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Are TMEM genes potential candidate genes for panic disorder?

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We analysed single nucleotide polymorphisms in two transmembrane genes (*TMEM98* and *TMEM132E*) in panic disorder (PD) patients and control individuals from the Faroe Islands, Denmark and Germany. The genes encode single-pass membrane proteins and are located within chromosome 17q11.2–q12, a previously reported candidate region for PD. Three single nucleotide polymorphisms (rs887231, rs887230 and rs4795942) located upstream and within *TMEM132E* showed a nominal significant association with PD primarily in the Danish cohort. No nominal significant associations were observed between *TMEM98* and PD. Our data indicate that *TMEM132E* might contribute moderately towards the risk of developing PD. *Psychiatr Genet* 24:37–41 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Panic disorder (PD) is a mental disorder influenced by genetic factors as well as environmental factors (Klauke *et al.*, 2010; Schumacher *et al.*, 2011). A variety of genetic studies of PD have been carried out. However, the results have been inconsistent (Maron *et al.*, 2010). Recently, we reported results from a genome-wide association study in a Faroese case–control cohort, suggesting chromosome 17q11.2–q12 comprising the amiloride-sensitive cation channel 1 (*ACCN1*) gene as a candidate region and gene for PD (Gregersen *et al.*, 2012). The 17q11.2–q12 region also comprises two transmembrane protein genes (*TMEM132E* and *TMEM98*). One of the microsatellite markers (D17S1842) associated with PD in our previous study is located within *TMEM132E* (Gregersen *et al.*, 2012). Interestingly, another TMEM gene, *TMEM132D* located on chromosome 12, has been identified as a novel candidate gene for PD (Erhardt *et al.*, 2011, 2012; Quast *et al.*, 2012). The TMEM genes encode single-pass membrane proteins and may have functions essential to neuronal cells (Oh-hashi, 2010). In the current study, we aimed to follow up on the 17q11.2–q12 region by analysing single nucleotide polymorphisms (SNPs) in *TMEM132E* and *TMEM98*. The genes were analysed in Faroese, Danish and German PD patients and control individuals.

Methods

We selected 11 tag-SNPs covering the gene region of *TMEM98* and 25 tag-SNPs covering the gene region of *TMEM132E* (Fig. 1). The tagging and genotyping procedure has been described previously (Gregersen *et al.*, 2012). The SNPs were analysed in three PD cohorts: 36 cases and 162 control individuals from the isolated population of the Faroe Islands, 243 cases and 649 control individuals from Denmark and 232 cases and 222 control individuals from Germany. The patients were diagnosed with PD or PD with agoraphobia and have been described before (Wang *et al.*, 2006; Kristensen *et al.*, 2009; Koefoed *et al.*, 2010; Erhardt *et al.*, 2011; Gregersen *et al.*, 2012). The controls were matched to the cases by country of origin.

Statistics

SNPs were tested for association with PD using the Cochran–Armitage trend test. For the three combined cohorts (Faroese–Danish, Danish–German and Faroese–Danish–German), allelic association was tested using the Cochran–Mantel–Haenszel test. Two-marker and three-marker haplotype associations were conducted using a sliding window approach as implemented in PLINK (Purcell *et al.*, 2007). *ACCN1* was included in the

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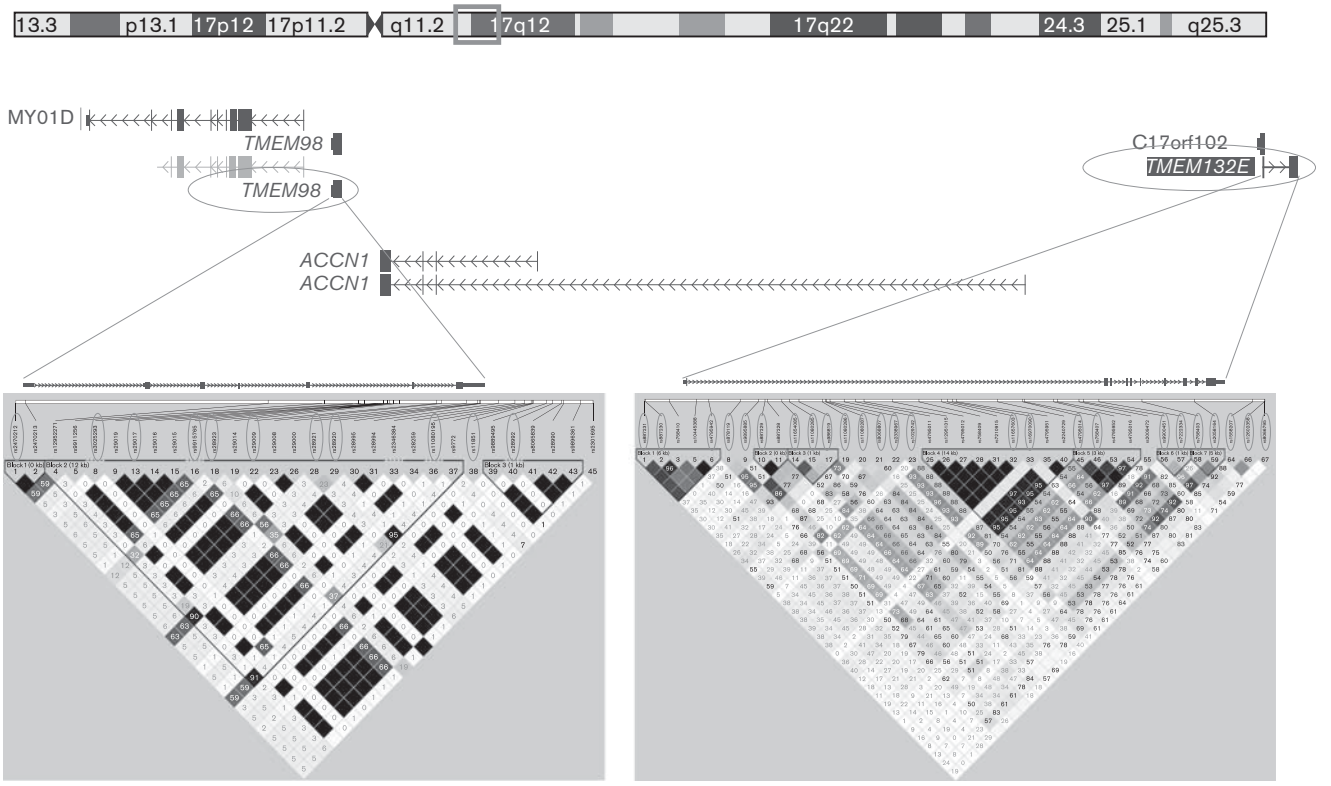
Fig. 1



The chromosome 17q11.2–q12 candidate region. *TMEM132E* and *TMEM98* are highlighted and single nucleotide polymorphisms are shown within the linkage disequilibrium plots.

Table 1 Significantly associated single nucleotide polymorphisms within *TMEM132E* analysed in Faroese, Danish and German panic disorder cases and controls

	rs887231 (C/T)			rs887230 (G/A)			rs4795942 (C/T)		
SNPs, allele frequency	MA (*F*)	*P*-value	OR (95 % CI)	MA (*F*)	*P*-value	OR (95 % CI)	MA (*F*)	*P*-value	OR (95 % CI)
FO									
Cases	6 (0.10)	0.201	0.57 (0.23–1.39)	4 (0.06)	0.051	0.34 (0.11–1.03)	6 (0.09)	**0.023**	**0.39** (0.16–0.96)
Controls	44 (0.18)			43 (0.16)			62 (0.24)		
DK									
Cases	68 (0.19)	**0.004**	**0.62** (0.45–0.87)	55 (0.13)	**0.036**	**0.62** (0.43–0.92)	98 (0.26)	**0.011**	**0.69** (0.51–0.93)
Controls	261 (0.29)			193 (0.18)			331 (0.35)		
FO + DK									
Cases	74 (0.17)	**0.001**	**0.63** (0.48–0.83)	59 (0.12)	**0.012**	**0.68** (0.50–0.92)	104 (0.23)	**0.002**	**0.68** (0.53–0.87)
Controls	305 (0.27)			236 (0.17)			393 (0.33)		
GE									
Cases	96 (0.30)	0.738	1.06 (0.77–1.44)	69 (0.18)	0.475	0.88 (0.62–1.25)	106 (0.32)	0.879	0.98 (0.71–1.33)
Controls	90 (0.28)			74 (0.21)			105 (0.33)		
DK + GE									
Cases	164 (0.24)	**0.043**	**0.80** (0.64–0.99)	124 (0.15)	**0.038**	**0.78** (0.62–0.99)	204 (0.28)	**0.039**	**0.81** (0.67–0.99)
Controls	351 (0.29)			267 (0.19)			436 (0.35)		
FO + DK + GE									
Cases	170 (0.23)	**0.020**	**0.78** (0.63–0.96)	128 (0.15)	**0.015**	**0.75** (0.59–0.94)	210 (0.27)	**0.010**	**0.78** (0.64–0.94)
Controls	394 (0.27)			309 (0.18)			497 (0.33)		

The minor allele (MA) is given in numbers and the minor allele frequencies (*F*) are in the parentheses. SNPs with $P \leq 0.05$ (nominal significance) are given in bold. Odds ratios (OR) are given with a 95% confidence interval (CI).
DK, Danish cohort; DK + GE, combined Danish and German cohort; FO, Faroese cohort; FO + DK, combined Faroese and Danish cohort; FO + DK + GE, combined Faroese, Danish and German cohort; GE, German cohort; SNP, single nucleotide polymorphism; *TMEM132E*, transmembrane protein 132E.

haplotype analyses because of its location between *TMEM98* and *TMEM132E* (Fig. 1); however, only in the analyses of the Faroese and Danish cohorts were *ACCN1* genotypes available. *P*-values 0.05 or less were considered as nominally significant. Each gene was tested for association at the level of the whole gene using

COMBASSOC (Curtis *et al.*, 2008). This program was further used to assess region-wide association by analysing the chromosome 17 region (17: 28.267.543–29.993.188) comprising *TMEM98*, *ACCN1* and *TMEM132E* (only for the Faroese and Danish cohorts). SNPs within the gene region of *TMEM98* and *TMEM132E* were imputed using MaCH 1.0 (Li and Abecasis, 2006). Fifty-six SNPs with a squared correlation above 0.3 and a quality score above 0.9 were analysed for association using PLINK.

Results

In total, 34 SNPs (11 within *TMEM98* and 23 within *TMEM132E*) were genotyped successfully with a call rate above 0.96 and a minor allele frequency above 0.01. No deviation from Hardy–Weinberg equilibrium ($P \leq 0.0015$) was observed among the controls. Thirty-eight of 1544 individuals were excluded from the analyses because of low genotyping.

No allelic or haplotypic association was detected between *TMEM98* and PD in any of the cohorts. One SNP (rs4795942) within *TMEM132E* was nominally significantly associated with PD in the Faroese cohort (Table 1). Three SNPs (rs887231, rs887230 and rs4795942) within the *TMEM132E* gene region showed a nominal significant association in the Danish cohort. The same three SNPs were also associated with PD when analysed in the combined samples of Faroese–Danish, Danish–German and Faroese–Danish–German cohorts (Table 1). rs887231 remained significantly associated after Bonferroni correction (correcting for 34 tests) in the combined Faroese–Danish cohort. None of the SNPs analysed showed an allelic association with PD in the German cohort. Nominally significantly associated two-marker and three-marker haplotypes were observed in all three cohorts and in the combined cohorts as shown in Table 2. Also, SNPs within the gene boundary of *ACCN1* and *TMEM132E* were included in significant associated two-marker and three-marker haplotypes in the Danish and combined Danish–Faroese cohorts (Table 2). In the subsequent imputation analyses, several of the SNPs showed a nominal allelic association in the Faroese and Danish cohorts (*P*-values ranging from 0.006 to 0.037) (data not shown). None of the imputed SNPs were associated with PD in the German cohort. Neither *TMEM132E* nor *TMEM98* showed a significant association at the level of the whole gene and no region-wise association was detected (data not shown).

Discussion

We followed up on the chromosome 17q11.2–q12 candidate region by analysing two TMEM genes (*TMEM98* and *TMEM132E*) for association with PD in three cohorts. *TMEM98* showed no association with PD. However, our data provided a trend for an association between *TMEM132E* and PD. Two SNPs (rs887231 and rs887230) located upstream and one SNP (rs4795942) located within *TMEM132E* showed both allelic and two-marker and three-marker haplotypic association in the Danish cohort. The minor allele frequencies of the three SNPs were over-represented in the control group, suggesting a protective effect of these variants. One of these SNPs (rs4795942) showed a nominal association in the Faroese cohort, with the minor allele frequency in the same direction as in the Danish cohort. The minor allele frequency of rs4795942 also indicated a protective effect in the German cohort. However, the results were not significant. After correcting for multiple comparisons, only rs887231 remained significantly associated in the combined Faroese and Danish cohort.

The two significantly associated upstream SNPs (rs887231 and rs887230) are located within the 3′ untranslated region and exon two of the predicted gene: *Homo sapiens* chromosome 17 open reading frame 102 (*C17orf102*). The *C17orf102* gene encodes the hypothetical protein LOC400591 and is located 1.4 kb upstream from *TMEM132E*.

The involvement of *TMEM132D* in the aetiology of PD has been reported recently (Erhardt *et al.*, 2011). A two-marker haplotype showed a significant association with PD in three independent German cohorts. Recently, this association has been replicated in a new independent cohort of European ancestry, in which the Danish PD cases and control individuals from our study were included (Erhardt *et al.*, 2012). Moreover, putatively functional and rare variants within *TMEM132D* have also been suggested to contribute towards the risk of developing PD (Quast *et al.*, 2012). *TMEM132D* and *TMEM132E* belong to the same gene family of five members: *TMEM132A*, *B*, *C*, *D* and *E*. Only two of the genes have been assigned a function. The *TMEM132A* gene product may play a role in cell survival by regulating stress-related genes in neuronal cells (Oh-hashi, 2010). The *TMEM132D* protein has been suggested to be involved in neural interconnecting and signalling (Nomoto *et al.*, 2003). Others have shown that *TMEM132D* is expressed in neurons and is colocalized with actin filaments (Walser *et al.*, 2011). As already indicated, the function of *TMEM132E* is unknown. However, a SNP located 38 kb downstream of *TMEM132E* has been associated with bipolar disorder; this association was replicated in the follow-up study (Sklar *et al.*, 2008). The downstream SNP was, however, not in linkage disequilibrium with the SNPs analysed in this study.

The results of the current study have to be interpreted with caution, especially when considering the small sample size of the Faroese cohort, as it may have affected the *P*-values. However, the Faroese population is presumed to have reduced genetic heterogeneity (Jorgensen *et al.*, 2002), which might justify the small sample size used. Furthermore, our results could be confounded

Table 2 Significantly associated two-marker haplotypes within *TMEM132E* analysed in Faroese, Danish and German panic disorder patients and controls

		FO			DK			FO + DK			GE			FO + DK + GE			DK + GE		
SNPs	Haplotype	Cases	Controls	*P*	Cases	Controls	*P*	Cases	Controls	*P*	Cases	Controls	*P*	Cases	Controls	*P*	Cases	Controls	*P*
*TMEM132*E	OMNIBUS			0.161			**0.012**			**0.012**			0.279			0.198			0.212
rs887231|rs887230	CG	0.06	0.13	0.084	0.11	0.15	**0.043**	0.11	0.15	**0.015**	0.16	0.17	0.533	0.13	0.16	0.076	0.14	0.16	0.123
	CA	0.03	0.01	0.367	0.04	0.07	**0.049**	0.04	0.06	0.138	0.07	0.05	0.125	0.06	0.06	0.903	0.06	0.06	0.496
	TA	0.91	0.85	0.195	0.84	0.78	**0.003**	0.85	0.79	**0.003**	0.77	0.78	0.755	0.81	0.79	0.103	0.81	0.78	0.080
rs887230|rs4795942	OMNIBUS			0.084			**0.044**			**0.015**			0.617			0.083			0.121
	GC	0.06	0.13	0.067	0.11	0.15	**0.039**	0.11	0.15	**0.013**	0.15	0.17	0.444	0.13	0.15	**0.047**	0.13	0.16	0.083
	AC	0.03	0.06	0.274	0.09	0.11	0.267	0.08	0.10	0.254	0.09	0.08	0.477	0.09	0.10	0.451	0.09	0.10	0.392
	AT	0.92	0.81	**0.026**	0.80	0.74	**0.014**	0.81	0.75	**0.005**	0.76	0.75	0.848	0.79	0.75	**0.031**	0.78	0.74	**0.043**
rs4795942|rs878119	OMNIBUS			0.171			**0.039**			**0.024**			0.988			0.130			0.108
	CG	0.02	0.01	0.738	0.02	0.02	0.762	0.02	0.02	0.839	0.03	0.03	0.971	0.02	0.02	0.964	0.02	0.02	0.996
	TG	0.22	0.20	0.702	0.14	0.11	0.095	0.15	0.12	0.189	0.12	0.11	0.719	0.13	0.12	0.284	0.13	0.11	0.117
	CA	0.07	0.18	**0.026**	0.18	0.25	**0.009**	0.17	0.23	**0.003**	0.21	0.22	0.923	0.19	0.23	**0.022**	0.20	0.24	**0.031**
	TA	0.69	0.61	0.198	0.66	0.63	0.205	0.67	0.63	0.093	0.64	0.65	0.867	0.65	0.63	0.225	0.65	0.64	0.397
rs11654085|rs11080285	OMNIBUS			0.837			0.170			0.265			**0.016**			**0.003**			**0.002**
	GG	0.39	0.43	0.562	0.48	0.44	0.186	0.47	0.44	0.271	0.49	0.49	0.901	0.48	0.45	0.208	0.48	0.46	0.195
	AC	0.47	0.45	0.718	0.43	0.44	0.793	0.44	0.44	0.881	0.46	0.41	0.118	0.45	0.43	0.431	0.45	0.43	0.427
	GC	0.14	0.13	0.750	0.09	0.12	0.091	0.10	0.12	0.136	0.05	0.10	**0.006**	0.08	0.11	**0.001**	0.07	0.11	**<0.001**
rs11080286|rs11080287	OMNIBUS			0.707			0.998			0.933			**0.010**			0.148			0.122
	TA	0.16	0.20	0.422	0.19	0.18	0.975	0.18	0.19	0.744	0.20	0.15	0.067	0.19	0.18	0.520	0.19	0.18	0.306
	CG	0.21	0.18	0.685	0.20	0.20	0.959	0.20	0.20	0.803	0.25	0.20	0.058	0.22	0.20	0.102	0.23	0.20	0.140
	TG	0.63	0.61	0.741	0.61	0.61	0.946	0.61	0.61	0.955	0.55	0.65	**0.002**	0.58	0.62	0.062	0.58	0.62	**0.041**
*ACCN1–TMEM132*E	OMNIBUS			0.417			**0.002**			**0.003**									
rs2097761|rs887231	GG	0.01	0.04	0.303	0.02	0.06	**<0.001**	0.02	0.06	**<0.001**									
	AG	0.08	0.11	0.414	0.14	0.16	0.266	0.13	0.15	0.265									
	GA	0.21	0.15	0.229	0.22	0.21	0.681	0.22	0.20	0.326									
	AA	0.70	0.70	0.981	0.62	0.57	**0.041**	0.63	0.59	0.128									

Further included are the two SNPs within the gene boundary of *ACCN1* and *TMEM132E*, respectively, but only for the Faroese and Danish cohorts.
SNPs with $P \leq 0.05$ (nominal significance) are given in bold.
Abbreviations are the same as in Table 1.

because of population stratification. To compensate for the possible population stratification between the different cohorts, the cases and controls were matched by country of origin.

However, a clear advantage of this study is the uniform phenotypes of the patients; we only included patients with PD or PD with agoraphobia. Phenotypic heterogeneity may be one of the reasons for the difficulties of replicating the results of association studies.

In conclusion, we suggest that an additional member of the *TMEM132* gene family, *TMEM132E*, may contribute moderately towards the risk of developing PD. However, further genetic studies are warranted.

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Conflicts of interest

There are no conflicts of interest.

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