

# KCND3 potassium channel gene variant confers susceptibility to electrocardiographic early repolarization pattern

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90 **Abstract**

91 Background. The presence of an early repolarization pattern (ERP) on the surface electrocardiogram  
92 (ECG) is associated with risk of ventricular fibrillation and sudden cardiac death. Family studies have  
93 shown that ERP is a highly heritable trait but molecular genetic determinants are unknown.

94 Methods. To identify genetic susceptibility loci for ERP, we performed a GWAS and meta-analysis in  
95 2,181 cases and 23,641 controls of European ancestry.

96 Results. We identified a genome-wide significant ( $p < 5E-8$ ) locus in the *KCND3* (potassium voltage  
97 gated channel subfamily D member 3) gene that was successfully replicated in additional 1,124 cases  
98 and 12,510 controls. A subsequent joint meta-analysis of the discovery and replication cohorts  
99 identified rs1545300 as the lead SNP at the *KCND3* locus (OR 0.82 per minor T allele,  $p = 7.7E-12$ ),  
100 but did not reveal additional loci. Co-localization analyses indicate causal effects of *KCND3* gene  
101 expression levels on ERP in both cardiac left ventricle and tibial artery.

102 Conclusions. In this study we identified for the first time a genome-wide significant association of a  
103 genetic variant with ERP. Our findings of a locus in the *KCND3* gene not only provide insights into the  
104 genetic determinants but also into the pathophysiological mechanism of ERP, discovering a promising  
105 candidate for functional studies.

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## 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(10). Ex vivo studies point towards a  
118 central role of the cardiac transient outward potassium current ( $I_{to}$ ) in the development of both, ERP  
119 and ERS(11). Furthermore, candidate genetic association studies have highlighted a role for several  
120 genes encoding cardiac ion channels in the development of ERP and ERS(12–15). These genes  
121 include gain-of-function variants in  $I_K$ -ATP channels (*KCNJ8*, *ABCC9*) and loss-of-function variants in  
122 cardiac L-type calcium channels (*CACNA1C*, *CACNB2b*, *CACNA2D1*) and sodium channels (*SCN5A*,  
123 *SCN10A*)(16). Interestingly, co-existence of two genetic variants in different ion channel genes with  
124 opposing effects can be observed leading to phenotypic incomplete penetrance of ERP(15). However,  
125 data from functional studies confirming causality are scarce(17).

126 Studies among relatives of sudden arrhythmic death syndrome show that ERP is more prevalent in the  
127 relatives than in controls indicating that ERP is an important potentially inheritable pro-arrhythmic  
128 trait(18, 19). Moreover, in family studies the heritability estimate for the presence of ERP was  $h^2=0.49$   
129 (20). However, estimates for common SNP heritability from unrelated individuals are lower(21). This  
130 may explain why the only GWAS on ERP to date failed to identify genetic variants reaching genome-  
131 wide significance(22), and indicates the need for larger GWAS with more power.

132 In order to identify genetic variations that convey susceptibility to ERP we performed a GWAS and  
133 meta-analysis in European ancestry individuals, comprising 2,181 ERP cases and 23,641 controls  
134 from eight cohorts that formed the discovery stage. The findings were taken forward to a replication  
135 stage in 1,124 cases and 12,510 controls from four additional cohorts. To maximize statistical power

136 for locus discovery, we subsequently performed a combined discovery and replication cohort GWAS  
137 meta-analysis of 3,305 ERP cases and 36,151 controls.

## 138 **Results**

139 Clinical characteristics of the study cohorts are depicted in **Table 1**. The proportion of ERP based on  
140 the definition by Haisaguerre and Macfarlane(6, 23) ranged from 6% to 14% which is in line with  
141 previously reported prevalence in the general population(2–4).

### 142 *Novel variants associated with ERP*

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

### 164 *Statistical finemapping of the associated locus*

165 All significantly associated SNPs of the combined GWAS meta-analysis were located within *KCND3*,  
166 the potassium voltage-gated channel subfamily D member 3 gene, and were intronic (**Table 3, Figure**  
167 **2**). We used these results to assess whether a single SNP or set of variants drive the association  
168 signal in *KCND3* (credible set). The 99% credible set was computed based on Approximate Bayes  
169 Factors for each SNP, resulting for each in a set of SNPs that with 99% posterior probability contained  
170 the variant(s) driving the association signal. For the associated locus at *KCND3* the credible set  
171 spanned 49 kb, and contained 19 variants. The two lead SNPs rs1545300 and rs12090194 had a  
172 posterior probability of 21% and 19%, respectively, whereas the former candidate SNP  
173 rs17029069(22) had a posterior probability of 2% (**Supplementary Table 2**).

174 To test whether the association in *KCND3* might be driven by heart rate or RR interval, we performed  
175 a sensitivity analysis in the 1,253 ERP cases and 11,463 controls of the Lifelines cohort adjusting the  
176 genetic association of rs1545300 additionally for these two traits in separate models. The effect  
177 estimates were virtually unchanged (OR=0.78) with  $p=1.2E-7$  for both adjustments. In addition, we  
178 assessed whether the association of rs1545300 might be related to a specific ERP subtype i.e. ST  
179 segment or ERP localization. In all subtype-stratified analyses the 95% confidence intervals of the  
180 effect sizes overlapped with the overall results not pointing to a subtype driven signal (**Supplementary**  
181 **Table 3**).

#### 182 *Expression quantitative trait locus (eQTL) and co-localization*

183 We searched the Genotype-Tissue Expression (GTEx) project database(24) to look for tissue-specific  
184 eQTLs including all genes in vicinity of  $\pm 1$ Mb of the lead SNP rs1545300 and found an association  
185 with *KCND3* expression levels in tibial artery ( $p=3.0E-6$ ,  $n=388$ ). Two additional eQTL associations of  
186 rs1545300 at  $FDR < 0.2$  across the 48 tissues tested were found with *KCND3* (ENSG00000171385.5)  
187 in the left ventricle ( $p=2.9E-4$ ,  $n=272$ ) of the human heart, and with *CEPT1* (ENSG00000134255.9) in  
188 the minor salivary gland ( $p=3.4E-4$ ,  $n=85$ ) (**Supplementary Table 4**).

189 Subsequent co-localization analyses of rs1545300 in these three tissues revealed also a significant  
190 correlation of gene expression pattern with ERP ( $p_{SMR} \leq 0.01$ ) (**Figure 3, Supplementary Table 5**),  
191 where for the left ventricle the correlation seems to be attributable to the same underlying causative  
192 variant ( $p_{HEIDI} \geq 0.05$ ), and for tibial artery the test was close to nominal significance ( $p_{HEIDI} = 0.05$ ).  
193 However, the significant  $p_{HEIDI} = 1.7E-3$  of *CEPT1* in the minor salivary gland points rather towards a  
194 pleiotropic effect of rs1545300 than to a causal effect of gene expression on ERP in this tissue. For all

195 three tissues, an increased gene expression level was associated with a higher risk of ERP  
196 **(Supplementary Table 5).**

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

198 To assess pleiotropic effects of the *KCND3* lead SNP rs1545300 or its proxies ( $r^2 > 0.8$ ), we looked for  
199 genome-wide significant associations in the NHGRI-EBI Catalog of published genome-wide  
200 association studies(25) (accessed: 07/30/2019). Pleiotropic associations were found for P-wave  
201 terminal force (rs12090194 and rs4839185)(26) and for reduced risk of atrial fibrillation per minor allele  
202 (rs1545300 and rs1443926)(27, 28). All these SNPs were in strong linkage disequilibrium ( $r^2 > 0.97$ )  
203 with the lead SNP. In addition, variants in low to moderate LD with rs1545300 were associated with P-  
204 wave duration (rs2798334,  $r^2 = 0.26$ )(29) and ST-T-wave amplitudes (rs12145374,  $r^2 = 0.60$ )(30). A  
205 phenome-wide lookup of rs1545300 in the association results of 778 traits available via the Gene  
206 ATLAS web portal (31) using 452,264 individuals of the UK Biobank cohort revealed an association of  
207 the ERP risk reducing minor T allele with reduced risk of heart arrhythmia (estimated OR=0.92,  
208  $p = 3.6E-6$ ). Of note, no other of the assessed traits reached significance after Bonferroni correction  
209 ( $p < 0.05/778 = 6.4E-5$ ).

#### 210 **Discussion**

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

219 To date, two competing theories explain the presence of J waves and ERP: the repolarization and the  
220 depolarization theory, both involving the  $I_{to}$  channel. On the basis of animal models evidence for the  
221 former is more compelling. Thus, J waves result from a transmural voltage gradient created by a more  
222 prominent epicardial phase 1 AP notch relative to the endocardial AP notch(11, 32). The  $I_{to}$  current  
223 notably influences the degree of the transmural heterogeneity of the phase 1 AP notch and

224 consecutively the magnitude of the J wave(11, 32). Pharmacological inhibition of the  $I_{to}$  current with 4-  
225 aminopyridine results in a reduction of the J wave amplitude(11). The depolarization theory is based  
226 on clinical overlap of ERP with Brugada syndrome, which has led to the suggestion of Brugada  
227 syndrome being a right ventricular variant of the ERP(33). In theory, deviation from the sequential  
228 activation of cardiac currents  $I_{Na}$ ,  $I_{to}$ , and  $I_{CaL}$  can lead to regional conduction slowing and appearance  
229 of inferior and/or lateral ERP(32, 34). In patients with ERS, distinct phenotypes of both delayed  
230 depolarization and early repolarization have been identified(35).

231 ERP is a highly heritable trait within families(3, 20), however limited heritability can be attributed to  
232 common SNPs in unrelated individuals(21). This might be a reason why the only GWAS to date which  
233 included 452 cases failed to replicate any genome-wide significant loci(22). In our study, which  
234 includes 3,334 cases, we discovered and replicated variants in the *KCND3* gene. Interestingly, one of  
235 these variants (rs17029069), which is in moderate LD ( $r^2=0.18$ ,  $D'=-1$ ) with our lead SNP rs1545300  
236 (**Supplementary Figure 3**) was reported as a candidate in the earlier GWAS meta-analysis(22).  
237 However, this variant did not replicate in their study, which the authors attributed to limited power  
238 based on the small sample size and/or heterogeneous phenotyping. In our study, experienced  
239 cardiologists evaluated more than 39,000 ECGs with high reproducibility ensuring a very high  
240 phenotyping quality(21). The resulting homogeneously assessed phenotype and the substantially  
241 increased number of cases are two aspects that elevated the statistical power of our GWAS meta-  
242 analysis. All detected variants cluster in intronic regions of the *KCND3* gene, without significant allelic  
243 heterogeneity. The annotation of the locus does not point to a direct pathogenic effect, i.e. a protein  
244 altering mutation, and also the statistical finemapping revealed no single SNP with a substantial  
245 posterior probability (e.g. >80%) of being causal. However, the latter approach has limitations of  
246 detecting rare causal variants due to imputation uncertainty and minimum minor allele frequency  
247 (MAF). Nevertheless, eQTL analysis suggested that the detected variants may affect gene expression  
248 of *KCND3*. Potential mechanisms include modification of gene expression via altered binding of  
249 transcription factors at *cis*-elements through enhancers or in DNaseI hypersensitivity regions (**Figure**  
250 **2**). This is supported by the results of the test for co-localization showing an increase of ERP risk due  
251 to increased gene expression levels of *KCND3* in tissues of the human heart and tibial artery. Similar,  
252 pharmacological ex vivo data predict gain of function mutations in the  $I_{to}$  current to increase the overall  
253 transmural outward shift, leading to an increased epicardial AP notch and thereby inducing ERP in the



254 surface ECG(32). Additionally, in close proximity to the lead SNP rs1545300 a long non-coding RNA  
255 (lncRNA), *KCND3 antisense RNA 1 (KCND3-AS1)* is described. lncRNAs have been shown to  
256 physiologically influence gene regulation through various mechanism e.g. chromatin remodeling,  
257 control of transcription initiation and post-transcriptional processing(36, 37). On the other hand,  
258 dysregulation of lncRNA control circuits can potentially impact development of disease(38): a very  
259 prominent example in cardiovascular diseases is the lncRNA *ANRIL*, which is a key effector of *9p21* in  
260 atherosclerotic risk and cardiovascular events(38–40).

261 Given the high prevalence of ERP in the general population and a high MAF of the identified genetic  
262 variants in our study the key question remains why only a very small subset of individuals develops  
263 severe ventricular arrhythmias and ERS. The fine interplay of a genetic predisposition and specific  
264 precipitating conditions might lead to an electrically vulnerable cardiac state. Insights into the potential  
265 origin of ventricular arrhythmias in ERS come from animal models and highlight the role of different ion  
266 channels including  $I_{to}$ (10). A pharmacological model of ERS in canine wedges from the inferior and  
267 lateral ventricular wall showed marked regional dispersion of repolarization (loss of phase 2 AP dome  
268 and AP shortening in some epicardial regions but not others). Presence of transmural repolarization  
269 heterogeneity allowed local re-excitation in form of closely coupled extrasystolic activity (phase 2 re-  
270 entry). The combination of an arrhythmogenic substrate, represented by regional electrical instability,  
271 and triggering premature ventricular beats resulted in ventricular fibrillation(10). Human data in ERS  
272 patients suggest that in a subgroup, the ERP is due to a pure repolarization phenotype and  
273 arrhythmia(35) is triggered by Purkinje fiber ectopic beats.

274 Genetic variants in various ion channel genes have been associated with ERS(16) including the  
275 *KCNJ8* and *ABCC9* genes encoding the Kir6.1 and ATP-sensing subunits of the  $K_{ATP}$  channel(6, 12,  
276 41, 42). The commonly implicated variant *KCNJ8*-p.S422L has a population frequency not consistent  
277 with ERS, and is predicted to be benign by multiple in silico algorithms according to the ClinVar  
278 database(43). A recent study by Chauveau *et al.* has, however, identified a de novo duplication of the  
279 *KCND3* gene in a patient who survived sudden cardiac death and in his 2-year-old daughter(13). Both  
280 exhibited marked ERP in the inferolateral leads that was augmented by bradycardia and pauses in  
281 heart rhythm, in keeping with a repolarization mechanism underlying the ERS phenotype. Studies  
282 have suggested that the inferior region of the left ventricle has a higher density of *KCND3* expression  
283 and higher intrinsic levels of  $I_{to}$ (10). This may explain the higher vulnerability of this region for the

284 development of ERS in the setting of a genetically mediated gain-of-function in the  $I_{to}$  current.  
285 Moreover, observational studies also identified different ERP subtypes including the occurrence of  
286 ERP in the inferior region and a horizontal/descending ST segment morphology to be associated with  
287 a higher risk of sudden arrhythmic death and cardiovascular mortality(2, 44, 45). However, in a  
288 subgroup of our study the association signal of ERP risk and *KCND3* variation was not dominated by a  
289 specific ERP high-risk subtype. Of note the formation of subgroups led to reduction in sample size and  
290 thus statistical power.

291 Taken together, the rare occurrence of ERS may be explained by different conditions. On the one  
292 hand, an underlying monogenic mutation may be found in some cases. On the other, no single causal  
293 mutation can be identified in the majority of ERS cases rendering the influence of multiple genes and  
294 environmental factors more likely i.e. a 'multi-hit condition'. Similar to other polygenic diseases, the  
295 sum of multiple minor effects of several common genetic variations together with specific external  
296 triggers may affect the occurrence of ERS. There is indeed evidence to suggest that common variants  
297 in the *KCND3* locus increase arrhythmogenicity. A phenome-wide lookup of our common lead SNP in  
298 more than 450,000 individuals from the UK Biobank linked the minor T allele associated with reduced  
299 ERP to a reduced risk of heart arrhythmia(31). Furthermore, additional data show an association of  
300 the same common variant with reduced risk of atrial fibrillation(27, 28). A small effect of a common  
301 SNP at *KCND3* does not necessarily mean that the variant is benign; rather a single risk allele is  
302 associated with a small but effective change in the gene expression level. Thus, the overall effects of  
303 the *KCND3* gene expression levels on the phenotype may appear much stronger compared to the  
304 small effect of rs1545300. Based on our results, it could be hypothesized that variation in *KCND3*  
305 gene expression levels and subsequently its encoded protein may affect the risk of ERP and  
306 eventually ERS. The positive effect direction of the change in *KCND3* gene expression levels in heart  
307 tissue on the risk of ERP estimated via the SMR test (**Supplementary Table 5**) suggests an elevated  
308 risk with increasing abundance of the *KCND3* encoded protein. Functional validation is necessary to  
309 validate this hypothesis and analyses of the *KCND3* gene in individuals with ERS is warranted to  
310 confirm the role of *KCND3* variation in arrhythmogenesis.

311 Our study has some limitations, which need to be acknowledged. Presence of ERP in the ECG can be  
312 variable, as it has been described to be dependent on age, heart rate, vagal activity and medication,  
313 although our findings were valid after adjusting for some of these factors. Therefore, we cannot

314 exclude that we have missed some individuals with ERP. Second, the tissue-specific gene expression  
315 data used for the co-localization analysis is based on a limited sample size. A larger gene expression  
316 sample or functional studies are needed to validate the revealed effect of *KCND3* expression on the  
317 ERP. Also, we analyzed only common and low-frequency SNPs with a MAF >1% missing rare variants  
318 and variants not included in the imputation panel. Finally, long-term outcome data identifying those  
319 individuals with ERP who suffer from ERS are not available. Further GWAS in large international  
320 collaborative cohorts of ERS patients are therefore necessary to determine the genetic risk.

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

## 327 **Methods**

### 328 *Study cohorts and SNP genotyping*

329 The discovery stage included 25,822 subjects (2,181 ERP cases) from eight independent cohorts with  
330 genetic and phenotypic data available for analyses: the British Genetics of Hypertension (BRIGHT)  
331 study, the Gutenberg Health Study (GHS1, GHS2), the Genetic Regulation of Arterial Pressure In  
332 humans in the Community (GRAPHIC) study, the Lifelines Cohort Study (Lifelines), the Study of  
333 Health in Pomerania (SHIP, SHIP-Trend), and TwinsUK. Additional 13,634 subjects (1,124 ERP  
334 cases) from four cohorts (Rotterdam Study I, II, III, and CHRIS) were used as independent replication:  
335 the Rotterdam Study (Rotterdam Study I, II, III), and the Cooperative Health Research In South Tyrol  
336 (CHRIS) study. The included subjects of all cohorts were of European ancestry, and all cohorts but  
337 BRIGHT (which sampled hypertensive cases) were population based (**Supplementary Table 6**). The  
338 determination of the discovery and replication cohorts was determined upfront based on the timeline of  
339 the availability of the genetic and ERP data.

### 340 *Electrocardiogram analysis and ERP evaluation*

341 12-lead ECGs of all 12 studies were obtained during a study visit in a supine position after  
342 approximately five minutes of rest and were analyzed manually by experienced and specifically trained

343 cardiologists for the presence of ERP. In detail, ECGs from TwinsUK and BRIGHT were evaluated in  
344 the UK (YJ, RB, ERB), ECGs from all other cohorts were evaluated in Germany (TT, BK, CH, WR).  
345 Paper-printed 12-lead ECGs were independently read by two experienced clinicians who were blinded  
346 with respect to age and sex. There was very high level of agreement between each pair of interpreters  
347 (95-98%)(20, 21). Cases of ambiguous or unequal phenotype were jointly reassessed by two readers,  
348 and a consensus decision was achieved. To determine interobserver variability between UK and  
349 German teams, a subset of ECGs was analyzed by both teams yielding a concordance of 96%(20,  
350 21).

351 The ERP phenotype was established according to the definition by Haissaguerre and Macfarlane(6,  
352 23). ERP was defined as elevation of the J-point above the level of QRS onset of  $\geq 0.1$  mV in at least  
353 two corresponding leads. To avoid confusion or overlap with Brugada syndrome or arrhythmogenic  
354 right ventricular dysplasia, leads V1 to V3 were excluded from ERP scoring. In case of presence of  
355 ERP, region, either inferior (leads II, III, aVF), antero-lateral (leads I, aVL, V<sub>4</sub>-V<sub>6</sub>), or both, and the  
356 maximum amplitude of J-point elevation was documented. Further, the morphology of ERP was  
357 assessed as either notching, slurring or both as well as the ST segment according to Tikkanen and  
358 colleagues(44) as either concave/rapidly ascending ( $>0.1$  mV elevation 100 ms after J-point peak or  
359 persistently elevated ST segment  $>0.1$  mV) or horizontal/descending ( $\leq 0.1$  mV elevation within 100 ms  
360 after J-point peak)(23, 44). In case of a QRS duration of  $>120$  ms or rhythm other than sinus rhythm  
361 (e.g. atrial fibrillation, pacemaker stimulation) ECGs were excluded from the analysis.

### 362 *Statistics*

363 Unless stated otherwise, the analyses were conducted and plotted using the R statistical software(46),  
364 a Z-test was applied, and all reported p-values are two-sided.

### 365 *GWAS in individual studies*

366 The GWAS in each study for both the discovery and replication stage was performed on autosomal  
367 imputed SNP genotypes using study-specific quality control protocols which are provided in detail in  
368 **Supplementary Table 6**. Association analyses were performed using logistic regression for ERP  
369 status as outcome and an additive genetic model on SNP dosages, thus taking genotype uncertainties  
370 of imputed SNPs into account. The analyses were adjusted for age, sex, and relevant study-specific  
371 covariates such as principal components for population stratification (**Supplementary Table 6**).

### 372 *Meta-analysis of individual study GWAS results*

373 The result files from individual studies GWAS underwent extensive quality control before meta-  
374 analysis using the `gwasqc()` function of the `GWAToolbox` package v2.2.4(47). The quality control  
375 included file format checks as well as plausibility and distributions of association results including  
376 effect sizes, standard errors, allele frequencies and imputation quality of the SNPs.

377 The meta-analyses were conducted using a fixed-effect inverse variance weighting as implemented in  
378 `Metal`(48). Monomorphic SNPs, SNPs with implausible association results (i.e.  $p \leq 0$ ,  $SE \leq 0$ ,  
379  $|\log(\text{OR})| \geq 1000$ ), and SNPs with an imputation quality score  $\leq 0.4$  were excluded prior to the meta-  
380 analyses resulting in a median of 12,839,202 SNPs per cohort (IQR: 10,756,073-13,184,807). During  
381 the meta-analysis, the study-specific results were corrected by their specific  $\lambda_{GC}$  if  $> 1$ . Results were  
382 checked for possible errors like use of incorrect association model by plotting the association p-values  
383 of the analyses against those from a z-score based meta-analysis for verifying overall concordance.  
384 SNPs that were present in  $< 75\%$  of the total sample size contributing to the respective meta-analysis  
385 or with a MAF  $\leq 0.01$  were excluded from subsequent analyses. Finally, data for up to 6,976,246 SNPs  
386 were available after the meta-analysis.

387 Quantile-quantile plots of the meta-analysis results are provided in **Supplementary Figure 2**. To  
388 assess whether there was an inflation of p-values in the meta-analysis results attributed to reasons  
389 other than polygenicity, we performed LD score regression(49). The LD score corrected  $\lambda_{GC}$  value of  
390 the discovery and replication combined meta-analysis was 1.01, supporting the absence of  
391 unaccounted population stratification. Genome-wide significance was defined as a p-value  $< 5E-8$ ,  
392 corresponding to a Bonferroni correction of one million independent tests(50). The  $I^2$  statistic was used  
393 to evaluate between-study heterogeneity(51).

394 To evaluate the presence of allelic heterogeneity within each locus, the GCTA stepwise model  
395 selection procedure (`cojo-slct` algorithm) was used to identify independent variants employing a step-  
396 wise forward selection approach(52). We used the genotype information of 4,081 SHIP individuals for  
397 LD estimation, and set the significance threshold for independent SNPs to  $5E-8$ .

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

#### 400 *Replication analysis*

401 To minimize the burden for multiple testing correction and thus maximizing the power for replication,  
402 the lead SNPs of genome-wide significant loci in the discovery stage were taken forward to the  
403 replication stage in independent samples (**Table 1**). SNPs were considered as replicated if the p-value

404 of a one-sided association test was  $<0.025$  which corresponds to a Bonferroni correction for the two  
405 lead SNPs tested at 5% significance level.

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

#### 408 *Gene expression based analyses*

409 The lead SNP rs1545300 of the *KCND3* locus of the combined discovery and replication GWAS meta-  
410 analysis was tested for *cis* eQTLs ( $\pm 1$ Mb window around the transcription start site) in 48 tissues  
411 available in the GTEx v7 database that included at least 70 samples. Significant associations were  
412 selected based on a Bonferroni corrected p-value  $<3.0E-5$  for the number of genes and tissues tested.  
413 Subsequently, the SNP rs1545300 was tested and plotted for co-localization in the three tissues with  
414 an eQTL  $FDR < 0.2$  by applying the SMR method(53) using the GWAS and GTEx eQTL summary  
415 statistics. The method includes a test whether the effect on expression observed at a SNP or at its  
416 proxies is independent of the signal observed in the GWAS, i.e. that gene expression and  $y$  are  
417 associated only because of a latent non-genetic confounding variable (SMR test), and a second test  
418 that evaluates if the eQTL and GWAS associations can be attributable to the same causative variant  
419 (HEIDI test). Significance for co-localization of the gene expression and the GWAS signals was  
420 defined by  $p_{SMR} < 0.01$ , where additionally a  $p_{HEIDI} \geq 0.05$  indicates the same underlying causal  
421 variant(53).

#### 422 *Data availability*

423 Summary association results of the combined GWAS meta-analysis have been submitted for full  
424 download to the CHARGE dbGaP website under accession phs000930  
425 [<https://www.ncbi.nlm.nih.gov/gap>].

#### 426 *Study approval*

427 All subjects gave written informed consent and all participating studies were approved by the local  
428 ethics committees and followed the recommendations of the Declaration of Helsinki.

#### 429 **Author Contributions**

430 Project design and analysis: W.R., T.T., A.T., M.D, E.R.B. Management of individual study: A.F.D.,  
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436 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  
437 review of the manuscript: all authors. The authorship order among co-first authors was set  
438 alphabetically.

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441 The authors have declared that no conflict of interest exists.

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563

564 **Figure Legends**

565 **Figure 1. GWAS results of the *KCND3* locus**

566 The results of the combined early repolarization pattern (ERP) GWAS results for the *KCND3* locus are  
567 shown for the replicated discovery stage lead SNP rs12090194 in n=38,811 individuals (A and B), and  
568 for the combined GWAS lead SNP rs1545300 in n=38,806 individuals (C and D). The regional  
569 association plots (A and C) show the association results in a  $\pm 500$  kb region around the lead SNP.  
570 SNPs are plotted on the x-axis according to their chromosomal position with the  $-\log_{10}(\text{p-value})$  of the  
571 GWAS association on the y-axis. Correlation with the lead SNP (purple) is estimated based on the  
572 1000 Genomes reference samples. Plots were generated using the website of LocusZoom(54).  
573 Genetic positions refer to GRCh37/hg19 coordinates. Forest plots of the respective lead SNPs are  
574 provided in (B) and (D), with odds ratios and their 95% confidence intervals plotted on the x-axis.  $I^2$  is  
575 the percentage of total variation across studies that is due to heterogeneity.

576 **Figure 2. Location of the significantly associated SNPs within the *KCND3* gene**

577 The top 43 SNPs with a genome-wide significance visualized by UCSC Genome Browser(55). All  
578 SNPs mapped into *KCND3* gene. The two leads SNPs rs1545300 and rs12090194 of the discovery  
579 and combined meta-analyses are reported with a red and an orange diamond, respectively. The  
580 H3K27Ac mark track (Layered H3K27Ac) shows the levels of enrichment of the H3K27Ac histone  
581 mark. Chemical modifications (e.g. methylation and acylation) to the histone proteins present in  
582 chromatin influence gene expression by changing how accessible the chromatin is to transcription.  
583 The H3K27Ac histone mark is thought to enhance transcription possibly by blocking the spread of the  
584 repressive histone mark H3K27Me3. The GeneHancer (GH) track set shows human regulatory  
585 elements, i.e. enhancers (gray) and promoters (red), containing tracks representing regulatory  
586 elements (Reg Elems), gene transcription start sites (TSS), associations between regulatory elements  
587 and genes (Interactions), and clustered interactions (Clusters). A gray box in the DNaseI  
588 Hypersensitivity Clusters track (DNase Clusters) indicates the extent of the hypersensitive region with  
589 darkness proportional to the maximum signal strength observed in any cell line. A gray box in the  
590 Transcription Factor ChIP-seq Clusters track (Txn Factor ChIP) indicates a cluster of transcription  
591 factor occupancy, with the darkness of the box being proportional to the maximum signal strength  
592 observed in any cell line contributing to the cluster.

593 **Figure 3. Co-localization results**

594 Illustration of the SMR test for the early repolarization pattern (ERP) risk and the expression  
595 quantitative trait loci (eQTLs) at the rs1545300 locus at chromosome 1p13.2 for (A) left ventricle of the  
596 heart, (B) tibial artery, and (C) minor salivary gland tissue. The sample size for the eQTLs are n=272,  
597 n=388 and n=85 in panels (A), (B) and (C), respectively. In each panel, the upper box shows the  
598 GWAS regional association plot with ERP risk of the combined GWAS (n=39,456), with level of  
599 significance of the SMR test (y-axis) for each transcript in the locus indicated by a diamond positioned  
600 at the center of the transcript. A significant SMR test represented by a purple diamond indicates an  
601 association of the transcript level of the respective genes (purple label) with the trait. For all three  
602 tissues, an increased gene expression level of a significant SMR test was associated with a higher risk  
603 of ERP. A filled purple diamond indicates a HEIDI test p-value >0.05, thus a likely co-localization. The  
604 lower box shows the regional association distribution with changes in expression of the highlighted  
605 (purple) gene transcript in the respective tissue. In both boxes, the x-axis refers to GRCh37/hg19  
606 genomic coordinates.

607

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

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

609  
610 ERP+: cases with early repolarization pattern; ERP-: controls

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

| SNP         | Chr:position  | A1/ A2 | Nearest gene | Discovery |             |                |                | Replication |             |         |                | Combined |             |                |                |       |
|-------------|---------------|--------|--------------|-----------|-------------|----------------|----------------|-------------|-------------|---------|----------------|----------|-------------|----------------|----------------|-------|
|             |               |        |              | 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] |                |                |       |

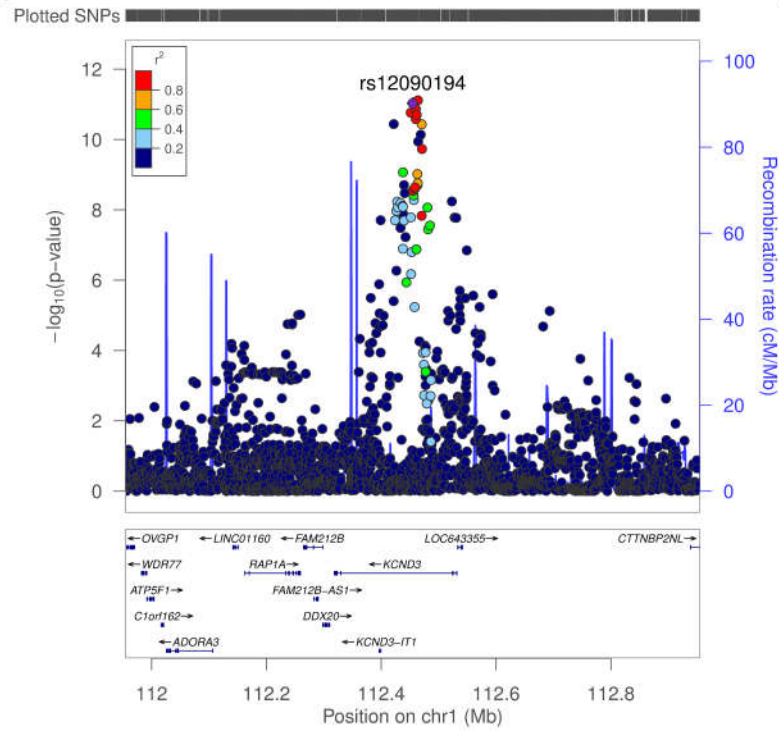
612  
613 A1: effect allele; AF1: allele frequency of A1; Chr: chromosome; position: position corresponding to  
614 build 37 (GRCh37); OR: odds ratio of A1 [95% confidence interval]; P: association p-value; I<sup>2</sup>:  
615 percentage of total variation across studies that is due to heterogeneity; N: sample size. Bold values  
616 indicate the lead SNP (lowest p-value) of a significantly associated locus in the corresponding meta-  
617 analysis stage.  
618

**Table 3: The 43 genome-wide significant SNPs of the *KCND3* locus**

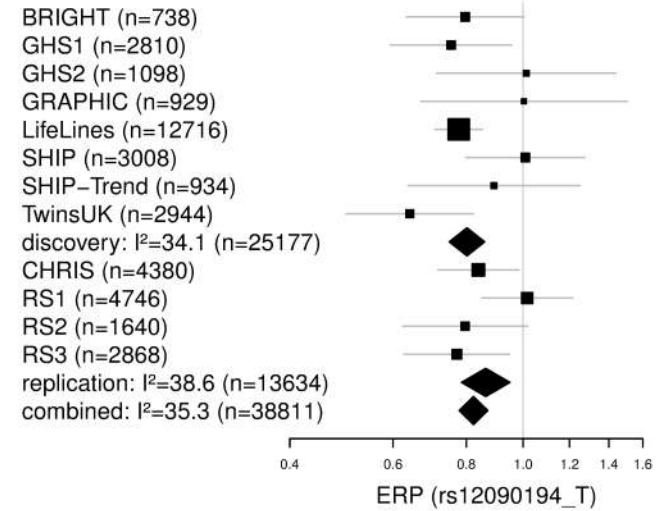
| SNP        | position    | location | A1/A2 | AF1  | OR               | P       |
|------------|-------------|----------|-------|------|------------------|---------|
| rs817972   | 112,399,057 | intron   | a/g   | 0.90 | 1.35 [1.22-1.50] | 2.0E-08 |
| rs583731   | 112,421,854 | intron   | t/c   | 0.09 | 0.69 [0.62-0.77] | 3.6E-11 |
| rs528779   | 112,424,077 | intron   | t/c   | 0.71 | 1.18 [1.11-1.25] | 2.0E-08 |
| rs612790   | 112,426,577 | intron   | c/g   | 0.29 | 0.84 [0.80-0.89] | 1.1E-08 |
| rs2813862  | 112,427,918 | intron   | c/g   | 0.29 | 0.84 [0.79-0.89] | 5.9E-09 |
| rs1767283  | 112,428,953 | intron   | t/c   | 0.29 | 0.84 [0.80-0.89] | 8.5E-09 |
| rs11102354 | 112,433,666 | intron   | a/g   | 0.31 | 1.18 [1.11-1.25] | 3.3E-08 |
| rs605604   | 112,433,863 | intron   | a/c   | 0.70 | 1.19 [1.12-1.26] | 6.4E-09 |
| rs2798334  | 112,437,344 | intron   | t/c   | 0.30 | 0.84 [0.79-0.89] | 7.7E-09 |
| rs2813864  | 112,437,853 | intron   | a/g   | 0.29 | 0.84 [0.79-0.89] | 8.2E-09 |
| rs2587368  | 112,437,907 | intron   | t/c   | 0.40 | 0.86 [0.81-0.90] | 1.7E-08 |
| rs2813865  | 112,437,956 | intron   | a/g   | 0.25 | 0.82 [0.77-0.87] | 8.6E-10 |
| rs894849   | 112,437,964 | intron   | t/c   | 0.41 | 0.86 [0.81-0.90] | 1.3E-08 |
| rs608673   | 112,439,757 | intron   | t/c   | 0.44 | 0.86 [0.81-0.91] | 2.0E-08 |
| rs7539683  | 112,439,770 | intron   | t/g   | 0.27 | 1.20 [1.13-1.27] | 2.0E-09 |
| rs1805222  | 112,440,983 | intron   | a/g   | 0.27 | 1.20 [1.13-1.27] | 3.3E-09 |
| rs2120436  | 112,451,447 | intron   | t/c   | 0.32 | 0.82 [0.78-0.87] | 1.7E-11 |
| rs2034124  | 112,451,681 | intron   | a/g   | 0.45 | 1.16 [1.10-1.22] | 1.6E-08 |
| rs12090194 | 112,454,822 | intron   | t/c   | 0.32 | 0.82 [0.78-0.87] | 9.3E-12 |
| rs72692596 | 112,455,415 | intron   | t/c   | 0.32 | 0.84 [0.79-0.89] | 2.8E-09 |
| rs72692597 | 112,455,442 | intron   | t/g   | 0.32 | 0.84 [0.79-0.89] | 2.9E-09 |
| rs4839182  | 112,456,538 | intron   | a/g   | 0.55 | 1.18 [1.12-1.25] | 3.9E-09 |
| rs4839183  | 112,456,882 | intron   | a/g   | 0.46 | 1.17 [1.11-1.23] | 5.3E-09 |
| rs72692602 | 112,458,833 | intron   | t/c   | 0.68 | 1.20 [1.13-1.27] | 2.5E-09 |
| rs72694603 | 112,458,893 | intron   | t/c   | 0.32 | 0.83 [0.79-0.89] | 2.3E-09 |
| rs4839184  | 112,460,221 | intron   | c/g   | 0.32 | 0.83 [0.78-0.87] | 2.6E-11 |
| rs4839185  | 112,460,262 | intron   | t/c   | 0.68 | 1.21 [1.15-1.28] | 1.4E-11 |
| rs1443926  | 112,461,902 | intron   | a/g   | 0.68 | 1.21 [1.15-1.28] | 2.0E-11 |
| rs6682872  | 112,462,984 | intron   | a/g   | 0.60 | 1.18 [1.12-1.24] | 9.6E-10 |
| rs4838926  | 112,463,323 | intron   | c/g   | 0.40 | 0.85 [0.81-0.90] | 2.0E-09 |
| rs4838927  | 112,463,617 | intron   | t/c   | 0.60 | 1.18 [1.12-1.24] | 1.7E-09 |
| rs1545300  | 112,464,004 | intron   | t/c   | 0.32 | 0.82 [0.78-0.87] | 7.7E-12 |
| rs17029069 | 112,464,376 | intron   | t/c   | 0.30 | 1.21 [1.14-1.28] | 1.1E-10 |
| rs12119724 | 112,468,814 | intron   | t/c   | 0.29 | 1.21 [1.14-1.28] | 7.3E-11 |
| rs11588747 | 112,470,474 | intron   | t/c   | 0.70 | 1.19 [1.12-1.27] | 1.5E-08 |
| rs2010749  | 112,470,581 | intron   | t/c   | 0.63 | 1.20 [1.14-1.27] | 3.7E-11 |
| rs1443927  | 112,471,029 | intron   | c/g   | 0.69 | 1.20 [1.14-1.28] | 1.9E-10 |
| rs12145374 | 112,480,536 | intron   | a/c   | 0.80 | 1.22 [1.14-1.30] | 8.6E-09 |
| rs72694622 | 112,481,667 | intron   | t/c   | 0.18 | 0.81 [0.75-0.87] | 3.6E-08 |
| rs12144965 | 112,484,962 | intron   | t/c   | 0.82 | 1.23 [1.14-1.32] | 2.8E-08 |
| rs3008527  | 112,523,095 | intron   | t/c   | 0.70 | 1.19 [1.12-1.26] | 5.8E-09 |
| rs3008528  | 112,527,869 | intron   | a/t   | 0.70 | 1.18 [1.11-1.25] | 1.7E-08 |
| rs2075811  | 112,530,626 | intron   | c/g   | 0.30 | 0.85 [0.80-0.90] | 1.7E-08 |

621 A1: effect allele; AF1: allele frequency of A1; position on chromosome 1 (build 37, GRCh37); OR:  
622 odds ratio of A1 [95% confidence interval]; P: association p-value. The SNPs are ordered by their  
623 position, and the results of the combined meta-analysis are given.

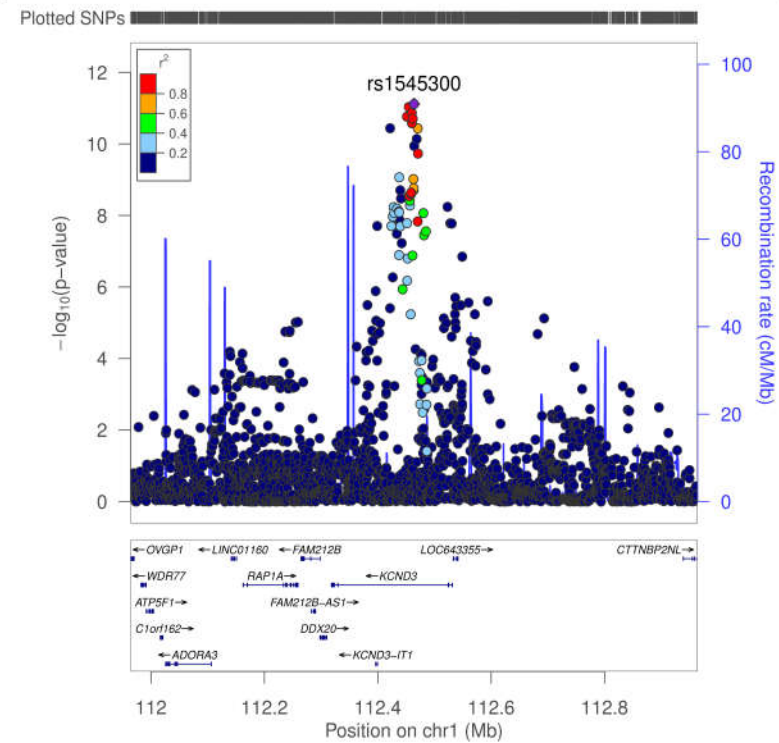
A



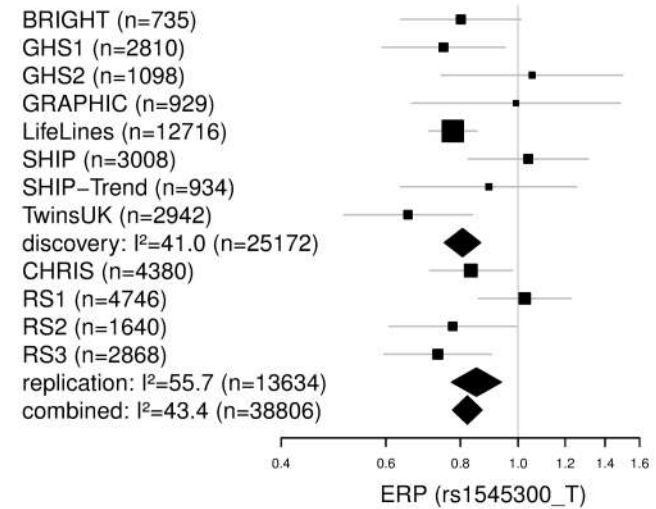
B

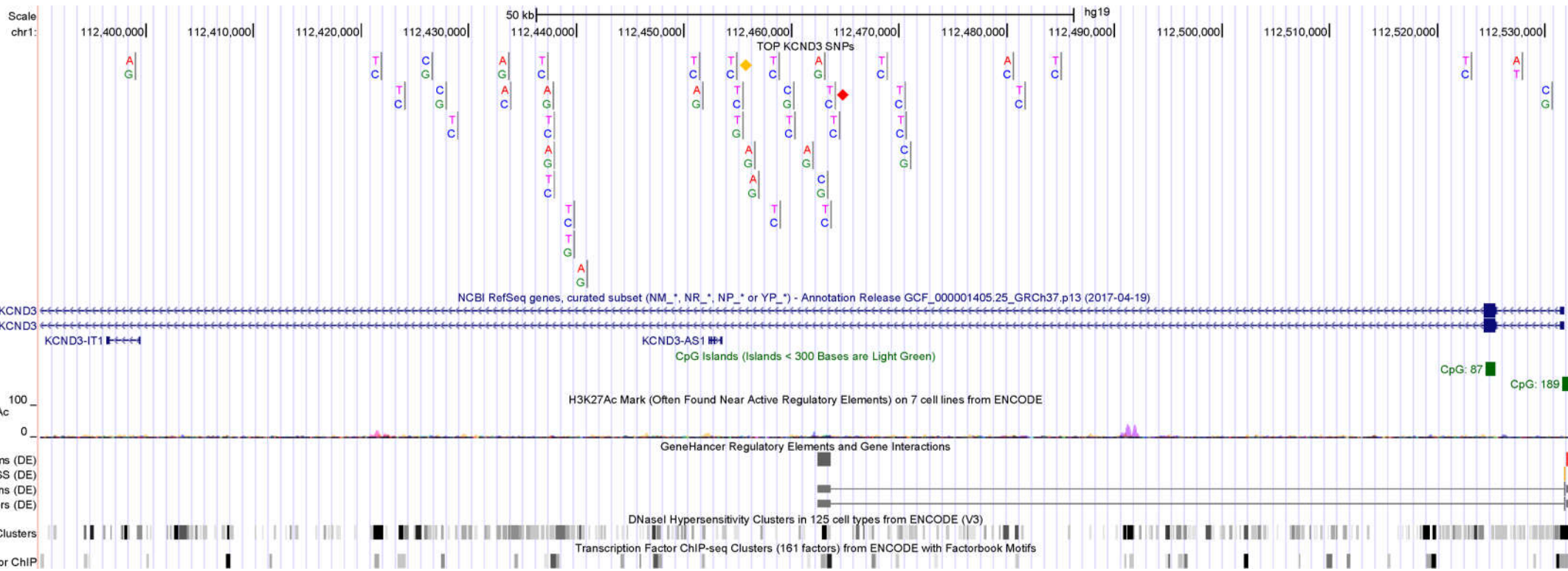


C



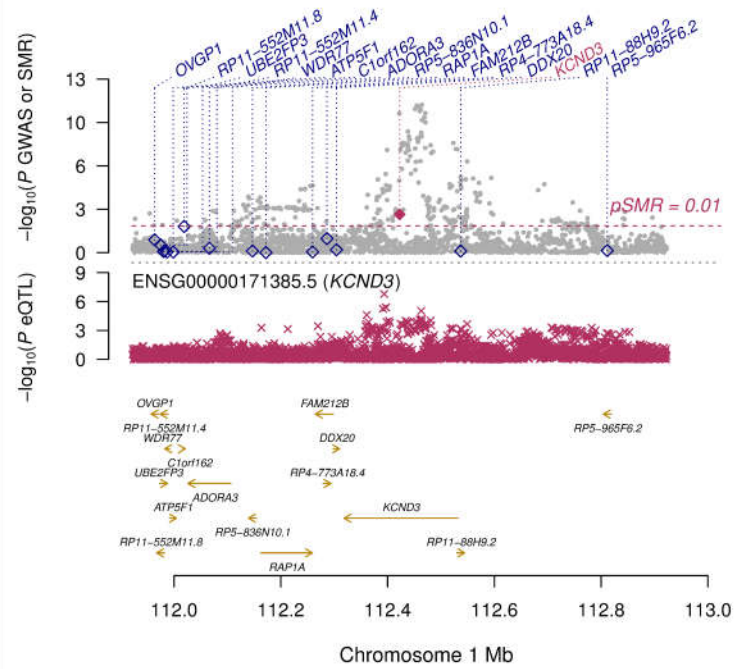
D



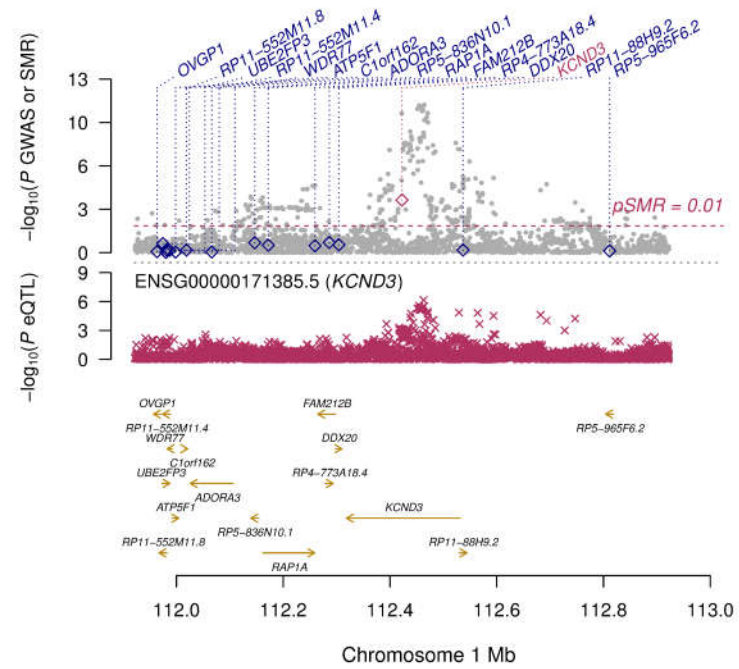




A



B



C

