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A Genome-Wide Study of Panic Disorder Suggests the Amiloride-Sensitive Cation Channel 1 as a Candidate Gene

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Abstract:

Panic disorder (PD) is a mental disorder with recurrent panic attacks that occur spontaneously and are not associated to any particular object or situation. There is no consensus on what causes PD. However, it is recognized that PD is influenced by environmental factors as well as genetic factors. Despite a significant hereditary component, genetic studies have only been modestly successful in identifying genes of importance for the development of PD. In this study, we conducted a genome-wide scan using microsatellite markers and PD patients and control individuals from the isolated population of the Faroe Islands. Subsequently, we conducted a fine-mapping, which revealed the amiloride-sensitive cation channel 1 (*ACCN1*) located on chromosome 17q11.2-q12 as a potential candidate gene for PD. The further analyses of the *ACCN1* gene using single-nucleotide polymorphisms (SNPs) revealed significant association with PD in an extended Faroese case-control sample. However, analyses of a larger independent Danish case-control sample yielded no substantial significant association. This suggests that the possible risk alleles associated in the isolated population are not those involved in the development of PD in a larger outbred population.

Keywords:

Panic disorder, genome-wide scan, isolated population, association analysis, *ACCN1*

Introduction:

Panic disorder (PD) is a common mental disorder in society[1,2]. It is characterized by recurrent, unprovoked and unpredictable panic attacks, followed by concern of subsequent attacks, resulting in a strong social and functional inhibition[3,4]. Estimates from family and twin studies ascribe a genetic contribution of approximately 40% to the disease etiology of the disorder[5-8]. However, the mechanism underlying PD is still unknown and presumably involves numerous susceptibility genes with major and/or minor effects[9,10]. Furthermore, the possibility of allelic heterogeneity, which most likely will reduce the power of performed studies to detect associated genes, exists. In this context, isolated populations are considered advantageous, as they possess highly beneficial features for diminishing genetic and allelic heterogeneity[11-13]. In the present study, we use the isolated population of the Faroe Islands to search for susceptibility genes for PD. The population history of this isolate in context of genetics has previously been described[14,15]. The isolate has formerly been used to locate chromosomal regions associated with other complex disorders[16,17], which has lead to identification of genes for schizophrenia and bipolar disorder in larger outbred populations[18-20].

We present the results from a three stage genetic investigation of PD (Figure 1). Firstly, we conducted a genome-wide scan on 13 distantly related patients with PD and 43 control individuals from the Faroe Islands. Secondly, we performed a fine-mapping of the chromosome regions observed to be significant associated with PD, using the same sample as in the genome-wide scan. In this paper we only report the results from the chromosome 17q11.2-q12 region. Lastly the amiloride-sensitive cation channel 1 (ACCN1) gene was analysed for association with PD in an extended Faroese case-control sample and in an outbred Danish case-control sample.

Materials and methods:

Subjects and Clinical Assessment

The Faroese sample: Thirteen patients with PD and 43 control individuals were included in the genome-wide scan. Recently, additional 18 patients and 119 control individuals were recruited to the Genetic Biobank of the Faroe Islands. All patients were interviewed using the Present State Examination (PSE)[21]. The inclusion criteria were PD with or without agoraphobia according to the ICD-10 diagnostic criteria[3]. The control individuals were evaluated to be healthy and were matched

82 to the cases by ethnicity. A detailed description of the sample has been described in Wang *et al.*[22].
83 The extended Faroese sample consisted of 31 PD patients and 162 healthy control individuals.
84
85 *The Danish sample:* The Danish sample consisted of 243 patients with PD and 645 healthy control
86 individuals. The sample was collected from two Danish cohorts, one in the area of Copenhagen[23]
87 and the other in Jutland[24]. The patients were diagnosed with PD with or without agoraphobia
88 according to the ICD-10 diagnostic criteria[3]. All the patients and controls were of Danish Caucasian
89 origin.
90

91 *Genotyping*

92 *Stage 1: The genome-wide scan*

93 Thirteen patients with PD and 43 control individuals from the Faroe Islands were genotyped using
94 approximately 500 microsatellite markers with an average inter-marker distance of 5 cM (range 0-14
95 cM). Primer sequences were obtained from the Human Genome Database (GDB) (primer sequences
96 are available on request). DNA fragments were amplified using standard PCR conditions in single or
97 multiplex reactions in a concentration of 6 ng/μL DNA. The PCR fragments were analysed on the
98 ABI 377 genetic analyser (Applied Biosystems, Foster City, CA, USA) and the alleles were
99 analysed using the Genemapper software version 3.7 (Applied Biosystems, Foster City, CA, USA).
100 Several chromosomal regions (4p16.1, 17q11.2-q12 and 19p13.2) showed significant association in
101 the genome-wide scan. These regions were submitted to further analyses in order to verify the
102 observation and to further delineate the IBD status of the regions.

103 *Stage 2: The 17q11.2-q12 region*

104 Given the results we have in the present study chosen to focus on the results from chromosome 17.
105 In order to verify the finding and to further delineate the identical by descended (IBD) status of the
106 17q11.2-q12 region, the marker density was increased from 20 to 42 microsatellite markers (29 in
107 the 17q11.2-q12 region), including the 5-HTTLPR repeat in the promoter region of the serotonin
108 transporter (*SLC6A4*) gene. The study sample and analytic procedures were the same as in the
109 genome-wide scan. Subsequently, the promoter region of the *ACCN1* gene was sequenced in 14
110 individuals using the ABI Big Dye Terminator 3.1 kit (Applied Biosystems, Foster City, CA, USA).
111 The sequencing revealed seven single nucleotide polymorphisms (SNPs) (rs28936, rs28935,

112 rs28933, rs62068265, rs9916605, rs7214382 and rs2228990), which were genotyped in the complete
113 sample from the genome-wide scan, using the SNaPshot protocol from Applied Biosystems and
114 analysed on an ABI 377 genetic analyser (Applied Biosystems, Foster City, CA, USA).
115

116 *Stage 3: ACCN1*

117 A bioinformatic search of the chromosome segment comprising the 17q11.2-q12 region suggested
118 the *ACCN1* gene as the most interesting gene, since acid-sensing ion channels (ASICs) may be
119 involved in anxiety related pathways [25-27]. The *ACCN1* gene comprises a genomic region of
120 approximately 1.1Mb and contains 10 coding exons. According to HapMap
121 (http://hapmap.ncbi.nlm.nih.gov/) approximately 500 tag-SNPs are required to capture the genetic
122 variation of this gene. However, we selected 55 tag-SNPs, covering exons, exon-intron boundaries,
123 and 3´and 5´ flanking regions, on the basis of the publicly available genotype data from the Centre
124 d'Etude du Polymorphisme Humain (CEPH) trios (http://www.cephb.fr/en/cephdb/), available in the
125 HapMap project dataset (phase II data freeze, assembly NCBI b36, dbSNP b126). The tagging
126 procedure was performed in Haploview, version 3.32[28]. Two SNPs (rs28936 and rs62068265) were
127 specifically selected to be included due to our positive findings in stage 2, while rs28935 was
128 tagged by rs8066566 ($D'= 1$, $r^2 = 1$). The SNPs were genotyped in the extended Faroese sample,
129 and additionally, in the Danish sample. The genotyping was performed using the Sequenom
130 platform as described by Nyegaard *et al.*[20]. One of the specifically selected SNPs (rs28936) failed to
131 be genotyped. Rs28936 is in very high linkage disequilibrium (LD) with rs28933 ($D'= 1$, $r^2 = 0.91$),
132 which subsequently was analysed using allele specific hybridization in the LightCycler® 480
133 System. Primers for rs28933 were designed and obtained from Tib molbiol (http://www.tib-
134 molbiol.de/de/) (primer sequences are available on request). Quality assurance was achieved by the
135 inclusion of two CEPH controls, which were genotyped for all the SNPs in this study, and showed a
136 100% concordance rate with the HapMap genotypes (http://hapmap.ncbi.nlm.nih.gov/).
137 Furthermore, all genotypes were doubled checked by two researchers.
138

139 *Statistical analyses:*

140

141 *Quality control:*

142 All markers were tested for deviation from Hardy-Weinberg Equilibrium (HWE) using Exact HWE
143 as implemented in PLINK[29] and discarded if the p-value was below 0.0001. Additionally, markers
144 with a call rate below 80% or a minor allele frequency (MAF) below 0.005 were excluded from
145 further analyses. Furthermore, individuals with missing genotype rate above 10% were excluded
146 from the analyses.
147
148 *Stage 1 and 2:*
149 Genotypes from the genome-wide scan and the fine-mapping of the 17q11.2-q12 region were tested
150 for association using Monte Carlo based tests as implemented in CLUMP[30]. The CLUMP statistics
151 presented in this paper are from the subtests T1 and T4. The T1 value is the standard Pearson Chi-
152 square test on the raw 2 x N contingency table, and the T4 value is obtained by reshuffling the
153 columns of the raw 2 x N table into new 2 x 2 tables, until the Chi-square value has reached a
154 maximum. Significance level of 0.05 was chosen, however to compensate for the lack of correction
155 for multiple testing and to ensure that the applied tests are not too conservative thresholds of 0.005
156 for single-locus analysis and 0.01 for two-locus analysis were applied. These different thresholds
157 were chosen mostly based on the different number of alleles/haplotypes analysed in the single-locus
158 and two-locus analyses and to prevent that tests are too conservative. Alleles and haplotypes of
159 markers with p-values below the threshold were, furthermore, tested with Fisher's exact test to
160 detect if specific alleles or haplotypes preferentially displayed a significant skewed distribution.
161 IBD_0 was calculated by use of the formula given by Houwen *et al.*[31]. IBD_0 indicates the probability
162 that the given haplotype/segment is inherited by chance with its observed frequency among the
163 cases, which are related to a known ancestor through a specific genealogical relationship - the 13
164 cases from the genome-wide scan share a known ancestor living 6.5 generations ago[22].
165
166 *Population structure analyses*
167 Analyses of the level of relatedness and inbreeding in the Faroese sample from stage 1 and 2 were
168 based on genome-wide multi locus data from 78 unlinked markers from the genome-wide scan.
169 Genetic differentiation among cases and controls were evaluated by Wright's F-statistic as
170 implemented in SPAGEDi 1.2[32], and inter and intra individual correlations were estimated to
171 evaluate any further genetic subclustering. Furthermore, Bayesian model-based clustering as
172 implemented in STRUCTURE[33], was applied to infer any potential cryptic substructure. Models

173 with and without admixture were applied without using any prior information on population
174 structure (i.e. disease status).
175
176 *Stage 3: ACCN1*
177 Allelic association for SNPs located within *ACCN1* was tested using the Cochran-Armitage trend
178 test for the Faroese and Danish samples. The Cochran-Mantel-Haenszel (CMH) test was used for
179 the combined Danish and Faroese sample in the open-source software PLINK[29]. Haplotype
180 association was performed using a sliding window approach of two- and three-marker haplotypes.
181 We report the nominal significant associations, i.e. p-values below 0.05. However, none of these
182 SNPs would withstand Bonferroni correcting for multiple testing, which would require a p-value
183 below 0.001. Association at the level of the whole gene or parts of the gene was assessed using the
184 program COMBASSOC performed with 9999 permutations[34]. COMBASSOC provides a single
185 measurement of significance by combining the p-values from all the SNP analyses and
186 subsequently by permutation testing assessing the empirical significance of the combined p-value.
187
188 *Imputation*
189 To infer missing genotypes and increase genomic coverage, SNP genotypes within the chromosome
190 17:28,363,219-29,509,938 region around *ACCN1* were imputed using MaCH 1.0[35] and the 1000
191 Genomes maps (Aug 2009) as reference haplotypes (CEPH population). Prior to the imputation
192 analysis, five A/T or G/C SNPs and seven SNPs, which were not genotyped in the 1000 Genome
193 project, were excluded. Thus, the imputation analysis was performed using 38 SNPs. Imputed
194 markers with a squared correlation (rsq) between imputed and true genotypes above 0.3 and a
195 quality score above 0.9 were accepted for further analyses. The imputed markers were subsequently
196 analysed for association (trend test and CMH test) using PLINK[29].
197

198 **Results:**

199 *Stage 1: The genome-wide scan*
200 In the genome-wide scan, 460 microsatellite markers were successfully genotyped. Several
201 chromosomal regions (4p16.1, 17q11.2-q12 and 19p13.2) showed significant association with PD
202 and increased haplotype sharing among the 13 Faroese patients. The present paper only reports the

203 association between PD and markers on chromosome 17. Using a threshold of 0.01 we detected a
204 significant association between PD and a two-marker segment (D17S1294-D17S1293) located in the
205 q11.2-q12 region on chromosome 17 (T1: p-value = 0.002; T4: p-value = 0.003).
206

207 *Analyses of genetic relatedness and population structure*

208 In summary, Wright's F statistics revealed no signs of genetic differentiation between the 13 cases
209 and 43 controls in the initial Faroese sample. The cluster analysis found it more likely that the data
210 belonged to a single cluster than to multiple clusters. Average within and between group kinship
211 coefficients did not differ significantly, indicating that the cases are not closer related to each other
212 than they are to the controls. In conclusion, we observed no significant stratification or cryptic
213 relatedness, and the samples might thus be considered sub-samples with the same genetic
214 background.

215 *Stage 2: The 17q11.2-q12 region*

216 Twenty-two microsatellites and seven SNPs within 17q11.2-q12 were successfully genotyped. The
217 CLUMP analyses showed a significant haplotypic association between the two-locus segment
218 D17S1293-D17S1842 and PD, using a threshold of 0.01 (T1: p-value = 0.007; T4: p-value = 0.007).
219 Fisher's exact test confirmed an overrepresentation of one particular haplotype (p-value = 0.003)
220 (Table 1). No significant association was observed between the 5-HTTLPR repeat within the
221 promoter region of the *SLC6A4* gene and PD. Single marker analysis of SNPs within *ACCN1*
222 revealed significantly association between PD and three SNPs using a threshold of 0.005: p-values
223 ranging from 0.001 to 0.003 (Table 1). The same three SNPs and an adjacent distal microsatellite
224 marker D17S1540 displayed, in addition, significant association in the two-marker analyses
225 performed using CLUMP and using a threshold of 0.01: p-values ranging from 0.003 to 0.0002
226 (Table 1). It appears very unlikely that the observed segments in the case group are inherited IBD by
227 chance through the known genealogy (Table 1).

228 *Stage 3: ACCN1*

229 A total of 50 SNPs were successfully genotyped with an average call rate of 0.98 (one SNP was
230 monomorphic and five SNPs failed to be genotyped). The statistical analyses in stage 3 were
231 performed separately for the three samples: the Faroese case-control samples, the Danish case-
232 control sample, and the combined sample of Danish and Faroese cases versus Danish and Faroese

233 controls. None of the SNPs showed significant deviation from HWE in the three samples of controls.
234 One SNP (rs11868226) was excluded due to frequency test (MAF) in the Faroese sample, whereas
235 none were excluded in the Danish and combined Faroese and Danish samples. Nineteen of 193
236 individuals (2 cases and 17 controls) in the Faroese sample, and 18 of 888 individuals (3 cases and
237 15 controls) in the Danish sample were removed due to low genotyping. No significant association
238 at the level of the whole gene was detected in any of the three samples, neither when including all
239 ten exons, nor when dividing the gene into smaller parts.
240
241 *The Faroese sample:* Six SNPs showed nominal significant allelic association with PD in the trend
242 test: p-values ranging from 0.016 to 0.044 (Table 2). Furthermore, the haplotype analysis showed
243 nominal significant association between PD and several haplotypes: p-values ranging from 0.009 to
244 0.047 for the two-marker haplotypes, and 0.0064 to 0.041 for the three-marker haplotypes (data not
245 shown). Several of the SNPs included in the significantly associated haplotypes were furthermore
246 allelic associated.
247
248 *The Danish samples:* The association analysis showed one SNP nominally significantly allelic
249 associated with PD (rs9915774; p-value = 0.031) (Table 2). The haplotype analyses revealed several
250 two- and three-marker haplotypes nominal significantly associated with PD: p-values ranging from
251 0.003 to 0.046 (data not shown). Rs9915774 was furthermore included in the significantly
252 associated two- and three-marker haplotypes.
253
254 *The combined Faroese and Danish sample:* Nominal significant allelic association was observed
255 between PD and rs9915774 (p-value = 0.007) (Table 2). The haplotype analyses revealed several
256 two- and three-marker haplotypes nominal significantly associated with PD: p-values ranging from
257 0.006 to 0.042 (data not shown). Rs9915774 was one of the SNPs in the significantly associated
258 haplotypes.
259

260 *Imputation*

261 In the 1.15Mb region around *ACCN1,* 3786 SNPs were imputed using 38 SNPs, and 415 of these
262 had an rsq above 0.3 and a quality score above 0.9. The genotype distribution for the imputed SNPs
263 did not deviate significantly from HWE. In the Faroese sample, the trend test revealed nominal
264 significant allelic association between PD and 69 of the imputed SNPs, including three SNPs

265 genotyped in this study: p-values ranging from 0.002 to 0.049 (data not shown). Furthermore, in the
266 Danish sample, the trend test showed nominal allelic association between PD and 39 of the imputed
267 SNPs, including one of which was genotyped in this study: p-values ranging from 0.004 to 0.038
268 (data not shown). No overlap of nominal significantly associated markers was observed between the
269 Faroese and Danish samples. In the combined Faroese and Danish sample, the CMH test showed
270 nominal significant allelic association between PD and 39 of the imputed SNPs including one of the
271 SNPs genotyped in this study: p-values ranging from 0.005 to 0.045 (data not shown).
272
273 **Discussion:**
274 In the search for susceptibility genes for PD, we conducted a genome-wide scan (stage 1) and a
275 subsequent fine-scaled follow-up study (stage 2) on PD patients and control individuals from the
276 Faroe Islands. The results revealed significant allelic association and increased haplotype sharing on
277 chromosome 17q11.2-q12, and a possible implication of the *ACCN1* gene located within this region.
278 In stage 3, we analysed *ACCN1* for association with PD in an extended Faroese case-control sample,
279 using tag-SNPs. Several SNPs within this gene were nominal associated with PD in this extended
280 sample. With the intention of replicating the findings in a larger outbred population, we analysed
281 *ACCN1* for association with PD in a Danish sample. The results revealed one significantly
282 associated SNP. The subsequent imputation analyses added no substantial significant association
283 between *ACCN1* and PD in any of the samples.
284
285 An important issue in mapping susceptibility genes for common complex disorders is whether the
286 genetic factors are likely to be common or rare in a population. Using an isolated population
287 provides increased power to our study to detect rare variants, which increasingly are being
288 identified for common complex disorders[36,37]. Isolated populations might pose an advantage over
289 outbreed populations in detecting rare variants[38], as only a low number of each risk alleles is likely
290 to be introduced into an isolated founder population. In this way, heterogeneity will be reduced, and
291 the LD surrounding the risk variant will be confined to the population history of the isolate[12].
292 Therefore, rare risk variants identified in isolated populations might not necessarily explain the
293 susceptibility of a disorder or be useful as diagnostic markers in outbred populations. However,
294 they could provide important clues about the mechanism underlying a disorder and thereby
295 contribute to the understanding of the etiology of a disorder like PD. In contrast, isolated
296 populations might not be beneficial in mapping common disease variants of low effect size.

297 Common alleles would not necessarily be enriched in the isolated population, since multiple
298 founders most likely have introduced the same risk allele into the founder population[39,40].
299
300 The results of this study should be interpreted in the context of several potential limitations. Firstly,
301 we did not correct for multiple testing in stage 1 and 2 using standardised methods. The Bonferroni
302 correction, which assumes independence between the individual tests, was considered too
303 conservative. Since many of the markers are in close proximity they are likely in LD[41] and the
304 association tests performed are hence not independent. However, in order to compensate for multiple
305 testing and reduce the type I error rate, we applied relatively low thresholds in stage 1 and 2.
306 Furthermore the combined approach with association analysis and IBD estimates should reduce the
307 number of stochastic single point observations. In stage 3, no correction for multiple testing was
308 performed, and therefore the nominal associated observations might represent false positives.
309 Secondly, even though we used an isolated population, which may justify the small sample size in
310 the Faroese sample, we cannot ignore that this may affect the p-values and the power to detect true
311 difference in allele frequencies between cases and controls. Thirdly, most of the association was
312 confined to the Faroese sample, which might suggest that possible risk alleles genotyped in the
313 present study are not necessarily those involved in the development of PD in a larger outbred
314 population. However, it is possible that low number of founders, isolation and genetic drift followed
315 by rapid exponential population growth has rendered the Faroese population homogenous enough to
316 be able to detect possible risk alleles not detectable in the larger outbred population. To confirm or
317 reject the trend for association observed between markers located within *ACCN1* and PD, it might be
318 of interest to analyse Norwegian and Scottish/Irish case-control samples, since these populations
319 most likely contributed much more to the founding of the Faroese population than the Danish[14,15].
320 Fourthly, we did not consider the possible population stratification in the extended Faroese sample
321 and the Danish sample. However, we detected no significant stratification between cases and
322 controls in the initial Faroese sample, which might apply to the extended Faroese sample as well,
323 considering the assumed reduced genetic heterogeneity in isolated populations[41]. But, we should not
324 ignore that even apparently homogeneous and isolated populations may have levels of population
325 stratification[42]. Fifthly, we excluded the large intron comprising 1Mb of the gene, and have therefore
326 not described all the genetic variation within *ACCN1*. Using the 50 tag-SNPs successfully genotyped
327 in this study we were able to describe the genetic variation of 120 SNPs. We find this strategy
328 sensible since 500 tag-SNPs would be required to capture all the genetic variation. The 17q11.2-q12

329 region, comprises a deletion, which recently has been associated with autism spectrum disorder and
330 schizophrenia[43], contains other interesting candidate genes. One of which is myosin 1D (*MYO1D*)
331 previously associated with major autism[44]. Furthermore, two transmembrane proteins -
332 transmembrane protein 98 (*TMEM98*), and transmembrane protein 132E (*TMEM132E*) (see figure
333 1) - are located in close proximity to *ACCN1*. Recent studies have shown a possible role of
334 transmembrane gene 132D (*TMEM132D*) in the etiology of PD[45,46]. It might therefore be relevant to
335 analyse these genes in future studies of PD.
336
337 In summary, we observed nominal association between PD and SNPs within the *ACCN1* gene, yet it
338 is unlikely from our data that *ACCN1* plays a major role in the general genetic susceptibility of PD.
339 We can therefore not confirm the involvement of ASICs in triggering panic attacks. This is
340 consistent with the inconclusive results from association analysis between anxiety spectrum
341 disorders and *ACCN2*[47]. It is still unknown whether there are susceptibility genes with major effects
342 in the etiology of PD, therefore *ACCN1* might be one of numerous susceptibility genes for PD each
343 contributing a moderate effect. Furthermore, most of the association was confined to the Faroese
344 sample, which might be due to the different population history of the study populations. Thus it
345 might be of interest to analyse *ACCN1* in Norwegian and Scottish/Irish case-control samples.
346
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352
353 **Conflict of Interest Statement:** Authors AGW, HAD, OM, TAK declare a potential financial
354 interest in a patent obtained by the Genetic Biobank of the Faroe Islands (Registration number in
355 Denmark: 2137539).
356
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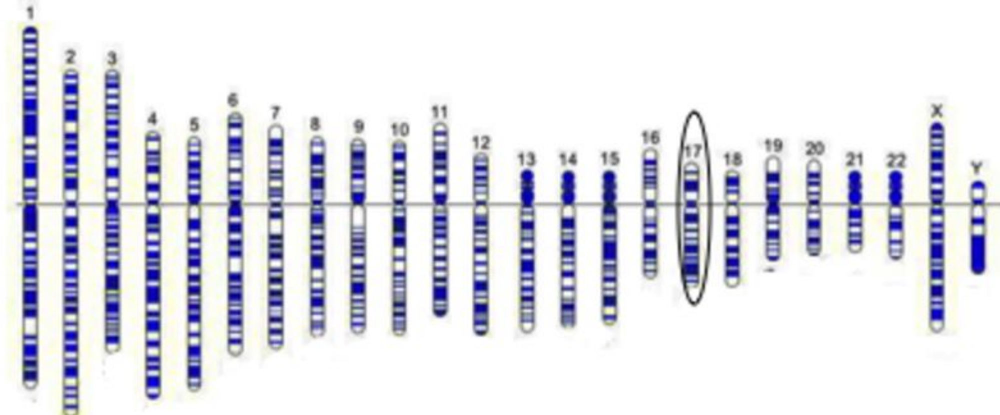
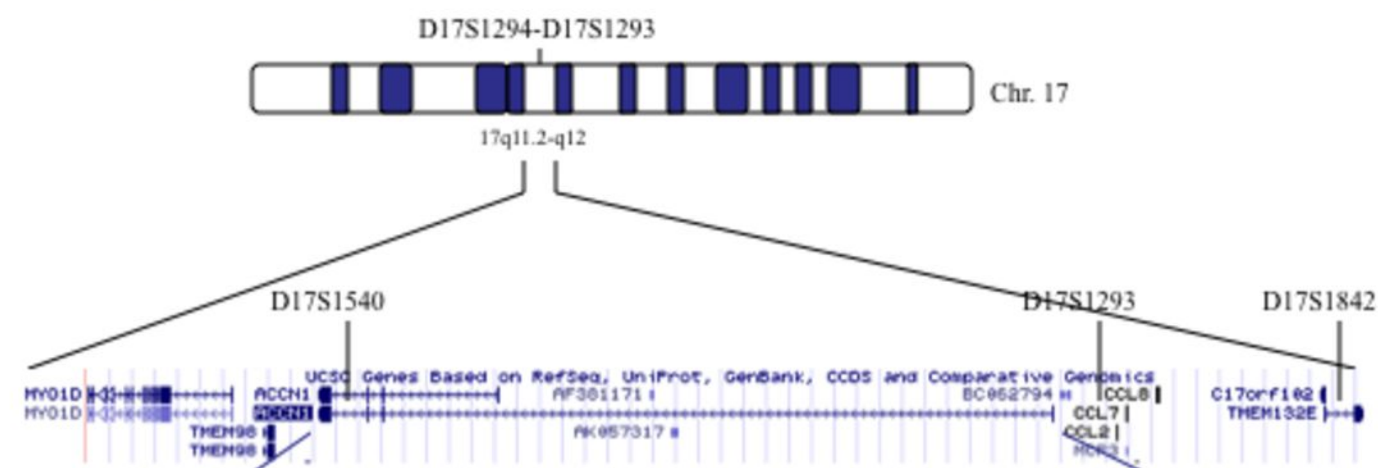
524 Figure 1. An overview of the study design: In stage 1 we conducted a genome-wide scan, which
525 detected significant association between PD and a two-marker segment (D17S1294-D17S1293) on
526 chromosome 17. In stage 2 we followed up on 17q11.2-q12, which revealed significant association
527 between PD and several markers (D17S1540, D17S1293 and D17S1842) within this region and
528 suggested *ACCN1* as a possible candidate gene. In stage 3 we analysed *ACCN1* for association with
529 PD using tag-SNPs.

530

Stage 1



Stage 2



Stage 3

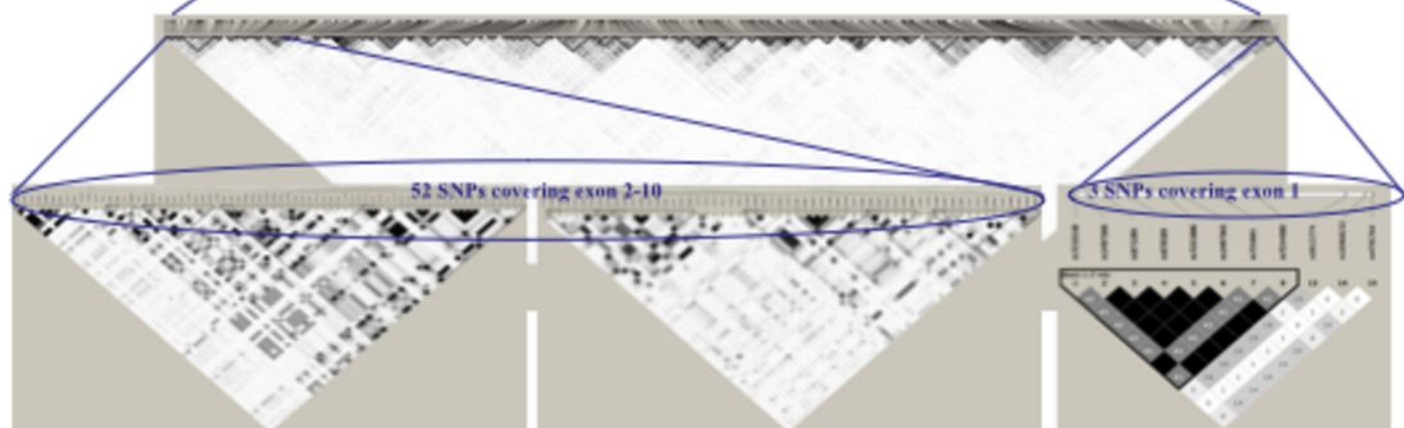


Table 1: Alleles and segments in 17q11.2-q12 showing significant association with PD (stage 2). Empirical CLUMP (T1 and T4 test statistics) for single- and two-marker analyses. Fisher's exact test for specific haplotypes/segments (alleles not shown). $IBD_{0\text{-sum}}$ shows the same probability summed over all 29 markers in 17q11.2-q12.

CLUMP									Fisher´s exact test				IBD by chance	
Single marker						Two markers								
Marker		**Cases**	**Controls**	**T1**	**T4**	**Marker**	**T1**	**T4**	**Segment**	**Cases**	**Controls**	**P**	**(P) IBD_0**	**(P) $IBD_{0\text{-sum}}$**
rs28936	G	15(0.58)	22(0.26)	0.0030	0.0030	rs28936-rs28935	0.0025	0.0028						
	A	11(0.42)	62(0.74)											
rs28935	A	15(0.58)	74(0.86)	0.0010	0.0010	rs28935-rs62068265	0.0033	0.0033	rs28935-rs62068265	9/26	12/86	0.0410	$2.587x10^{-11}$	$5.493x10^{-9}$
	G	11(0.42)	12(0.14)											
rs62068265	G	24(0.92)	84(0.98)	0.1400	0.0140	rs62068265-rs28933	0.0006	0.0002	rs62068265-rs28933	15/26	18/84	0.0011	$1.230x10^{-22}$	$2.673x10^{-19}$
	C	2(0.08)	2(0.02)											
rs28933	A	15(0.58)	18(0.21)	0.0010	0.0010	rs28933-D17S1540	0.0007	0.0008	rs28933-D17S1540	8/18	4/72	0.0002	$3.521x10^{-11}$	$5.615x10^{-9}$
	G	11(0.42)	66(0.79)											
D17S1540	*	9(0.35)	9(0.11)	0.0180	0.0200									
		17(0.65)	71(0.89)											
D17S1293	*	7(0.27)	9(0.10)	0.1480	0.1300	D17S1293-D17S1842	0.0070	0.0070	D17S1293-D17S1842	4/18	1/86	0.0030	$1.179x10^{-4}$	$1.03x10^{-2}$
		19(0.63)	77(0.90)											
D17S1842	*	16(0.62)	60(0.70)	0.0670	0.0590				rs28936-rs28935- rs62068265	8/20	9/76	0.0069	$9.940x10^{-11}$	$1.541x10^{-8}$
		10(0.48)	26(030)						rs28935- rs62068265-rs28933	8/18	5/78	0.0003	$3.521x10^{-11}$	$5.501x10^{-9}$
									rs62068265-rs28933-D17S1540	8/20	4/70	0.0005	$9.940x10^{-11}$	$1.541x10^{-8}$

*The allele frequency is given for the the allele showing the most skewed distribution against all the other alleles.

Table 2: Significantly associated SNPs within *ACCN1* analysed in the extended Faroese (FO) and Danish (DK) case-control samples and in the combined sample between Faroese and Danish cases vs. Faroese and Danish controls (DK+FO) (stage 3). The allele counts are given in numbers and the frequency are shown in brackets.

	FO	FO		DK	DK		FO+DK	FO+DK	
SNP	Cases	Controls	P_{trend}	Cases	Controls	P_{trend}	Cases	Controls	P
RS8066566									
A	16(0.28)	41(0.14)	0.016	62(0.13)	192(0.15)	0.245	78(0.15)	233(0.15)	0.805
G	42(0.72)	245(0.86)		414(0.87)	1066(0.85)		456(0.85)	1311(0.85)	
RS16589									
A	10(0.17)	93(0.32)	0.020	164(0.34)	434(0.34)	0.895	174(0.32)	527(0.34)	0.392
G	48(0.83)	195(0.68)		316(0.66)	824(0.66)		364(0.68)	1019(0.66)	
RS16585									
G	2(0.03)	45(0.16)	0.016	47(0.10)	145(0.12)	0.297	49(0.09)	190(0.12)	0.072
A	56(0.97)	245(0.84)		433(0.90)	1115(0.88)		489(0.91)	1360(0.88)	
RS12451625									
A	1(0.02)	28(0.10)	0.044	37(0.08)	102(0.08)	0.784	38(0.07)	130(0.08)	0.354
G	57(0.98)	262(0.90)		443(0.92)	1158(0.92)		500(0.93)	1420(0.92)	
RS4289044									
G	17(0.29)	47(0.16)	0.026	116(0.24)	278(0.22)	0.346	133(0.25)	325(0.21)	0.094
C	41(0.71)	243(0.84)		362(0.76)	980(0.78)		403(0.75)	1223(0.79)	
RS8070997									
G	7(0.12)	12(0.04)	0.018	78(0.16)	175(0.14)	0.229	85(0.16)	187(0.12)	0.069
A	51(0.88)	278(0.96)		402(0.84)	1085(0.86)		453(0.84)	1363(0.88)	
RS9915774									
A	5(0.09)	53(0.19)	0.072	58(0.12)	204(0.16)	0.033	63(0.12)	257(0.17)	0.006
G	53(0.91)	233(0.81)		422(0.88)	1054(0.84)		475(0.88)	1287(0.83)	