}=1.4E-6$ , **Table 2**) as described in the Methods. The  
146 combined meta-analysis of all 12 cohorts including up to 39,456 individuals revealed only the locus at  
147 *KCND3* to be genome-wide significantly associated with ERP (**Supplementary Figure 1**). The lead  
148 SNP was rs1545300 (EAF 31.9%, OR=0.82,  $p=7.7E-12$ ), followed by the discovery stage lead SNP  
149 rs12090194 being in strong linkage disequilibrium with rs1545300 ( $r^2=0.96$ ,  $D'=1$ ) (**Figure 1**). Both  
150 SNPs were imputed at very high confidence (imputation quality score  $>0.97$ ) in all cohorts. The  
151 quantile-quantile plots did not show any inflation (individual study  $\lambda_{GC}$  between 0.81 and 1.03, median:  
152 0.91), and overall meta-analysis  $\lambda_{GC}=1.02$  (linkage disequilibrium [LD] score regression intercept: 1.01,  
153 see Methods) (**Supplementary Figure 2**). Summary statistics based conditional analysis to select  
154 independent hits did not reveal any secondary signals.

155 *Statistical finemapping of the associated locus*

156 All significantly associated SNPs were located within *KCND3*, the potassium voltage-gated channel  
157 subfamily D member 3 gene and were intronic. We used the discovery and replication stage combined  
158 GWAS results to assess whether a single SNP or set of variants drive the association signal in  
159 *KCND3* (credible set). The 99% credible set was computed based on Approximate Bayes Factors for  
160 each SNP, resulting for each in a set of SNPs that with 99% posterior probability contained the  
161 variant(s) driving the association signal. For the associated locus at *KCND3* the credible set spanned  
162 49 kb, and contained 19 variants. The two lead SNPs rs1545300 and rs12090194 had a posterior  
163 probability of 21% and 19%, respectively, whereas the former candidate SNP rs17029069(18) had a  
164 posterior probability of 2% (**Supplementary Table 2**).

165 To test whether the association in *KCND3* might be driven by heart rate or RR interval, we performed  
166 a sensitivity analysis in the 1,253 ERP cases and 11,463 controls of the Lifelines cohort adjusting the

167 genetic association of rs1545300 additionally for these two traits in separate models. The effect  
168 estimates were virtually unchanged (OR=0.78) with  $p=1.2E-7$  for both adjustments. In addition, we  
169 assessed whether the association of rs1545300 might be related to a specific ERP subtype i.e ST  
170 segment or ERP localization. In all subtype-stratified analyses the 95% confidence intervals of the  
171 effect sizes overlapped with the overall results not pointing to a subtype driven signal (**Supplementary**  
172 **Table 3**).

### 173 *eQTL and co-localization*

174 We searched the Genotype-Tissue Expression (GTEx) project database(20) to look for tissue-specific  
175 eQTLs including all genes in vicinity of  $\pm 1$ Mb of the lead SNP rs1545300 and found an association  
176 with *KCND3* expression levels in tibial artery ( $p=3.0E-6$ ,  $n=388$ ). Two additional eQTL associations of  
177 rs1545300 at a false discovery rate (FDR)  $<0.2$  across the 48 tissues tested were found with *KCND3*  
178 (ENSG00000171385.5) in the left ventricle ( $p=2.9E-4$ ,  $n=272$ ) of the human heart, and with *CEPT1*  
179 (ENSG00000134255.9) in the minor salivary gland ( $p=3.4E-4$ ,  $n=97$ ) (**Supplementary Table 4**).

180 Subsequent co-localization analyses of rs1545300 in these three tissues revealed also a significant  
181 correlation of gene expression pattern with ERP ( $p_{SMR} \leq 0.01$ ) (**Figure 2, Supplementary Table 5**),  
182 where for the left ventricle the correlation seems to be attributable to the same underlying causative  
183 variant ( $p_{HEIDI} \geq 0.05$ ), and for tibial artery the test was close to nominal significance ( $p_{HEIDI}=0.05$ ).  
184 However, the significant  $p_{HEIDI}=1.7E-3$  of *CEPT1* in the minor salivary gland points rather towards a  
185 pleiotropic effect of rs1545300 than to a causal effect of gene expression on ERP in this tissue. For all  
186 three tissues, an increased gene expression level was associated with a higher risk of ERP  
187 (**Supplementary Table 5**).

### 188 *Pleiotropic effects of the lead SNPs*

189 To assess pleiotropic effects of the *KCND3* lead SNP rs1545300 or its proxies ( $r^2 > 0.8$ ), we looked for  
190 genome-wide significant associations in the NHGRI-EBI Catalog of published genome-wide  
191 association studies(21) (accessed: 05/03/2019). Pleiotropic associations were found for P-wave  
192 terminal force (rs12090194 and rs4839185)(22) and for atrial fibrillation (rs1545300 and  
193 rs1443926)(23, 24). All these SNPs were in strong linkage disequilibrium ( $r^2 > 0.97$ ) with the lead SNP.  
194 In addition, variants in low to moderate LD with rs1545300 were associated with P-wave duration  
195 (rs2798334,  $r^2=0.26$ )(25) and ST-T-wave amplitudes (rs12145374,  $r^2=0.60$ )(26).

196 **Discussion**

197 In this GWAS meta-analysis comprising 3,305 cases and 36,151 controls including independent  
198 replication samples, we describe an association of ERP with a locus on chromosome 1 in the *KCND3*  
199 gene. This is the first study identifying a robust genome-wide significant association between genetic  
200 variants and ERP. Our findings form the genetic basis for further functional studies examining the  
201 pathophysiological mechanism of ERP and potentially ERS. The *KCND3* gene encodes the main  
202 pore-forming alpha subunit of the voltage-gated rapidly inactivating A-type potassium channel. In the  
203 cardiac ventricle *KCND3* contributes to the fast cardiac transient outward potassium current ( $I_{to}$ ), which  
204 plays a major role in the early repolarization phase 1 of the cardiac action potential (AP).

205 To date, two competing theories explain the presence of J waves and ERP: the repolarization and the  
206 depolarization theory, both involving the  $I_{to}$  channel. On the basis of animal models evidence for the  
207 former is more compelling. Thus, J waves result from a transmural voltage gradient created by a more  
208 prominent epicardial phase 1 AP notch relative to the endocardial AP notch(10, 27). The  $I_{to}$  current  
209 notably influences the degree of the transmural heterogeneity of the phase 1 AP notch and  
210 consecutively the magnitude of the J wave(10, 27). Pharmacological inhibition of the  $I_{to}$  current with 4-  
211 aminopyridine results in a reduction of the J wave amplitude(10). The depolarization theory is based  
212 on clinical overlap of ERP with Brugada syndrome, which has led to the suggestion of Brugada  
213 syndrome being a right ventricular variant of the ERP(28). In theory, deviation from the sequential  
214 activation of cardiac currents  $I_{Na}$ ,  $I_{to}$ , and  $I_{CaL}$  can lead to regional conduction slowing and appearance  
215 of inferior and/or lateral ERP(27, 29). In patients with ERS, distinct phenotypes of both delayed  
216 depolarization and early repolarization have been identified(30).

217 ERP is a highly heritable trait within families(3, 16), however limited heritability can be attributed to  
218 common SNPs in unrelated individuals(17). This might be a reason why the only GWAS to date which  
219 included 452 cases failed to replicate any genome-wide significant loci(18). In our study, which  
220 includes 3,334 cases, we discovered and replicated variants in the *KCND3* gene. Interestingly, one of  
221 these variants (rs17029069), which is in moderate LD ( $r^2=0.18$ ,  $D'=-1$ ) with our lead SNP rs1545300  
222 (**Supplementary Figure 3**) was reported as a candidate in the earlier GWAS meta-analysis(18).  
223 However, this variant did not replicate in their study, which the authors attributed to limited power  
224 based on the small sample size and/or heterogeneous phenotyping. In our study, experienced  
225 cardiologists centrally adjudicated more than 39,000 ECGs with high reproducibility ensuring a very



226 high phenotyping quality(17). The resulting homogenously assessed phenotype and the substantially  
227 increased number of cases are two aspects that elevated the statistical power of our GWAS meta-  
228 analysis. All detected variants cluster in intronic regions of the *KCND3* gene, without significant allelic  
229 heterogeneity. The annotation of the locus does not point to a direct pathogenic effect, i.e. a protein  
230 altering mutation, and also the statistical finemapping revealed no single SNP with a substantial  
231 posterior probability (e.g. >80%) of being causal. However, the latter approach has limitations of  
232 detecting rare causal variants due to imputation uncertainty and minimum minor allele frequency  
233 (MAF). Nevertheless, eQTL analysis suggested that the detected variants may affect gene expression  
234 of *KCND3*. Potential mechanisms include modification of gene expression via altered binding of  
235 transcription factors at *cis*-elements. This is supported by the results of the test for co-localization  
236 showing an increase of ERP risk due to increased gene expression levels of *KCND3* in tissues of the  
237 human heart and tibial artery. Similar, pharmacological *ex vivo* data predict gain of function mutations  
238 in the  $I_{to}$  current to increase the overall transmural outward shift, leading to an increased epicardial AP  
239 notch and thereby inducing ERP in the surface ECG(27). Additionally, in close proximity to the lead  
240 SNP rs1545300 a long non-coding RNA (lncRNA), *KCND3* antisense RNA 1 (*KCND3-AS1*) is  
241 described. lncRNAs have been shown to physiologically influence gene regulation through various  
242 mechanism e.g. chromatin remodeling, control of transcription initiation and post-transcriptional  
243 processing(31, 32). On the other hand, dysregulation of lncRNA control circuits can potentially impact  
244 development of disease(33): a very prominent example in cardiovascular diseases is the lncRNA  
245 *ANRIL*, which is a key effector of *9p21* in atherosclerotic risk and cardiovascular events(33–35).

246 Given the high prevalence of ERP in the general population and a high MAF of the identified genetic  
247 variants in our study the key question remains why only a very small subset of individuals develops  
248 severe ventricular arrhythmias and ERS. The fine interplay of a genetic predisposition and specific  
249 precipitating conditions might lead to an electrically vulnerable cardiac state. Insights into the potential  
250 origin of ventricular arrhythmias in ERS come from animal models and highlight the role of different ion  
251 channels including  $I_{to}$ (36). A pharmacological model of ERS in canine wedges from the inferior and  
252 lateral ventricular wall showed marked regional dispersion of repolarization (loss of phase 2 AP dome  
253 and AP shortening in some epicardial regions but not others). Presence of transmural repolarization  
254 heterogeneity allowed local re-excitation in form of closely coupled extrasystolic activity (phase 2 re-  
255 entry). The combination of an arrhythmogenic substrate, represented by regional electrical instability,



256 and triggering premature ventricular beats resulted in ventricular fibrillation(36). Human data in ERS  
257 patients suggest that in a subgroup, the ERP is due to a pure repolarization phenotype and  
258 arrhythmia(30) is triggered by Purkinje fiber ectopic beats.

259 Genetic variants in various ion channel genes have been associated with ERS(37) including the  
260 *KCNJ8* and *ABCC9* genes encoding the Kir6.1 and ATP-sensing subunits of the  $K_{ATP}$  channel(6, 11,  
261 38, 39). The commonly implicated variant *KCNJ8*-p.S422L has a population frequency not consistent  
262 with ERS, and is predicted to be benign by multiple in silico algorithms according to the ClinVar  
263 database(40). A recent study by Chaveau *et al.* has, however, identified a de novo duplication of the  
264 *KCND3* gene in a patient who survived sudden cardiac death and in his 2-year-old daughter(12). Both  
265 exhibited marked ERP in the inferolateral leads that was augmented by bradycardia and pauses in  
266 heart rhythm, in keeping with a repolarization mechanism underlying the ERS phenotype. Studies  
267 have suggested that the inferior region of the left ventricle has a higher density of *KCND3* expression  
268 and higher intrinsic levels of  $I_{to}$ (36). This may explain the higher vulnerability of this region for the  
269 development of ERS in the setting of a genetically mediated gain-of-function in the  $I_{to}$  current. The  
270 findings of our study therefore suggest that common variation may play a role in the expression of  
271 *KCND3* and the  $I_{to}$  current and that it is likely to be relevant in ERS as well. This may in part explain  
272 the minimal yield of pathogenic variants in ERS cases. Further GWAS in large collaborative cohorts of  
273 ERS patients are therefore necessary to determine the importance of polygenic risk. A systematic  
274 evaluation of pleiotropic effects demonstrated known associations of the identified *KCND3* SNPs with  
275 ECG phenotypes only. The lead SNP rs1545300 or its proxies in strong LD ( $r^2 > 0.97$ ) were found to be  
276 related with P-wave terminal force(22) and atrial fibrillation(23). This highlights the sharing of  
277 underlying mechanisms between *KCND3* variation and cardiac electrical activity.

278 Our study has some limitations, which need to be acknowledged. Presence of ERP in the ECG can be  
279 variable, as it has been described to be dependent on age, heart rate, vagal activity and medication,  
280 although our findings were valid after adjusting for some of these factors. Therefore, we cannot  
281 exclude that we have missed some individuals with ERP. Second, the tissue-specific gene expression  
282 data used for the co-localization analysis is based on a limited sample size. A larger gene expression  
283 sample or functional studies are needed to validate the revealed effect of *KCND3* expression on the  
284 ERP.

285 In conclusion, we show for the first time, a robust association of genetic variants with the ERP in a  
286 large GWAS of individuals of European ancestry. The locus in the *KCND3* ion channel gene is an  
287 intuitive candidate and supports the theory that at least a proportion of ERS is a pure channelopathy.  
288 Intensive future research will be needed to extend the discovery of ERP susceptibility loci to  
289 individuals of non-European ancestry, and to improve identification and risk stratification of the subset  
290 of individuals with the ERP who are at highest risk for potentially lethal ventricular arrhythmias.

## 291 **Methods**

### 292 *Study cohorts and SNP genotyping*

293 The discovery stage included 25,822 subjects (2,181 ERP cases) from eight independent cohorts: the  
294 British Genetics of Hypertension (BRIGHT) study, the Gutenberg Health Study (GHS1, GHS2), the  
295 Genetic Regulation of Arterial Pressure In humans in the Community (GRAPHIC) study, the Lifelines  
296 Cohort Study (Lifelines), the Study of Health in Pomerania (SHIP, SHIP-Trend), and TwinsUK.  
297 Additional 13,634 subjects (1,124 ERP cases) from four cohorts (Rotterdam Study I, II, III, and CHRIS)  
298 were used as independent replication: the Rotterdam Study (Rotterdam Study I, II, III), and the  
299 Cooperative Health Research In South Tyrol (CHRIS) study. The included subjects of all cohorts were  
300 of European ancestry, and all cohorts but BRIGHT (which sampled hypertensive cases) were  
301 population based (**Supplementary Table 1**). All subjects gave written informed consent and the  
302 studies were approved by the local ethics committees.

### 303 *Electrocardiogram analysis and ERP evaluation*

304 12-lead ECGs of all 12 studies were analyzed manually by experienced and specifically trained  
305 cardiologists for the presence of ERP according to the established definition by Haissaguerre and  
306 Macfarlane(6, 19). In case of a QRS duration of >120 ms or rhythm other than sinus rhythm (e.g. atrial  
307 fibrillation, pacemaker stimulation) ECGs were excluded from the analysis. The methodology  
308 employed and robustness of inter-observer correlations have been presented elsewhere(17).

309 In detail, ERP was defined as elevation of the J-point above the level of QRS onset of  $\geq 0.1$  mV in at  
310 least two corresponding leads. To avoid confusion or overlap with Brugada syndrome or  
311 arrhythmogenic right ventricular dysplasia, leads V1 to V3 were excluded from ERP scoring. In case of  
312 presence of ERP, region, either inferior (leads II, III, aVF), antero-lateral (leads I, aVL, V<sub>4</sub>-V<sub>6</sub>), or both,  
313 and the maximum amplitude of J-point elevation was documented. Further, the morphology of ERP

314 was assessed as either notching, slurring or both as well as the ST segment according to Tikkanen  
315 and colleagues(41) as either concave/rapidly ascending ( $>0.1$  mV elevation 100 ms after J-point peak  
316 or persistently elevated ST segment  $>0.1$  mV) or horizontal/descending ( $\leq 0.1$  mV elevation within 100  
317 ms after J-point peak)(19, 41).

#### 318 *GWAS in individual studies*

319 The GWAS in each study for both the discovery and replication stage was performed on autosomal  
320 imputed SNP genotypes using study-specific quality control protocols which are provided in detail in  
321 **Supplementary Table 1**. Association analyses were performed using logistic regression for ERP  
322 status as outcome and an additive genetic model on SNP dosages, thus taking genotype uncertainties  
323 of imputed SNPs into account. The analyses were adjusted for age, sex, and relevant study-specific  
324 covariates such as principal components for population stratification (**Supplementary Table 1**).

#### 325 *Statistical methods for meta-analysis*

326 The result files from individual studies GWAS underwent extensive quality control before meta-  
327 analysis using the `gwasqc()` function of the `GWAToolbox` package v2.2.4(42). The quality control  
328 included file format checks as well as plausibility and distributions of association results including  
329 effect sizes, standard errors, allele frequencies and imputation quality of the SNPs.

330 The meta-analyses were conducted using a fixed-effect inverse variance weighting as implemented in  
331 `Metal`(43). Monomorphic SNPs, SNPs with implausible association results (i.e.  $p \leq 0$ ,  $SE \leq 0$ ,  
332  $|\log(OR)| \geq 1000$ ), and SNPs with an imputation quality score  $\leq 0.4$  were excluded prior to the meta-  
333 analyses resulting in a median of 12,839,202 SNPs per cohort (IQR: 10,756,073-13,184,807). During  
334 the meta-analysis, the study-specific results were corrected by their specific  $\lambda_{GC}$  if  $>1$ . Results were  
335 checked for possible errors like use of incorrect association model by plotting the association p-values  
336 of the analyses against those from a z-score based meta-analysis for verifying overall concordance.  
337 SNPs that were present in  $<75\%$  of the total sample size contributing to the respective meta-analysis  
338 or with a MAF  $\leq 0.01$  were excluded from subsequent analyses. Finally, data for up to 6,976,246 SNPs  
339 were available after the meta-analysis.

340 Quantile-quantile plots of the meta-analysis results are provided in **Supplementary Figure 2**. To  
341 assess whether there was an inflation of p-values in the meta-analysis results attributed to reasons  
342 other than polygenicity, we performed LD score regression(44). The LD score corrected  $\lambda_{GC}$  value of  
343 the discovery and replication combined meta-analysis was 1.01, supporting the absence of

344 unaccounted population stratification. Genome-wide significance was defined as a p-value  $<5E-8$ ,  
345 corresponding to a Bonferroni correction of one million independent tests. Unless stated otherwise, all  
346 reported p-values are two-sided. The  $I^2$  statistic was used to evaluate between-study  
347 heterogeneity(45).

348 To evaluate the presence of allelic heterogeneity within each locus, the GCTA stepwise model  
349 selection procedure (cojo-slc algorithm) was used to identify independent variants employing a step-  
350 wise forward selection approach(46). We used the genotype information of 4,081 SHIP individuals for  
351 LD estimation, and set the significance threshold for independent SNPs to  $5E-8$ .

352 All loci were named according to the nearest gene of the lead SNP. Genomic positions correspond to  
353 build 37 (GRCh37).

#### 354 *Replication analysis*

355 To minimize the burden for multiple testing correction and thus maximizing the power for replication,  
356 the lead SNPs of genome-wide significant loci in the discovery stage were taken forward to the  
357 replication stage in independent samples (**Table 1**). SNPs were considered as replicated if the p-value  
358 of a one-sided association test was  $<0.025$  which corresponds to a Bonferroni correction for the two  
359 lead SNPs tested at 5% significance level.

360 Finally, the GWAS results from the discovery and replication studies were meta-analyzed to search for  
361 additional genome-wide significant loci by maximizing the statistical power for locus discovery.

#### 362 *Gene expression based analyses*

363 The lead SNP rs1545300 of the *KCND3* locus of the combined discovery and replication GWAS meta-  
364 analysis was tested for *cis* eQTLs ( $\pm 1$ Mb window around the transcription start site) in 48 tissues  
365 available in the GTEx v7 database that included at least 70 samples. Significant associations were  
366 selected based on a Bonferroni corrected p-value  $<3.0E-5$  for the number of genes and tissues tested.  
367 Subsequently, the SNP rs1545300 was tested and plotted for co-localization in the three tissues with  
368 an eQTL  $FDR < 0.2$  by applying the SMR method(47) using the GWAS and GTEx eQTL summary  
369 statistics. The method includes a test whether the effect on expression observed at a SNP or at its  
370 proxies is independent of the signal observed in the GWAS, i.e. that gene expression and y are  
371 associated only because of a latent non-genetic confounding variable (SMR test), and a second test  
372 that evaluates if the eQTL and GWAS associations can be attributable to the same causative variant  
373 (HEIDI test). Significance for co-localization of the gene expression and the GWAS signals was

374 defined by  $p_{SMR} < 0.01$ , where additionally a  $p_{HEIDI} \geq 0.05$  indicates the same underlying causal  
375 variant(47).

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### 378 **Author Contributions**

379 Project design and analysis: W.R., T.T., A.T., M.D, E.R.B. Management of individual study: A.F.D.,  
380 C.F., C.P., E.R.B., K.J.L., M.A.I., M.D., M.G., M.Z., N.P., N.V., P.B.M., P.P., P.v.d.H., S.A., S.B.F.,  
381 T.D.S., T.M., T.T., W.R., Y.J. Recruitment of individual study subjects: A.F.D., G.S., H.S., I.D., M.D.,  
382 M.Z., N.V., P.v.d.H., S.A., S.B.F. Drafting of the manuscript: A.R., A.T., B.H.S., E.R.B., M.D.B.,  
383 M.E.v.d.B., T.T., W.R. Statistical methods and analysis of individual study: A.T., C.C., C.F., H.R.W.,  
384 H.S., I.M.N., K.S., M.E.v.d.B., S.P. Genotyping of individual study: A.G.U., C.F., M.N., P.B.M., U.V.  
385 Interpretation of the results: A.R., A.T., B.K., C.H., E.R.B., M.D., M.D.B., T.K., T.T., W.R. Critical  
386 review of the manuscript: all authors.

### 387 **Competing Financial Interests**

388 The authors declare no competing financial interests.

### 389 **Data availability**

390 Summary genetic association results have been submitted for full download to the CHARGE dbGaP  
391 website under accession phs000930 [<https://www.ncbi.nlm.nih.gov/gap>].

### 392 **Figure Legends**

#### 393 **Figure 1. GWAS results of the *KCND3* locus**

394 The results of the combined ERP GWAS results for the *KCND3* locus are shown for the replicated  
395 discovery stage lead SNP rs12090194 (A and B), and for the combined GWAS lead SNP rs1545300  
396 (C and D). The regional association plots (A and C) show the association results in a  $\pm 500$  kb region  
397 around the lead SNP. SNPs are plotted on the x-axis according to their chromosomal position with the  
398  $-\log_{10}(p\text{-value})$  of the GWAS association on the y-axis. Correlation with the lead SNP (purple) is

399 estimated based on the 1000 Genomes reference samples. Plots were generated using the website of  
400 LocusZoom (Pruim, R. J. *et al.* Bioinformatics, 2010). Genetic positions refer to GRCh37/hg19  
401 coordinates. Forest plots of the respective lead SNPs are provided in (B) and (D), with odds ratios and  
402 their 95% confidence intervals plotted on the x-axis.  $I^2$  is the percentage of total variation across  
403 studies that is due to heterogeneity.

#### 404 **Figure 2. Co-localization results**

405 Illustration of the SMR test for ERP risk and expression QTLs at the rs1545300 locus at chromosome  
406 1p13.2 for (A) left ventricle of the heart, (B) tibial artery, and (C) minor salivary gland tissue. In each  
407 panel, the upper box shows the GWAS regional association plot with ERP risk, with level of  
408 significance of the SMR test (y-axis) for each transcript in the locus indicated by a diamond positioned  
409 at the center of the transcript. A significant SMR test represented by a purple diamond indicates an  
410 association of the transcript level of the respective genes (purple label) with the trait. For all three  
411 tissues, an increased gene expression level of a significant SMR test was associated with a higher risk  
412 of ERP. A filled purple diamond indicates a HEIDI test p-value  $>0.05$ , thus a likely co-localization. The  
413 lower box shows the regional association distribution with changes in expression of the highlighted  
414 (purple) gene transcript in the respective tissue. In both boxes, the x-axis refers to GRCh37/hg19  
415 genomic coordinates.

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- 521

**Table 1: Baseline characteristics of the study populations**

Study (discovery stage)	BRIGHT		GHS1		GHS2		GRAPHIC	
	ERP+	ERP-	ERP+	ERP-	ERP+	ERP-	ERP+	ERP-
Number of samples (n)	189	1173	182	2628	70	1028	57	893
Females (n)	105	747	60	1358	26	536	18	457
Age in years (mean±SD)	57.6±12.1	59.4±12.3	54.5±10.0	55.6±10.9	54.0±10.2	54.9±10.9	52.3±3.9	52.8±4.5
Heart rate in bpm (mean±SD)	61.7±9.9	63.7±11.2	67.6±11.5	69.1±10.8	67.1±11.4	68.7±10.8	63.5±8.0	64.1±9.8
BMI (mean±SD)	27.7±3.4	27.4±3.8	26.8±4.4	27.1±4.7	27.5±5.5	27.2±4.9	27.4±4.0	27.4±4.3
Study (discovery stage)	Lifelines		SHIP		SHIP-Trend		TwinsUK	
	ERP+	ERP-	ERP+	ERP-	ERP+	ERP-	ERP+	ERP-
Number of samples (n)	1253	11463	173	2835	86	848	171	2773
Females (n)	639	6902	79	1508	38	494	150	2651
Age in years (mean±SD)	48.0±11.5	47.9±11.3	46.6±16.1	48.5±15.8	49.8±14.5	49.7±13.4	51.7±13.2	52.7±12.4
Heart rate in bpm (mean±SD)	66.3±10.9	68.4±11.5	70.5±11.6	73.7±11.6	64.4±8.9	65.9±9.6	64.1±10.3	66.8±10.4
BMI (mean±SD)	25.7±3.8	26.4±4.3	25.9±4.2	27.3±4.9	26.9±4.4	27.3±4.6	25.3±4.4	25.7±4.6
Study (replication stage)	CHRIS		Rotterdam Study I		Rotterdam Study II		Rotterdam Study III	
	ERP+	ERP-	ERP+	ERP-	ERP+	ERP-	ERP+	ERP-
Number of samples (n)	427	3953	308	4438	164	1476	225	2643
Females (n)	159	2318	182	2739	84	825	116	1541
Age in years (mean±SD)	45.2±16.3	45.7±16.1	66.4±7.6	66.3±7.7	64.1±7.3	64.4±7.5	56.7±5.6	57.0±6.7
Heart rate in bpm (mean±SD)	60.3±8.9	62.5±8.8	68.7±11.6	69.2±11.9	67.5±10.6	68.8±10.8	69.0±11.7	69.6±10.5
BMI (mean±SD)	25.4±4.2	25.6±4.6	27.5±7.4	27.1±6.9	27.5±4.1	27.5±4.1	27.6±4.9	27.5±5.0

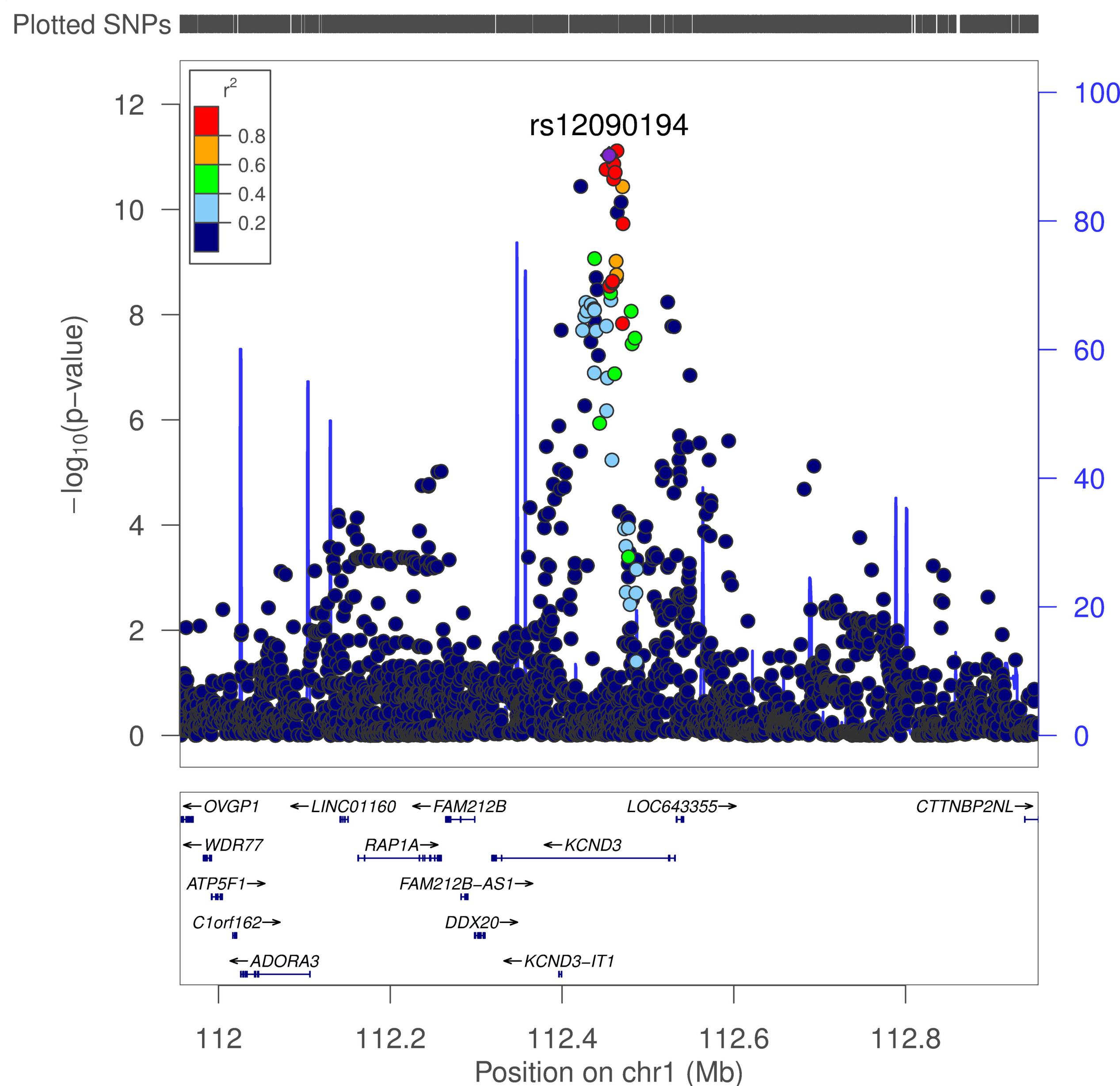
ERP+: cases with early repolarization pattern; ERP-: controls; SD: standard deviation; bpm: beats per minute; BMI: body mass index

**Table 2: Lead SNPs of the GWAS association results**

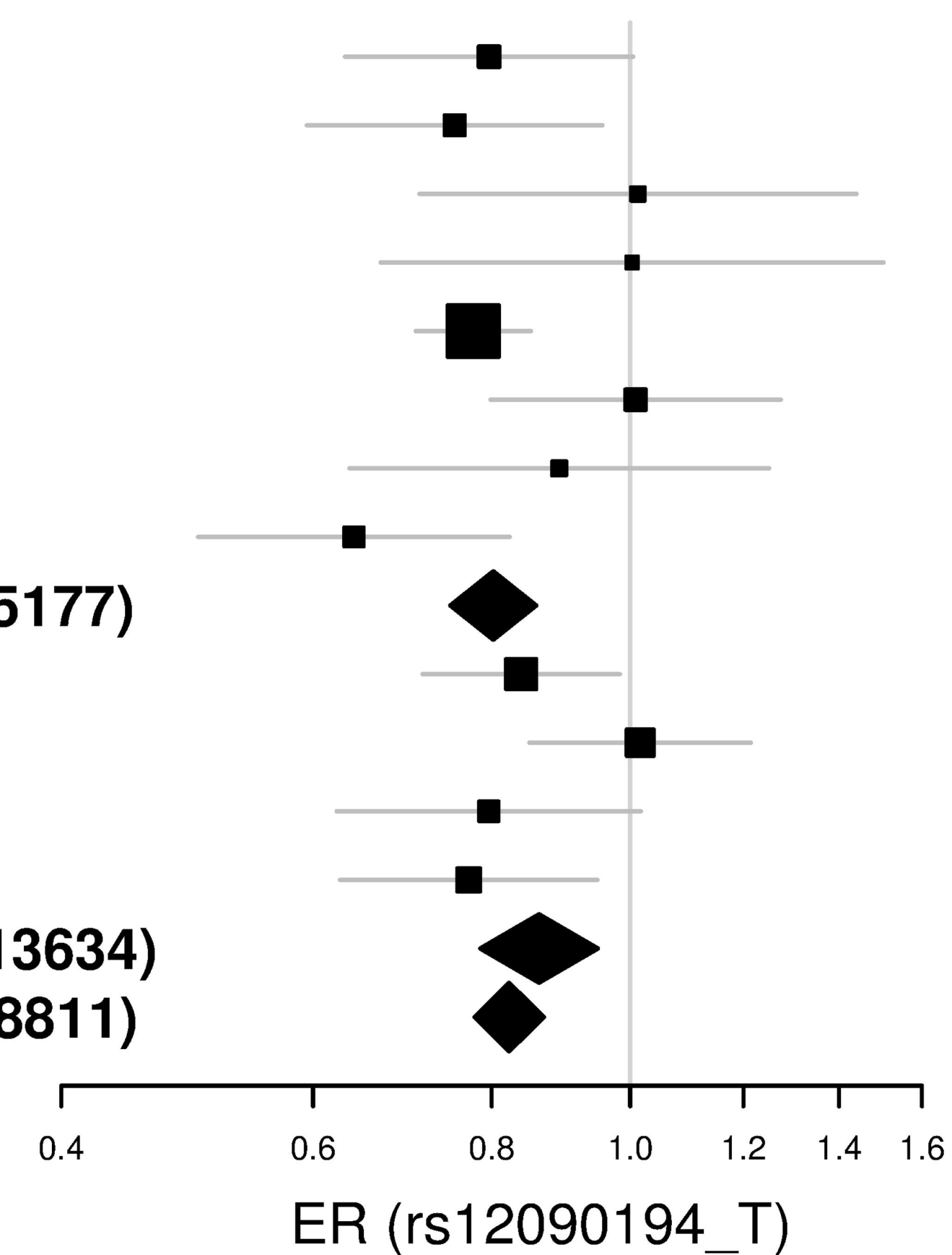
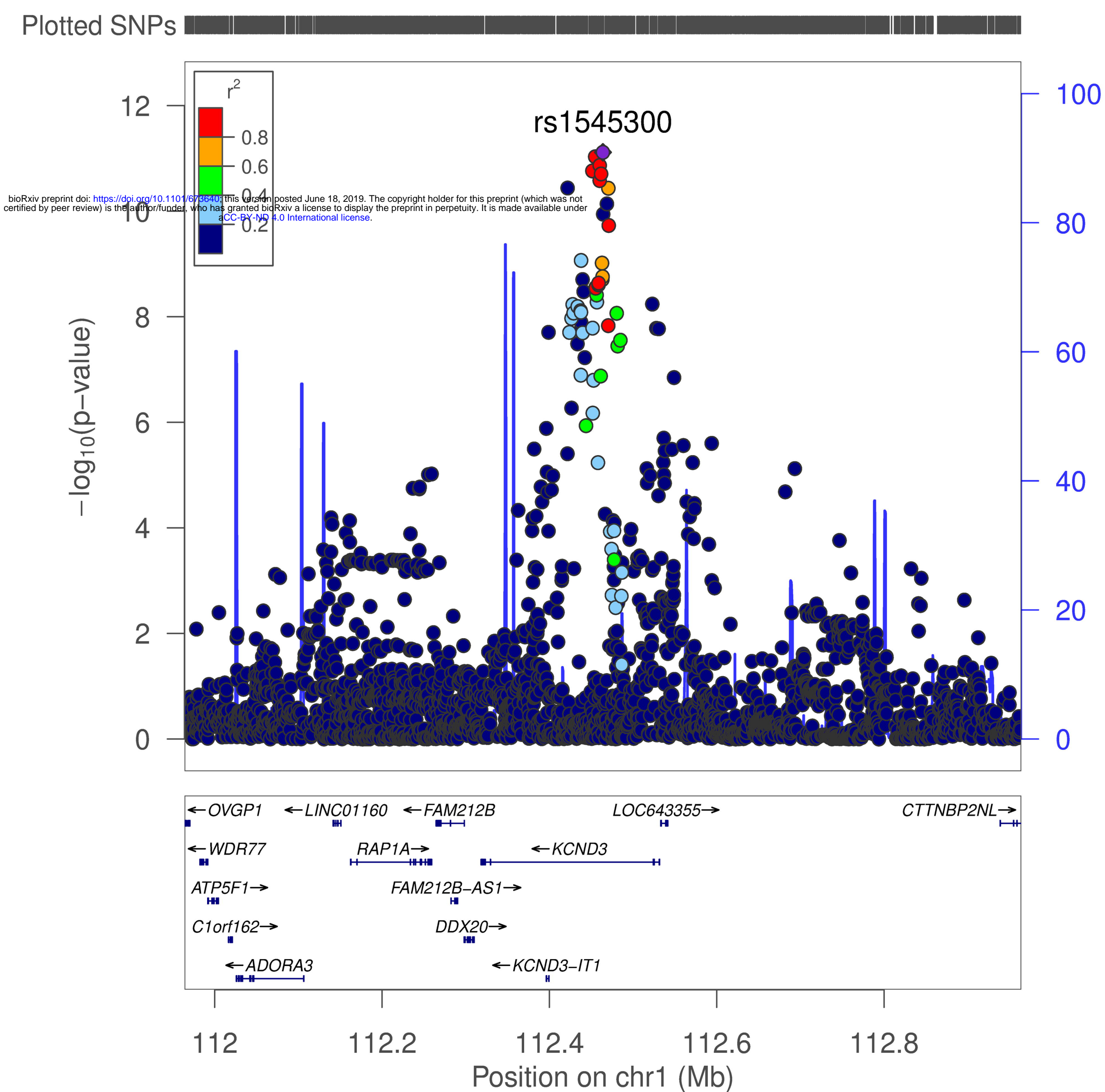
variant information				discovery					replication				combined			
SNP	chr:position	A1/A2	nearest gene	AF1	OR	P	I <sup>2</sup>	N	OR	P	I <sup>2</sup>	N	OR	P	I <sup>2</sup>	N
rs12090194	1:112,454,822	t/c	KCND3	0.32	0.80	<b>4.6E-10</b>	34	25177	0.86	2.5E-03	39	13634	0.82	9.3E-12	35	38811
					[0.75-0.86]				[0.79-0.95]				[0.78-0.87]			
rs1545300	1:112,464,004	t/c	KCND3	0.32	0.81	1.4E-09	41	25172	0.85	9.4E-04	56	13634	0.82	<b>7.7E-12</b>	43	38806
					[0.75-0.86]				[0.77-0.94]				[0.78-0.87]			
rs139772527	16:208,761	t/c	HBZ	0.01	2.57	<b>2.0E-08</b>	0	21495	1.21	2.8E-01	0	13634	1.81	1.4E-06	11	35129
					[1.85-3.58]				[0.85-1.73]				[1.42-2.31]			

A1: effect allele; AF1: allele frequency of A1; OR: odds ratio of A1 [95% confidence interval]; P: association p-value; I<sup>2</sup>: percentage of total variation across studies that is due to heterogeneity; N: sample size. Bold values indicate the lead SNP (lowest p-value) of a significantly associated locus in the corresponding meta-analysis stage.

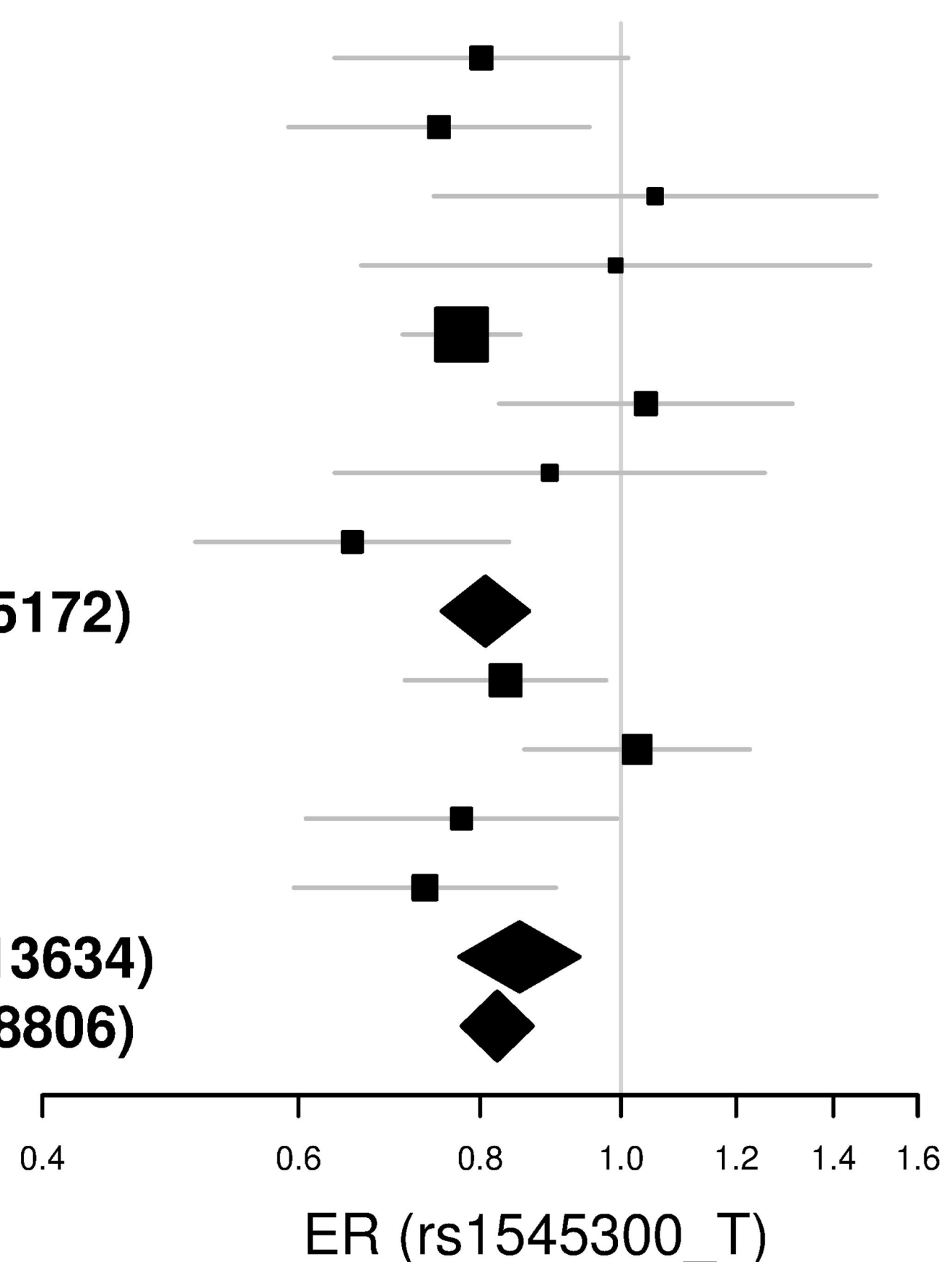


**A****B**

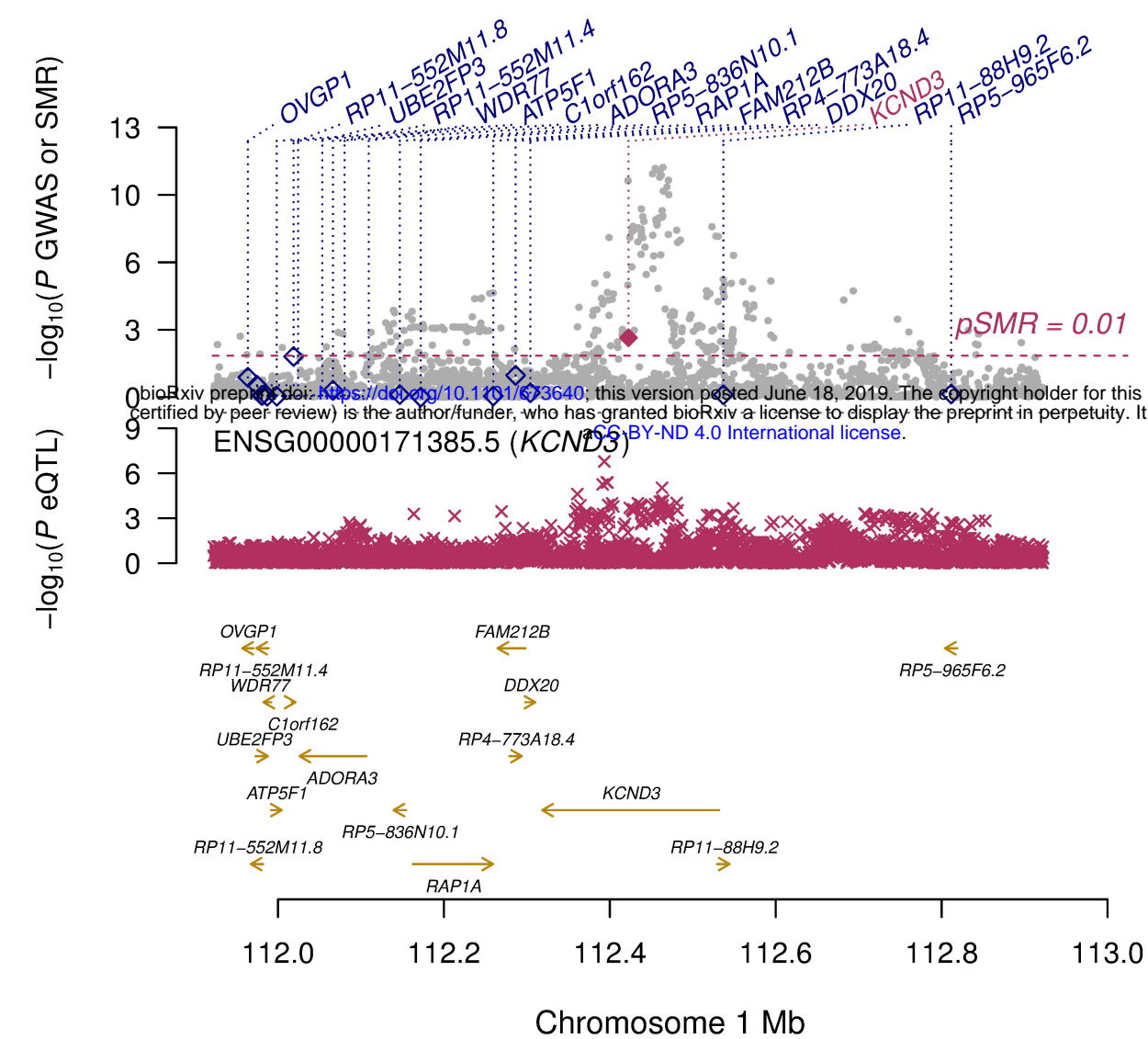
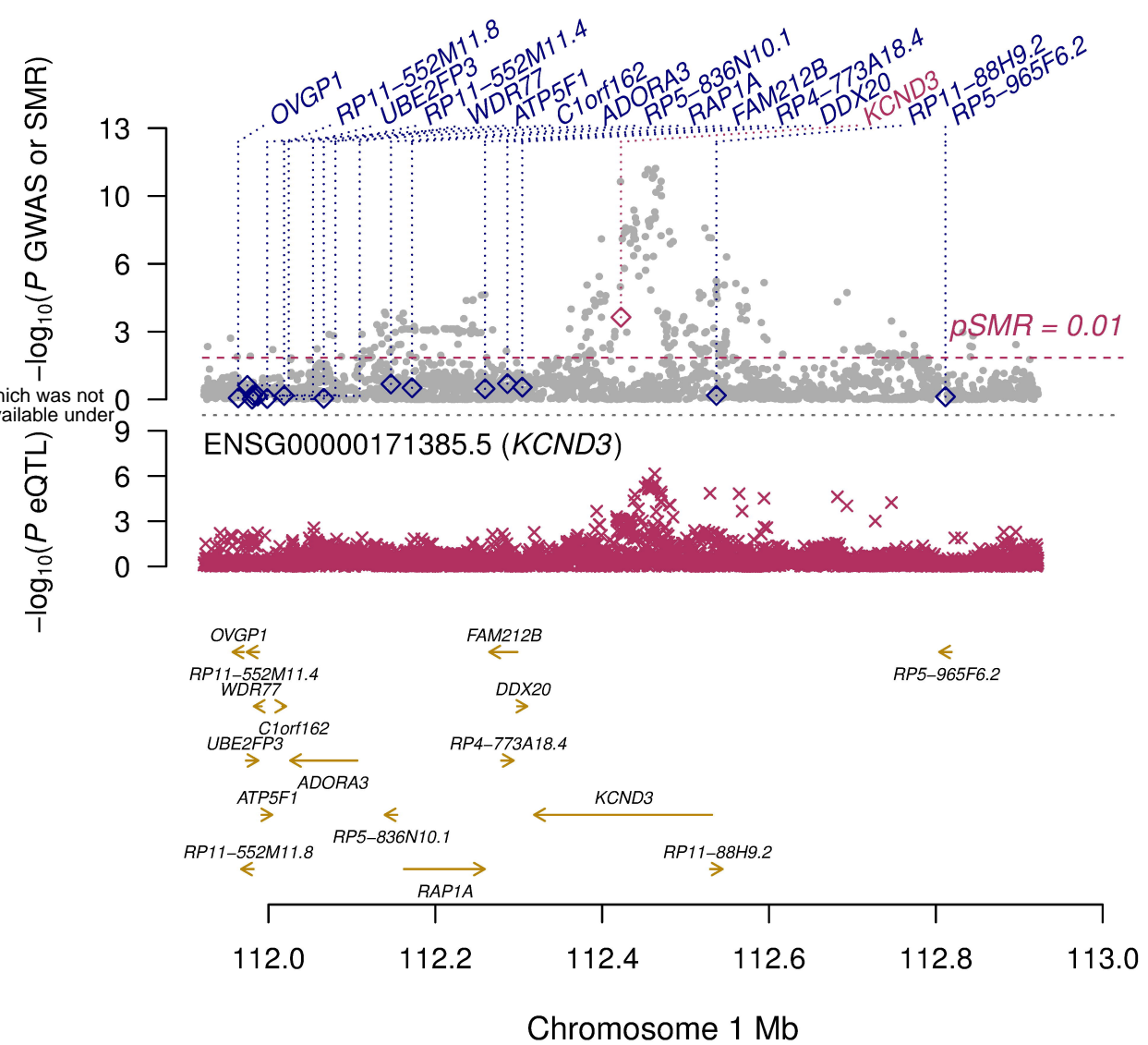
BRIGHT (n=738)  
 GHS1 (n=2810)  
 GHS2 (n=1098)  
 GRAPHIC (n=929)  
 LifeLines (n=12716)  
 SHIP (n=3008)  
 SHIP-Trend (n=934)  
 TwinsUK (n=2944)  
**discovery:  $I^2=34.1$  (n=25177)**  
 CHRIS (n=4380)  
 RS1 (n=4746)  
 RS2 (n=1640)  
 RS3 (n=2868)  
**replication:  $I^2=38.6$  (n=13634)**  
**combined:  $I^2=35.3$  (n=38811)**

**C****D**

BRIGHT (n=735)  
 GHS1 (n=2810)  
 GHS2 (n=1098)  
 GRAPHIC (n=929)  
 LifeLines (n=12716)  
 SHIP (n=3008)  
 SHIP-Trend (n=934)  
 TwinsUK (n=2942)  
**discovery:  $I^2=41.0$  (n=25172)**  
 CHRIS (n=4380)  
 RS1 (n=4746)  
 RS2 (n=1640)  
 RS3 (n=2868)  
**replication:  $I^2=55.7$  (n=13634)**  
**combined:  $I^2=43.4$  (n=38806)**





**A****B****C**