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No association between the *PCM1* gene and schizophrenia: A multi-center case-control study and a meta-analysis

Ryota Hashimoto [a,b,c,*], Kazutaka Ohi [b,c], Yuka Yasuda [b,c], Motoyuki Fukumoto [b,c], Hidenaga Yamamori [b,c,d], Kouzin Kamino [b,e], Takashi Morihara [b], Masao Iwase [b], Hiroaki Kazui [b], Shusuke Numata [f], Masashi Ikeda [c,g], Shu-ichi Ueno [h], Tetsuro Ohmori [f], Nakao Iwata [c,g], Norio Ozaki [c,i], Masatoshi Takeda [a,b]

[a] *Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine Osaka Japan*
[b] *Department of Psychiatry, Osaka University Graduate School of Medicine, Suita, Osaka, Japan*
[c] *CREST (Core Research for Evolutionary Science and Technology), JST (Japan Science and Technology Agency), Kawaguchi, Saitama, Japan*
[d] *Department of Molecular Neuropsychiatry, Osaka University Graduate School of Medicine, Suita, Osaka, Japan*
[e] *Shoraiso National Hospital, Yamatokoriyama, Nara, Japan*
[f] *Department of Psychiatry, Tokushima University Graduate School of Medicine, Kuramoto, Tokushima, Japan*
[g] *Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi, Japan*
[h] *Department of Psychiatry, Ehime University Graduate School of Medicine, Toon, Ehime, Japan*
[i] *Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan*

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ABSTRACT

Alterations in centrosomal function have been suggested in the pathology of schizophrenia. The molecule pericentriolar material 1 (PCM1) is involved in maintaining centrosome integrity and in the regulation of the microtubule cytoskeleton. PCM1 forms a complex at the centrosome with the disrupted-in-schizophrenia 1 (DISC1) protein, which is a major susceptibility factor for schizophrenia. The association between genetic variants in the *PCM1* gene and schizophrenia has been reported by several case-control studies, linkage studies and a meta-analysis. The aims of this study are to replicate the association between four single-nucleotide polymorphisms (SNPs) in the *PCM1* gene and schizophrenia in a Japanese population (1496 cases and 1845 controls) and to perform a meta-analysis of the combined sample groups (3289 cases and 3567 controls). We failed to find a significant association between SNPs or haplotypes of the *PCM1* gene and schizophrenia in the Japanese population ($P>0.28$). The meta-analysis did not reveal an association between the four examined SNPs and schizophrenia. Our data did not support genetic variants in the *PCM1* gene as a susceptibility locus for schizophrenia.

1. Introduction

Schizophrenia is a common and complex psychiatric disease. The lifetime morbidity rate is 0.5–1.0% across distinct populations. Family, twin and adoption studies of schizophrenia have indicated that there are strong genetic factors implicated in the etiopathogenesis of the condition. Indeed, the estimated heritability is approximately 80% (Cardno and Gottesman, 2000). Several genetic linkage analyses of the 8p22-21 region in independent schizophrenia family samples have confirmed linkage in schizophrenia (Kendler et al., 1996; Blouin et al., 1998; Brzustowicz et al., 1999; Gurling et al., 2001). The region includes several genes implicated in the etiology of schizophrenia, such as pericentriolar material 1 (*PCM1*), *PPP3CC*, *SLC18A1* and *FZD3*.

The *PCM1* gene has been implicated in schizophrenia across multiple studies. Gurling et al. (2006) found that the marker D8S261 within *PCM1* showed both linkage and transmission disequilibrium with schizophrenia in a family sample of 13 UK and Iceland families affected with schizophrenia and that this marker was also associated with schizophrenia in a US sample of 100 trios but not in a Scottish sample of 200 cases and 200 controls from Edinburgh. They performed a follow-up study on these results with a University College of London (UCL) sample group of 450 cases and 450 controls and found that markers within *PCM1* were associated with schizophrenia. The associated markers were rs445422, rs13276297, D8S261 and rs370429. Two other markers, D8S2616 and rs3214087, showed a trend towards association. In addition, Gurling et al. (2006) have found that *PCM1*-associated patients with schizophrenia had a significant reduction in the volume of the grey matter of the orbitofrontal cortex in comparison with non-*PCM1*-associated

* Corresponding author at: Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, D3, 2-2, Yamadaoka, Suita, Osaka, 5650871, Japan. Tel./fax: +81 668793074.
E-mail address: hashimor@psy.med.osaka-u.ac.jp (R. Hashimoto).

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patients with schizophrenia. Kamiya et al. (2008) have performed mutation screening of 39 exons and flanking splice sites of the *PCM1* gene in 32 patients with schizophrenia. Although they found two known missense mutations (rs412750 and rs370429) in the cohort, they failed to find any association between these SNPs (single-nucleotide polymorphisms) and schizophrenia (32 cases and 219 controls). Additionally, Kamiya et al. (2008) have found a mutation (E1353X) of the gene in schizophrenia. The allele was not present in any of the controls, whereas the allele was present in a family (a proband and the two affected relatives). Datta et al. (2010) have reported a significant replication of the *PCM1* associations in a Scottish sample group of 858 cases and 591 controls from Aberdeen (rs445422) and in combined UCL and Aberdeen sample groups (rs208747, rs370429 and rs445422). More recently, a meta-analysis has shown evidence for an association between the *PCM1* gene and schizophrenia (Moens et al., 2010).

The centrosome plays a role in organizing microtubules and contributes to cell cycle progression, cell polarization, and ciliogenesis (Badano et al., 2005). The centrosome is required for proper neurodevelopment, especially in the cerebral cortex (Higginbotham and Gleeson, 2007). The PCM1 protein is a component of centriolar satellites and acts as a scaffold to target several proteins to the centrosome in a dynein motor-dependent manner. It also regulates microtubular dynamics and neuronal cell growth (Kubo et al., 1999). The PCM1 protein interacts directly with the disrupted-in-schizophrenia 1 (*DISC1*), one of the major susceptibility factors for schizophrenia, and Bardet–Biedl syndrome 4 (*BBS4*) proteins (Kamiya et al., 2008). DISC1 and BBS4 are required for targeting PCM1 and other cargo proteins to the centrosome in a synergistic manner. In the developing cerebral cortex, the suppression of PCM1 leads to neuronal migration defects, which are phenocopied by the suppression of either DISC1 or BBS4 and are exacerbated by the concomitant suppression of both (Kamiya et al., 2008). Several BBS proteins localize primarily to the centrosome and the basal body of ciliated cells where they contribute to the maintenance of microtubular dynamics as well as intracellular transport and ciliary function (Ansley et al., 2003). These findings suggest that PCM1 plays a role in centrosomal functions in cortical development and that the perturbation of centrosomal function contributes to the development of schizophrenia. In this study, we first investigated whether the *PCM1* gene is associated with schizophrenia in a Japanese population and we then performed a meta-analysis.

2. Methods

2.1. Subjects

The subjects in our genetic association study consisted of 1496 patients with schizophrenia (54.7% males (818/678); mean age ± SD: 46.9 ± 15.1 years) and 1845 healthy controls (51.1% males (942/903); mean age ± SD: 45.1 ± 20.0 years). The sex ratio and the mean age differed significantly between the groups (sex ratio: $\chi^2 = 4.35$, $P = 0.037$; mean age: $z = 5.15$, $P < 0.001$). All subjects were biologically unrelated Japanese and were recruited at three geographic regions in Japan: Osaka, Aichi and Tokushima (Ohi et al., 2009). Patients were recruited among both the outpatient and inpatient populations at university and psychiatric hospitals. Each patient with schizophrenia in the study had been diagnosed by at least two trained psychiatrists according to the criteria of the *Diagnostic and Statistical Manual of Mental* Disorders, fourth edition (DSM-IV), based on an unstructured clinical interview. Controls were recruited through local advertisements. Psychiatrically healthy controls were evaluated using unstructured interviews to exclude individuals who had current or past contact with psychiatric services. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with the World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University, Fujita Health University, Nagoya University and Tokushima University.

2.2. SNP selection and SNP genotyping

We first selected two SNPs in the *PCM1* gene, rs208747 and rs445422, which had been associated with schizophrenia in the reported meta-analysis comprising 1794 patients and 1553 controls (Moens et al., 2010) to replicate the association in the Japanese population (1463 patients and 1795 controls). Then, we chose an additional six SNPs: rs370429, rs454755, rs13276297, rs3780103, rs6991775 and rs3214087; the association of these SNPs with schizophrenia had been examined in reported studies (Gurling et al., 2006; Kamiya et al., 2008; Datta et al., 2010; Moens et al., 2010). Venous blood was collected from the subjects, and genomic DNA was extracted from whole blood according to standard procedures. SNPs were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, California, USA), as described previously (Hashimoto et al., 2006, 2007). TaqMan probe and primers for rs454755 was failed to design by the manufacturer (the Custom TaqMan® SNP Genotyping Assays: Applied Biosystems, Foster City, California, USA). Detailed information on the PCR conditions is available upon request. We did not find polymorphic variations in rs208747 within our sample groups (480 patients and 643 controls), which is consistent with the HapMap JPT data. While genotyping call rates were 95.9% (rs13276297) and 98.8% (rs6991775), we chose the four remaining SNPs for further analysis that had genotyping call rates greater than 99% (rs445422: 99.2% , rs3780103: 99.4%, rs3214087: 99.7%, and rs370429: 99.4%). No deviation from the Hardy–Weinberg equilibrium (HWE) in the examined four SNPs was detected in the patients with schizophrenia or the healthy controls ($P > 0.05$). The positions of the four SNPs analyzed in the present study are shown in Supplementary Fig. 1.

2.3. Power analysis

We performed power calculations using the Power Calculator for Two Stage Association Studies (http://www.sph.umich.edu/csg/abecasis/CaTS/ (Skol et al., 2006)). Power estimates were based on an allele frequency of 0.0135 (rs445422); an odds ratio of 1.78 (rs445422), as indicated by Moens et al. (2010); and an alpha level of 0.05. Power was calculated with a prevalence of 0.01 using a multiplicative model.

2.4. Meta-analysis of the PCM1 association studies

The studies included in the meta-analysis were selected using the Schizophrenia Research Forum (http://www.schizophreniaforum.org) and PubMed with the search terms "PCM1" and "Schizophrenia". The analyzed data encompass all publications up to Nov 2010.

2.5. Statistical analyses

Statistical analyses were performed using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan) and PASW Statistics 18.0 software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls were analyzed using χ^2 tests for sex and the Mann–Whitney *U*-test for age. Deviation from HWE was tested separately in cases and controls using χ^2 tests for goodness of fit. The allelic and genotypic distributions of the *PCM1* polymorphisms between patients and controls were analyzed using χ^2 tests. Pairwise linkage disequilibrium (LD) analyses, expressed using r^2, were applied to detect the intermarker relationship in each group using Haploview 4.1 software (http://www.broad.mit.edu/mpg/haploview/contact.php). Haplotype analysis was performed as

Table 1
Comparison of genotype and allele distributions for SNPs in the *PCM1* gene between patients with schizophrenia and controls.

Marker				SCZ			CON			Genotypic	SCZ	CON	Allelic	OR (95% CI)
db SNP IDs	Position[a]	M/m[b]	Location	M/M	M/m	m/m	M/M	M/m	m/m	*p* value (*df*=2)	MAF		*p* value (*df*=1)	
rs445422	17837373	C/T	Intron 2	0.90	0.10	0.004	0.90	0.10	0.003	0.81	0.05	0.05	0.89	1.02 (0.82–1.26)
rs3780103	17863852	C/T	Exon 16 (P784P)	0.72	0.25	0.023	0.73	0.25	0.022	0.99	0.15	0.15	0.89	1.01 (0.88–1.16)
rs3214087	17871541	C/–	Intron 22	0.46	0.44	0.10	0.46	0.44	0.10	0.88	0.32	0.32	0.90	1.01 (0.91–1.12)
rs370429	17893427	C/T	Exon 28 (T1543I)	0.86	0.13	0.007	0.85	0.15	0.009	0.56	0.07	0.08	0.28	0.91 (0.76–1.09)

SCZ, patients with schizophrenia; CON, healthy controls; M, major allele; m, minor allele; MAF, minor allele frequency; OR, odds ratio. All alleles are represented according to the + strand DNA sequence to make them comparable with the previously published data.
[a] db SNP build 129.
[b] The first shown alleles are major alleles.

described previously (Ohi et al., 2009). We used a two- to four-window fashion analysis.

The meta-analyses were performed using the Comprehensive Meta Analysis software (Version 2.0, BIOSTAT, Englewood, NJ, USA). Cochran's χ^2-based Q statistical test was performed to assess possible heterogeneity among the individual studies. The random-effect model described by DerSimonian and Laird was applied in the presence of the heterogeneity of the genetic effects ($P \leq 0.05$), while the fixed-effect model described by Mantel–Haenszel was applied in the absence of heterogeneity ($P > 0.05$). The significance of the pooled odds ratios (ORs) was assessed using a χ^2 test. Statistical tests were two-tailed, and the significance level was set at $P < 0.05$.

3. Results

3.1. Genetic association analysis

The genotype and allele frequencies of four SNPs located in the *PCM1* gene are summarized in Table 1. Our study size of 1496 cases and 1845 controls had sufficient power (>0.8) to detect an effect at an odds ratio of 1.78 or larger, as described in the previous report for rs445422 (Moens et al., 2010). No significant difference in the genotype or allele frequency between patients and controls was observed in four of the SNPs analyzed in our Japanese population ($P > 0.28$). Haplotype analysis also showed no significant association with schizophrenia (global $P > 0.40$) (Supplementary Table 1). The LD relationships between markers in our Japanese sample group are provided in Supplementary Fig. 1. The LD pattern observed in our controls was nearly identical to that observed in our patients, which is similar to the HAPMAP data for the Japanese population. However, the LD pattern was different from those previously reported for the UCL and Aberdeen samples (Datta et al., 2010). The strong LD patterns observed between rs445422 and rs370429 were observed in both Japanese and Caucasian populations. Although there was strong LD with rs3780103 and rs3214087 in the Caucasian population, weak LD was observed between rs3780103 and rs3214087 in the Japanese population.

3.2. Meta-analysis

We selected eight studies (six case-control and two family-based studies) using the Schizophrenia Research Forum and MEDLINE (Gurling et al., 2006; Kamiya et al., 2008; Datta et al., 2010; Moens et al., 2010). The demographics of the combined study population are shown in Table 2. A case-control and two family-based samples (studies 2, 8 and 9) were excluded from the present study because they only examined associations between schizophrenia and microsatellite markers, including D8S2615, D8S2616 and D8S261. The subjects (study 4) studied by Datta et al. (2010) were identical to those (study 1) used by Gurling et al. (2006). Thus, we included four case-control samples (studies 1, 3, 5, 6 and 7) (3289 patients and 3567 controls). The meta-analysis showed no association between any SNP and schizophrenia in the overall population (Table 3). We found no evidence of heterogeneity among studies in the overall population in rs3780103 and rs3214087, however, probabilities of heterogeneity in rs445422 ($P = 0.058$, $\chi^2 = 7.48$) and rs370429 ($P = 0.022$, $\chi^2 = 9.62$) were less than the traditional threshold for the heterogeneity test ($P = 0.1$) (Table 3). Detailed information concerning allele frequencies for each *PCM1* polymorphism in each study is shown in Supplement Table 2.

4. Discussion

Although several publications have provided evidence for the association between the polymorphisms within *PCM1* and schizophrenia (Gurling et al., 2006; Kamiya et al., 2008; Datta et al., 2010; Moens et al., 2010), we failed to replicate the association in a Japanese population. Our meta-analysis also demonstrated no significant

Table 2
Demographics of the combined studies.

	Authors	Ethnicities	Patients	Controls	Diagnostic criteria
Case-control studies					
1	Gurling et al. (2006)	UK (white English, Irish, Welsh or Scottish descent)	450	450	ICD-10
2	Gurling et al. (2006)[a]	Scottish	200	200	ICD-10
3	Kamiya et al. (2008)	USA	32	219	DSM-IV
4	Datta et al. (2010)[b]	UK (English, Irish, Welsh or Scottish descent)	450	450	ICD-10
5	Datta et al. (2010)	Aberdeen	858	591	DSM-III or IV
6	Moens et al. (2010)	Swedish	486	512	DSM-IV
7	Hashimoto et al. (present study)	Japanese	1496	1845	DSM-IV
Family-based studies					
8	Gurling et al. (2006)[a]	UK, Iceland	13 families		DSM-III
9	Gurling et al. (2006)[a]	USA	100 families		DSM-III

[a] These samples (2, 8 and 9) were excluded from the present study because these studies only examined associations between schizophrenia and microsatellite markers, such as D8S2615, D8S2616 and D8S261.
[b] These subjects (4) were identical to those (1) used by Gurling et al. (2006).

Table 3
Meta-analysis of the genetic association studies for each SNP.

db SNP ID	M/m	Number of studies[a]	Q Statistic (heterogeneity) p value (Q)	p value (z)	OR (95% CI)
rs445422 (T)	C/T	4	**0.058 (7.48)**	0.11 (1.56)	1.17 (0.96–1.41)
rs3780103 (T)	C/T	4	0.57 (2.00)	0.30 (1.04)	1.04 (0.96–1.13)
rs3214087 (−)	C/−	4	0.55 (2.11)	0.56 (−0.58)	0.98 (0.90–1.06)
rs370429 (T)	C/T	4	**0.022 (9.62)**	0.31 (1.02)[b]	1.31 (0.78–2.18)

Fixed- or random-effects p value (z): chi-square test was used to determine the significance of the overall OR. Q statistic (heterogeneity) p value (Q): Cochran's Q test was used to assess heterogeneity. A random-effects model was applied in the presence of the heterogeneity of the genetic effects ($p \leq 0.05$), while a fixed-effects model was applied in the absence of heterogeneity ($p > 0.1$). Significant p values are shown in bold.

[a] The number of studies included in each meta-analysis is indicated.

[b] These analyses were performed using a random-effects model.

association between any of the SNPs in the *PCM1* gene and schizophrenia in the overall populations.

The inability to replicate genetic association is a common problem in attempts to detect genetic polymorphisms contributing to susceptibility to a complex human disease. A number of reasons for this have been discussed, including population stratification, genetic heterogeneity, clinical assessment, publication bias, sample size and random error (Cardon and Palmer, 2003; Colhoun et al., 2003). First, the most likely reason for heterogeneity is ethnic stratification. Significant heterogeneity among studies was observed in rs370429, which was associated with schizophrenia in previous studies (Gurling et al., 2006; Datta et al., 2010). The allele frequencies for each SNP in the present study were not similar to those in the Caucasian sample groups (Gurling et al., 2006; Kamiya et al., 2008; Datta et al., 2010). Indeed, polymorphic variation of rs208747, which was associated with schizophrenia in the reported meta-analysis, was not found in the examined Japanese population (more than 1000 subjects). The LD patterns within the *PCM1* gene reported for the JPT HapMap sample are not similar to those reported for the CEU and YRI HapMap samples. Thus, the significant heterogeneity observed in our meta-analysis could result from ethnic stratification in the combined samples. Second, there were differences in the criteria used to diagnose schizophrenia among the studies. Patients were diagnosed according to the DSM-III, DSM-IV or ICD-10 criteria for each study. This difference between the studies may have been one of the reasons for the observed heterogeneity. Third, the combined sample population (3289 cases and 3567 controls) in our meta-analysis had sufficient power (>0.80) to detect a genetic effect at ORs of 1.285 or greater for rs445422 when the allele frequency was input as 0.034. However, the sample size had insufficient power (<0.80) to detect a small effect with ORs of 1.12–1.16 in the three risk SNPs for schizophrenia in the genome-wide association study and in the subsequent replication studies using total of 16,726 subjects (O'Donovan et al., 2008). To achieve 80% power, greater than 10,000 cases and comparable controls are needed to detect small effects of the OR (less than 1.12–1.16) reported by O'Donovan et al. (2008). Other variables, such as differences in age and sex, might increase the heterogeneity.

In conclusion, we failed to find an association between the *PCM1* gene and schizophrenia in a Japanese population. These findings are supported by meta-analyses of previously published studies and the present study. The heterogeneity among studies observed in our meta-analysis might be due to differences in ethnic heterogeneity, phenotypic heterogeneity or the sample size of each study. Factors such as inadequate power, as well as allelic and locus heterogeneity could all affect the ability to detect genetic associations. Further replication studies in distinct populations are required to confirm the ethnic stratification of the association between the *PCM1* gene and schizophrenia.

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Contributors

R. Hashimoto supervised the entire project, collected the data, wrote the manuscript, was critically involved in the design, analysis and interpretation of the data and was responsible for performing the literature review. K. Ohi was critically involved in the collection and analysis of the data, and contributed to the editing of the final manuscript and contributed intellectually to the interpretation of the data. Y. Yasuda, M. Fukumoto, H. Yamamori, K. Kamino, T. Morihara, M. Iwase, H. Kazui, S. Numata, M. Ikeda, S. Ueno, T. Ohmori, N. Iwata, N. Ozaki, and M. Takeda were heavily involved in the collection of the majority of the data and contributed intellectually to the interpretation of the data. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

Supplementary materials related to this article can be found online at doi:10.1016/j.schres.2011.03.024.

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Impact on schizotypal personality trait of a genome-wide supported psychosis variant of the *ZNF804A* gene

Yuka Yasuda[a,b], Ryota Hashimoto[a,b,c,*], Kazutaka Ohi[a,b], Motoyuki Fukumoto[a,b], Satomi Umeda-Yano[d], Hidenaga Yamamori[a,d], Tomo Okochi[e], Masao Iwase[a], Hiroaki Kazui[a], Nakao Iwata[e], Masatoshi Takeda[a]

[a] *Department of Psychiatry, Osaka University Graduate School of Medicine, Osaka, Japan*
[b] *CREST (Core Research for Evolutionary Science and Technology) of JST (Japan Science and Technology Agency), Saitama, Japan*
[c] *Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, Osaka, Japan*
[d] *Department of Molecular Neuropsychiatry, Osaka University Graduate School of Medicine, Osaka, Japan*
[e] *Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi, Japan*

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ABSTRACT

Schizophrenia is a complex disorder with a high heritability. Relatives with schizophrenia have an increased risk not only for schizophrenia but also for schizophrenia spectrum disorders, such as schizotypal personality disorder. A single nucleotide polymorphism (SNP), rs1344706, in the Zinc Finger Protein 804A (*ZNF804A*) gene, has been implicated in susceptibility to schizophrenia by several genome-wide association studies, follow-up association studies and meta-analyses. This SNP has been shown to affect neuronal connectivities and cognitive abilities. We investigated an association between the *ZNF804A* genotype of rs1344706 and schizotypal personality traits using the Schizotypal Personality Questionnaire (SPQ) in 176 healthy subjects. We also looked for specific associations among *ZNF804A* polymorphisms and the three factors of schizotypy—cognitive/perceptual, interpersonal and disorganization—assessed by the SPQ. The total score for the SPQ in carriers of the risk T allele was significantly higher than that in individuals with the G/G genotype ($p = 0.042$). For the three factors derived from the SPQ, carriers with the risk T allele showed a higher disorganization factor ($p = 0.011$), but there were no differences in the cognitive/perceptual or interpersonal factors between genotype groups ($p > 0.30$). These results suggest that the genetic variation in *ZNF804A* might increase susceptibility not only for schizophrenia but also for schizotypal personality traits in healthy subjects.

Schizophrenia is a common and complex psychiatric disease with a lifetime morbidity rate of 0.5–1.0%. Family, twin, and adoption studies of schizophrenia have indicated that there are strong genetic factors associated with schizophrenia, with an estimated heritability of approximately 80%, and that the risk of occurrence is increased approximately 10-fold in first-degree relatives with schizophrenia [3,28].

Since a genome wide association study (GWAS) for schizophrenia identified a single-nucleotide polymorphism (SNP), rs1344706, in the Zinc Finger Protein 804A (*ZNF804A*) gene as one of the strongest risk genes for schizophrenia [16], this gene has been the subject of intense research activity. The ZNF804A gene is located on chromosome 2q32.1 and consists of four exons and three introns, spanning 341 kb. Several subsequent genome wide association and follow-up case-control studies for schizophrenia have supported association with the same T risk allele [19,22]. In addition, meta-analysis of a robust data set (schizophrenia/schizoaffective disorder, $n = 18{,}945$; schizophrenia plus bipolar disorder, $n = 21{,}274$; and controls $n = 38{,}675$) has provided evidence for association between rs1344706 in the *ZNF804A* gene and schizophrenia and psychotic disorders (schizophrenia and bipolar disorder) [31]. Despite an extensive search for other functional

Abbreviations: ANOVA, one-way analysis of variance; ANCOVA, one-way analysis of covariance; DSM, Diagnostic and Statistical Manual of Mental Disorders; GWAS, genome wide association study; SPD, schizotypal personality disorder; SPQ, Schizotypal Personality Questionnaire; SNP, single nucleotide polymorphism; *ZNF804A*, Zinc Finger Protein 804A.

* Corresponding author at: Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, D3, 2-2, Yamadaoka, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6879 3074; fax: +81 6 6879 3059.
E-mail address: hashimor@psy.med.osaka-u.ac.jp (R. Hashimoto).

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variants at this locus in the study, rs1344706 remains the most strongly associated variant [31]. There is a difference of allelic distributions of this SNP among ethnic groups, e.g. T allele frequencies in Japan: 42%, in China: 52%, in UK: 59%, in Germany (Munich): 58%, in USA: 61%, respectively (SzGene database: http://www.szgene.org). Rs1344706 is located on intron near the 3′ end of the gene and lies in approximately 30 bp of conserved mammalian sequence. The *ZNF804A* mRNA expression level in subjects with the T allele of rs1344706 was higher than that in subjects with the G allele in prefrontal cortex [19]. The region of this SNP contains zinc ion and DNA binding domains and predicted binding sites for the brain-expressed transcription factors MYT1l and POU3F1/OCT-6 include the T allele of this SNP. Thus, rs1344706 may have a possible role in regulation of gene expression.

Although the biological function of the *ZNF804A* gene remains unclear, several clues about the gene's function have been gathered from cognitive neuroscience studies. In these studies, rs1344706 has been associated with variance of the functional brain connectivity during n-back tasks [5], neural activation during theory-of-mind tasks [29], and neuropsychological performances, such as visual memory, episodic and working memory and attention [2,9,30]. These functions are impaired in patients with schizophrenia.

Schizotypal personality disorder (SPD) is characterized by social avoidance, ideas of reference, vagueness, magical thinking, odd speech, illusions and paranoid ideation. Relatives of individuals with schizophrenia show such personality traits at increased rates in comparison with relatives of individuals with other psychiatric disorders or in mentally healthy subjects [24]. These traits were subsequently incorporated into the *Diagnostic and Statistical Manual of Mental Disorders* (DSM)-III criteria for SPD and are listed in the DSM-IV-TR on Axis II. These traits can be identified by means of a well-validated questionnaire, the Schizotypal Personality Questionnaire (SPQ) [18]. In line with converging evidence from adoption, family and twin studies [11,12,27], genetic linkage patterns to schizotypy and schizophrenia have been reported to be similar [6]. Furthermore, several studies have demonstrated that individuals with SPD scores similar to patients with schizophrenia show abnormalities in a very wide range of neuropsychological tests and in cerebral gray matter volumes [4,15]. Cognitive deficits and smaller gray-matter volumes in individuals with SPD are very similar to, but somewhat less pronounced than, those in patients with schizophrenia, indicating that SPD is in a genetic continuum with schizophrenia.

Little is known about the influence of susceptibility genes for schizophrenia on schizotypal personality traits. Association studies have shown correlations between the Val158 allele with high activity in the *COMT* gene and high scores on schizotypal personality traits in healthy individuals [1,21]. Other molecular genetic studies have reported associations between the *NRG1* [13], *DTNBP1* [26], *RGS4* [25] and *DAAO* [26] genes and schizotypy components. In this study, we investigated whether the genome-wide supported psychosis variant in the *ZNF804A* gene is associated with schizotypal personality traits in healthy subjects.

The subjects in this study consisted of 176 healthy individuals [47.2% males (83/93), 36.8 ± 11.5 years old]. All subjects were biologically unrelated and were Japanese. They were recruited through local advertisements at Osaka University. Psychiatrically, medically and neurologically healthy controls were evaluated using the structured clinical interview from the DSM-IV non-patient version, to exclude individuals who had current or past contact with psychiatric services or had received psychiatric medication [32]. Subjects were excluded from this study if they had neurological or medical conditions that could potentially affect the central nervous system, such as atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, active cancer, cerebrovascular disease, epilepsy, seizures or mental retardation. Subjects who had first- or second-degree relatives with psychiatric disorders or who were receiving psychotropic medication were also excluded. Full scale IQ is assessed using the Wechsler Adult Intelligence Scale, Revised or Third edition. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with the World Medical Association's Declaration of Helsinki and was approved by the Osaka University Research Ethics Committee.

For assessing schizotypal personality traits, a full Japanese version of the SPQ was administered to all subjects [10,23]. The SPQ is a 74-item self-report questionnaire with a "yes/no" response format [17]. All items answered "yes" are scored 1. The SPQ measures nine subscales of specific schizotypal features, i.e., ideas of reference, odd beliefs/magical thinking, unusual perceptual experiences, suspiciousness/paranoid ideation, social anxiety, no close friends, constricted affect, eccentric/odd behavior, and odd speech. The total SPQ score is obtained by simply adding scores from all of the items together. The three schizotypal trait factors—cognitive/perceptual, interpersonal and disorganization—are derived by summation of the related subscale raw scores according to the three-factor model of Raine et al. [18]. We examined the factor structure of the SPQ for our sample using a confirmatory factor analysis in Amos 19.0 (IBM SPSS Amos for Japan) to determine whether the three-factor solution (cognitive-perceptual, interpersonal and disorganized) provides better fit to our sample or not. Several indices were selected to assess the fit of the three-factor model for the nine subscales for the full 74-item SPQ to our sample, such as the Goodness of Fit Index (GFI), the Adjusted GFI (AGFI), the Comparative Fit Index (CFI) and the Root Mean Square Error of Approximation (RMSEA). Indices of the fit of the three-factor model to our sample were 0.90 (GFI), 0.81 (AGFI), 0.87 (CFI) and 0.13 (RMSEA). Values greater than 0.9 for GFI, 0.8 for AGFI and 0.9 for CFI indicate a good fit. While RMSEA values < 0.05 indicate very good goodness of fit, RMSEA values > 0.1 are a sign of poor goodness of fit. GFI and AGFI values for three-factor model were greater than 0.9 and 0.8, while CFI and RMSEA values were lesser than 0.9 and greater than 0.10. These data suggests that the three-factor model moderately fits for our sample, as reported previously [10,18,20].

We selected rs1344706 in the *ZNF804A* gene because this variant has been found to be associated with schizophrenia and bipolar disorder in genome-wide association and follow-up studies [16] and to be associated with functional brain connectivity, visual memory, episodic and working memory and attention [2,5,9,30]. Venous blood was collected from the subjects, and genomic DNA was extracted from whole blood according to standard procedures. The SNP was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, CA, USA), as described previously [7,8]. Detailed information on the PCR conditions is available upon request.

Statistical analyses were performed using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan) and PASW Statistics 18.0 software (SPSS Japan Inc., Tokyo, Japan). The differences in the clinical characteristics between genotype groups were analyzed using χ^2 tests for categorical variables and the Mann–Whitney *U*-test for continuous variables. The presence of Hardy–Weinberg equilibrium was examined using the χ^2 test for goodness of fit. No deviation from Hardy–Weinberg equilibrium was detected in the subjects ($p > 0.05$). The effects of the *ZNF804A* genotype on the total score and on the three factors of the SPQ were analyzed by a one-way analysis of variance (ANOVA). To control confounding factors, the effects of the *ZNF804A* genotype on the total score and the three factors of the SPQ were analyzed by a one-way analysis of covariance (ANCOVA), with age, sex and education years as covariates, because the score and factors have been correlated with

Table 1
Demographic variables for subjects.

Variables	Total (n = 176)	T carrier (n = 125)	G/G (n = 51)	p values	(z)
Age (years)	36.8 ± 11.5	37.3 ± 11.8	35.7 ± 11.0	0.49	−0.69
Sex (male/female)[a]	83/93	64/61	19/32	0.09	2.83
Education (years)	15.4 ± 2.4	15.3 ± 2.4	15.5 ± 2.3	0.68	0.41
IQ	109.3 ± 11.9	110.1 ± 11.6	107.1 ± 12.6	0.21	−1.25

Means ± SD are shown.
[a] χ^2 test.

Table 2
Impact of the risk variant in the *ZNF804A* gene on the schizotypal personality trait.

SPQ	T carrier (n = 125)	G/G (n = 51)	Cohen's d	Genotype effect		
Variables				$F_{1,171}$	p values	η^2
Total score	11.7 ± 9.5	8.7 ± 7.2	0.36	4.19	**0.042**	0.024
Cognitive/perceptual	3.5 ± 3.9	2.8 ± 3.6	0.19	1.10	0.30	0.006
Interpersonal	5.3 ± 4.8	4.2 ± 3.5	0.26	2.20	0.14	0.013
Disorganization	3.6 ± 3.6	2.2 ± 2.7	0.44	6.59	**0.011**	0.037

SPQ: schizotypal personality questionnaire. Means ± SD are shown. The effect sizes are typically categorized as small (d = 0.20, η^2 = 0.01), medium (d = 0.50, η^2 = 0.06) or large (d = 0.80, η^2 = 0.14). To control the confounding factors, the effect of the *ZNF804A* genotype on schizotypal traits was analyzed by a one-way analysis of covariance with age, sex and years of education as covariates. Significant p-values are shown in boldface and underlined.

these confounding factors [14]. Given the relatively low number of homozygous risk T allele individuals, these analyses focused on a comparison of homozygous carriers of one or two copies of the T allele (a combined T/T and T/G genotype group) versus homozygous non-risk G/G genotype carriers. Bonferroni correction was applied for multiple testing on three factors of the SPQ to avoid type I error. Standardized effect sizes were calculated using Cohen's d method (http://www.uccs.edu/faculty/lbecker). The significant level for statistical tests was set at two-tailed $p < 0.05$.

We examined possible associations between the *ZNF804A* genotype (T carrier vs. G/G genotype) and schizotypal traits in healthy subjects. Demographic variables, age, sex, years of education and IQ were not significantly different between genotype groups ($p > 0.09$) (Table 1). We first examined the possible effect of the *ZNF804A* SNP on the total SPQ score and found a significant effect of genotype on the total SPQ score ($F_{1,174} = 4.21$, $p = 0.042$; adjusted $F_{1,171} = 4.19$, $p = 0.042$) (Table 2). Then, we further investigated the genotype effects on the three SPQ factors: cognitive/perceptual, interpersonal and disorganization. There was a significant genotype effect on the disorganization factors ($F_{1,174} = 6.36$, $p = 0.013$; adjusted $F_{1,171} = 6.59$, $p = 0.011$), whereas there was no significant genotype effect on the cognitive/perceptual or interpersonal factors ($p > 0.30$). The effect of genotype on the disorganization factors remained positive after correction for multiple tests (corrected p value, disorganization: $p = 0.033$). The risk T allele carriers of rs1344706 showed higher scores on schizotypal traits, particularly disorganization factors, compared with subjects with the G/G genotype (Fig. 1). These effect sizes of the total score and disorganization factor were 0.36 and 0.44, respectively. When the two genotypes were divided into three genotype groups (subjects with T/T genotype, T/G genotype and G/G genotype), we found a marginal genotype effect on the total SPQ score (adjusted $F_{2,170} = 2.64$, $p = 0.074$) and a significant genotype effect on the disorganization factors (adjusted $F_{2,170} = 3.33$, $p = 0.038$) (Supplementary Table 1). Post hoc analysis revealed that the subjects with the T/G genotype showed marginally higher scores on the total SPQ score than subjects with G/G genotype (post hoc corrected $p = 0.071$) and significantly higher scores on disorganization factors than subjects with G/G genotype (post hoc corrected $p = 0.039$). There was no difference of the scores between subjects with T/T genotype and T/G genotype or subjects with T/T genotype and G/G genotype. When we compared schizotypal traits between different two genotype groups (T/T vs. G carrier), there was no significant difference between the genotype groups in total score or factors (Supplementary Table 2).

In this study, we found an association between the genetic variant in the *ZNF804A* gene and schizotypal personality traits, particularly disorganization factors, in healthy subjects. Individuals with the risk T allele scored higher on schizotypal personality traits and disorganization factors than those with non-risk alleles. Thus, the *ZNF804A* gene could affect personality and neurocognitive performance. SPD has a strong familial relationship with schizophrenia [12]. The SPQ is useful in screening for SPD in the general population and in researching the correlates of individual schizotypal traits [17]. The *ZNF804A* gene that increases the risk for schizophrenia might also increase the risk for the schizotypal personality traits measured by the SPQ, consistent with a continuum model of schizophrenia.

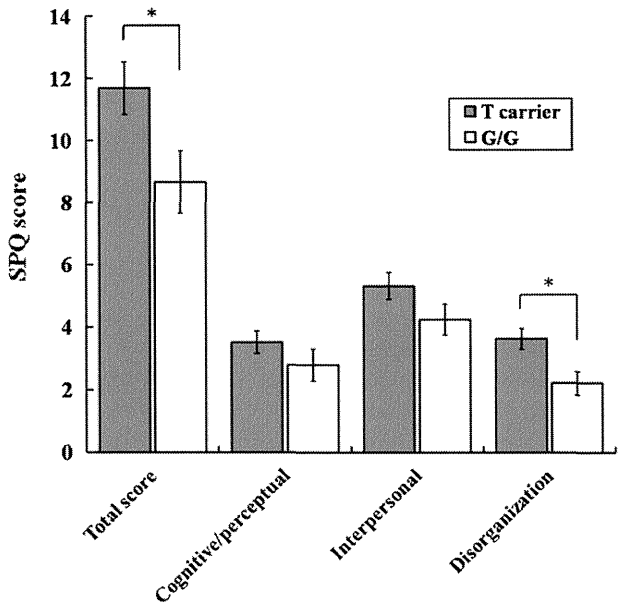

Fig. 1. The association between the risk-associated *ZNF804A* genotype and the total score and the three SPQ factors. Gray bars represent individuals who are T-carriers (T/T and T/G genotypes) of rs1344706. White bars represent individuals with the G/G genotype of the SNP. Error bars represent standard errors of the mean. *$p < 0.05$.

Because the odds ratio of rs1344706 between patients with schizophrenia and controls was 1.10 [31], the genetic variation makes a small contribution to the risk for schizophrenia. However, the effect size of the risk SNP on the schizotypal personality traits is medium. Therefore, the larger effects size of schizotypal personality traits than that for the diagnosis of schizophrenia suggests that schizotypal personality traits could be more powerful tools to detect an association with schizophrenia. In other words, it might be possible to require a smaller sample size of schizotypal personality traits to find an association with schizophrenia. It is necessary to carry out further investigations to confirm our findings in other sample groups with much larger sample sizes, in different ethnicities or in relatives with schizophrenia.

There were several limitations in this study. As this personality assessment is based on self-report, it is not an objective measurement. It might not be representative of the general population because the subjects were recruited from single place (a university) and the sample size of this study was small. A false-positive association could not be excluded in our study, despite relatively homogenous population and correction for multiple testing of this study. As the function of this SNP is unknown, other variants, which are in linkage disequilibrium with this SNP, might be truly associated with the personality trait.

In conclusion, we found for the first time that in healthy subjects, risk T carriers had higher scores for self-reported schizotypal personality traits in comparison with individuals with the G/G genotype. This finding is in agreement with reported findings on schizophrenia, and it adds to the body of evidence that the *ZNF804A* gene might be involved in the pathogenesis of schizophrenia. It may be useful to investigate schizotypal personality traits and the *ZNF804A* gene for prevention of and early intervention in schizophrenia. The SPQ could be used to assess the potential for developing schizophrenia.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2011.03.069.

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The *SIGMAR1* gene is associated with a risk of schizophrenia and activation of the prefrontal cortex

Kazutaka Ohi [a,b], Ryota Hashimoto [a,b,c,*], Yuka Yasuda [a,b], Motoyuki Fukumoto [a,b], Hidenaga Yamamori [a,d], Satomi Umeda-Yano [d], Kouzin Kamino [a,e], Koji Ikezawa [a], Michiyo Azechi [a], Masao Iwase [a], Hiroaki Kazui [a], Kiyoto Kasai [f], Masatoshi Takeda [a,c]

[a] *Department of Psychiatry, Osaka University Graduate School of Medicine, Osaka, Japan*
[b] *Core Research for Evolutionary Science and Technology (CREST) of Japan Science and Technology Agency (JST), Saitama, Japan*
[c] *Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, Osaka, Japan*
[d] *Department of Molecular Neuropsychiatry, Osaka University Graduate School of Medicine, Osaka, Japan*
[e] *National Hospital Organization Shoraiso Hospital, Nara, Japan*
[f] *Department of Neuropsychiatry, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*

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ABSTRACT

Several studies have identified the possible involvement of sigma non-opioid intracellular receptor 1 (*SIGMAR1*) in the pathogenesis of schizophrenia. The Gln2Pro polymorphism in the *SIGMAR1* gene has been extensively examined for an association with schizophrenia. However, findings across multiple studies have been inconsistent. We performed a meta-analysis of the association between the functional Gln2Pro polymorphism and schizophrenia using combined samples (1254 patients with schizophrenia and 1574 healthy controls) from previously published studies and our own additional samples (478 patients and 631 controls). We then used near-infrared spectroscopy to analyze the effects of the Gln2Pro genotype, a schizophrenia diagnosis and the interaction between genotype and diagnosis on activation of the prefrontal cortex (PFC) during a verbal fluency task (127 patients and 216 controls). The meta-analysis provided evidence of an association between Gln2Pro and schizophrenia without heterogeneity across studies (odds ratio = 1.12, $p = 0.047$). Consistent with previous studies, patients with schizophrenia showed lower bilateral activation of the PFC when compared to controls ($p < 0.05$). We provide evidence that Pro carriers, who are more common among patients with schizophrenia, have significantly lower activation of the right PFC compared to subjects with the Gln/Gln genotype ($p = 0.013$). These data suggest that the *SIGMAR1* polymorphism is associated with an increased risk of schizophrenia and differential activation of the PFC.

1. Introduction

Schizophrenia is a common and complex psychiatric disease with strong genetic components. Schizophrenia has an estimated heritability of approximately 80% (Cardno and Gottesman, 2000; Tsuang, 2000) and many genes have been implicated in the pathogenesis of schizophrenia (Sun et al., 2008).

Sigma-1 receptors are relatively small single transmembrane proteins located on the plasma and subcellular membranes, particularly in the endoplasmic reticulum; the protein plays a role in modulating intracellular calcium signaling (van Waarde et al., 2010). Sigma-1 receptors are also involved in modulating the activity of some ion channels and in several neurotransmitter systems such as glutamatergic and dopaminergic neurotransmission (Hayashi and Su, 2004). Several drugs targeted to the central nervous system, including antipsychotics (haloperidol, chlorpromazine and nemonapride), selective serotonin reuptake inhibitors (fluvoxamine and sertraline) and acetylcholinesterase inhibitors (donepezil), show high to moderate affinities for sigma-1 receptors (Cobos et al., 2008). Of the antipsychotics, only haloperidol is known to act as an antagonist for sigma-1 receptor (Cobos et al., 2008). This affinity between antipsychotic drugs and sigma-1 receptors suggests that the receptors play a substantial role in the pathogenesis of schizophrenia. Sigma-1 antagonists improve the behavior of animals in models based on the

Abbreviations: *SIGMAR1*, sigma non-opioid intracellular receptor 1; PFC, prefrontal cortex; NIRS, near-infrared spectroscopy; SNP, single nucleotide polymorphism; VFT-letter, letter version of the verbal fluency test; oxyHb, oxygenated hemoglobin; OR, odds ratio; ANCOVA, analysis of covariance.

* Corresponding author at: Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, D3, 2-2, Yamadaoka, Suita, Osaka, 565-0871, Japan. Tel./fax: +81 6 6879 3074.
E-mail address: hashimor@psy.med.osaka-u.ac.jp (R. Hashimoto).

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motor effects of dopaminergic stimulants or NMDA antagonists (Cobos et al., 2008). In clinical trials, putative selective antagonists of sigma receptors showed antipsychotic effects for schizophrenia (Cobos et al., 2008).

The sigma non-opioid intracellular receptor 1 (*SIGMAR1*) gene is located on chromosome 9p13. This gene contains cytokine and steroid responsive elements. In genetic association studies, Ishiguro et al. (1998) detected associations between schizophrenia and two functional polymorphisms of the *SIGMAR1* gene, Gln2Pro and GC-241-240TT. These two polymorphisms were in near complete linkage disequilibrium with each other and resulted in two haplotypes, Pro2/TT241-240 and Gln2/GC-241-240 (Ishiguro et al., 1998). The transcriptional activity of the TT-241-240 haplotype, which was in near complete linkage disequilibrium with Pro2 in *SIGMAR1* gene, was significantly reduced compared with that of the GC-241-240 haplotype (Miyatake et al., 2004). The Gln2Pro polymorphism is part of the N-terminus amino acid sequence motif MQWAVGRR, which is a putative endoplasmic reticulum retention signal (Schutze et al., 1994). The functional polymorphism has been extensively examined for an association with schizophrenia. However, the findings of multiple studies have been inconsistent (Ohmori et al., 2000; Satoh et al., 2004; Uchida et al., 2003). A meta-analysis of Gln2Pro in *SIGMAR1* has found no evidence for a significant association between the genetic variant and schizophrenia, although the Pro allele was marginally more frequent in schizophrenia patients (32%) than in controls (29%) ($p=0.06$) (Uchida et al., 2003). The lack of association identified in the meta-analysis may be the result of a type II error stemming from a small sample size (779 patients with schizophrenia and 636 healthy controls).

Many attempts have been made to minimize clinical and genetic heterogeneity for schizophrenia. A strategy for gene discovery proposes using quantitative neurobiological traits as intermediate phenotypes instead of the diagnosis of schizophrenia (Meyer-Lindenberg and Weinberger, 2006; Tan et al., 2008). This strategy has the potential to reduce clinical and genetic heterogeneity by applying intermediate phenotypes that reflect underlying genetic vulnerability better than diagnostic categorization. Combined imaging and genetic studies have shown that brain function, as assessed by neuroimaging techniques, is a sensitive intermediate phenotype that bridges the gap between genotype and diagnostic categorization (Weinberger et al., 2001). Near-infrared spectroscopy (NIRS) is a functional neuroimaging technology used to noninvasively assess changes in cerebral blood volume. Verbal fluency, a classic test of executive function, is the most reliable task currently used to induce prominent and wide-spread frontotemporal activation in normal subjects that can be well-differentiated from that of patients with schizophrenia (Ikezawa et al., 2009; Takizawa et al., 2008). Structural and functional abnormalities of the prefrontal cortex (PFC) are well known to exist in patients with schizophrenia (Ragland et al., 2009; Segall et al., 2009). Sigma-1 receptors are widely expressed in the mammalian brain tissues (Kekuda et al., 1996; Kitaichi et al., 2000). The chronic administration of the preferential sigma-1 receptor ligand is able to modify levels of several glutamate subunits in the rat PFC (Guitart et al., 2000). Postmortem study comparing normal controls to patients with schizophrenia revealed that schizophrenics have a reduced density of sigma binding sites in the frontal cerebral cortex (Simpson et al., 1991). There is evidence that activation of the PFC during the verbal fluency task, as assessed using multi-channel NIRS, is significantly lower in Pro carriers of the *SIGMAR1* gene than in individuals with the Gln/Gln genotype (Takizawa et al., 2009a). We hypothesized that the lower *SIGMAR1* expression modulated by the Gln2Pro polymorphism might be related to hypoactivation of the PFC in schizophrenia via impaired regulation of NMDA receptor-mediated glutamatergic neurotransmission.

In this study, we first attempted to replicate the association between a functional single nucleotide polymorphism (SNP) in the *SIGMAR1* gene and schizophrenia in our samples; we then added our samples to the available samples from previous studies and performed a meta-analysis. We next examined the influence of the Gln2Pro polymorphism on prefrontal hemodynamic activation during a verbal fluency task using a noninvasive neuroimaging technique, two-channel NIRS, in patients with schizophrenia and in healthy volunteers.

2. Methods

2.1. Subjects

Subjects for the genetic association study included 478 unrelated patients with schizophrenia [48.5% males (232 males/246 females), mean age ± SD: 48.3 ± 15.7 years] and 631 unrelated healthy controls [46.9% males (296/335), mean age ± SD: 58.7 ± 21.4 years]. The sex ratio did not differ significantly between cases and controls ($p=0.56$), while the mean age of schizophrenia patients was significantly lower than that of controls ($p<0.001$). Cases were recruited from both outpatients and inpatients at Osaka University Hospital and the psychiatric hospitals. Each subject with schizophrenia had been diagnosed by at least two trained psychiatrists based on an unstructured clinical interview; diagnoses were made based on the criteria of the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). Controls were recruited through local advertisements. Psychiatrically healthy controls were evaluated using unstructured interviews to exclude individuals who had current or past contact with psychiatric services.

The subjects who underwent NIRS analysis were 127 unrelated patients with schizophrenia [55.1% males (70/57), mean age ± SD: 36.9 ± 12.3 years] and 216 unrelated healthy controls [44.4% males (96/120), mean age ± SD: 36.8 ± 11.6 years]. These subjects were included in the genetic association study and agreed to receive the examination using NIRS. These subjects in the present NIRS analysis included subjects in our two previous NIRS studies (Azechi et al., 2010; Ikezawa et al., 2009). All subjects were biologically unrelated Japanese. Subjects were excluded from this analysis if they had neurological or medical conditions that could potentially affect the central nervous system, such as atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, cancer with active stage, cerebrovascular disease, epilepsy, seizures, substance-related disorders or mental retardation. Cases were recruited from both outpatients and inpatients at Osaka University Hospital. Each patient with schizophrenia had been diagnosed by a trained psychiatrist according to the DSM-IV criteria based on Structured Clinical Interview for DSM-IV (SCID). Controls were recruited through local advertisements at Osaka University. Psychiatrically, medically and neurologically healthy controls were evaluated using the SCID-Non-Patient version to exclude individuals who had current or past contact with psychiatric services or had received psychiatric medication (Hashimoto et al., 2010; Ohi et al., 2009). Current symptoms of schizophrenia were evaluated using the five syndrome models of the positive and negative syndrome scale (PANSS) (Lindenmayer et al., 1994). There were 15 patients taking haloperidol, which has a high affinity and acts as an antagonist for sigma-1 receptor (Cobos et al., 2008), at the NIRS measurement [Pro carrier $N=10$, mean chlorpromazine equivalents (CPZeq) of haloperidol ± SD: 398.8 ± 338.2 mg/day, Gln/Gln $N=5$, 500.0 ± 326.0 mg/day]. We found no differences in CPZeq of haloperidol in subjects taking haloperidol between the genotype groups ($p=0.46$). The sex ratio and mean age did not differ significantly between cases and controls ($p>0.06$), while years of education, estimated premorbid intelligence quotient (IQ) and performance score on the letter version of the verbal fluency test (VFT-letter) during the NIRS measurement were significantly lower in patients with schizophrenia than in controls ($p<0.001$) (Table 1). When the genotype groups were compared, we found no differences in demographic variables, age, sex, years of education, estimated premorbid IQ, performance score on VFT-letter, CPZeq of total antipsychotics, ratio of subjects taking haloperidol, age at onset of

Table 1
Demographic variables for subjects included in the NIRS analysis.

Gln2Pro (rs1800866)	Schizophrenia ($N=127$)			Control ($N=216$)			Group difference
Variables	Pro carrier ($N=70$)	Gln/Gln ($N=57$)	p values (z)	Pro carrier ($N=115$)	Gln/Gln ($N=101$)	p values (z)	p values (z)
Age (years)	36.6 ± 11.5	37.2 ± 13.4	0.92 (−0.10)	37.7 ± 12.2	35.8 ± 10.9	0.30 (−1.03)	0.95 (0.06)
Sex (Male/Female)	38/32	32/25	0.83 (0.04)[b]	51/64	45/56	0.98 (<0.01)[b]	0.06 (3.65)[b]
Education (years)	13.7 ± 2.6	14.2 ± 2.2	0.27 (1.09)	15.3 ± 2.4	15.1 ± 2.3	0.57 (−0.56)	3.62×10^{-6} (−4.63)
Estimated premorbid IQ[a]	101.0 ± 10.0	100.4 ± 10.3	0.85 (−0.19)	106.7 ± 8.4	106.7 ± 7.8	0.94 (−0.08)	2.96×10^{-8} (−5.54)
Performance score of VFT-letter	13.1 ± 5.0	12.7 ± 4.3	0.68 (−0.41)	17.4 ± 4.8	17.2 ± 4.6	0.99 (<0.01)	5.03×10^{-14} (−7.53)
CPZeq (mg/day)	616.1 ± 492.9	640.3 ± 623.3	0.68 (−0.41)	–	–	–	–
Subjects taking haloperidol (+/−)	10/60	5/52	0.34 (0.92)[b]	–	–	–	–
Age at onset (years)	23.9 ± 8.3	23.8 ± 9.5	0.66 (−0.44)	–	–	–	–
Duration of illness (years)	12.6 ± 10.4	13.4 ± 11.5	0.81 (0.25)	–	–	–	–
PANSS, Positive	14.4 ± 4.4	15.2 ± 5.4	0.31 (−1.02)	–	–	–	–
PANSS, Negative	17.7 ± 6.2	16.5 ± 6.1	0.35 (−0.93)	–	–	–	–
PANSS, Cognitive	12.2 ± 3.9	11.9 ± 3.9	0.94 (−0.07)	–	–	–	–
PANSS, Excitement	8.3 ± 2.9	8.0 ± 3.6	0.39 (−0.86)	–	–	–	–
PANSS, Depression/Anxiety	10.0 ± 3.1	9.4 ± 3.7	0.20 (−1.28)	–	–	–	–

CPZeq; CPZeq of total antipsychotics. Five syndrome model of PANSS proposed by Lindenmayer et al. (1994). Means ± SD are shown. [a]Pro carriers ($N=69$); Gln/Gln ($N=56$), [b]χ^2 test. Significant p-values are shown in boldface and underlined.

illness, duration of illness, or PANSS scores (Table 1). Written informed consent was obtained from all subjects after the procedures had been fully explained. This study was carried out in accordance with the World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University.

2.2. SNP selection and SNP genotyping

We selected Gln2Pro (rs1800866) in the *SIGMAR1* gene to examine the association between the Gln2Pro polymorphism and schizophrenia and to investigate the association between the polymorphism and prefrontal hemodynamic responses. Gln2Pro is located within exon 1. Venous blood was collected from the subjects and genomic DNA was extracted from whole blood according to standard procedures. The SNP was genotyped using the custom designed TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, California, USA) as previously described (Hashimoto et al., 2006; Hashimoto et al., 2007b). No deviation from Hardy–Weinberg equilibrium in the examined SNP was detected in the patients or controls ($p>0.05$).

2.3. Meta-analysis of the SIGMAR1 association studies

The studies included in the meta-analysis were selected using the Schizophrenia Research Forum (http://www.schizophreniaforum.org) and PubMed with the search terms "SIGMAR1" and "Schizophrenia". The analyzed data encompass all publications up to December 2010.

2.4. Analysis of activation of the prefrontal cortex by NIRS

Activation of the PFC during the VFT-letter task was analyzed according to methods that have been previously described (Azechi et al., 2010; Ikezawa et al., 2009). The test consisted of a pre-task period (30 s), a task period (60 s) and a post-task period (60 s). In the VFT-letter task, subjects were instructed to generate as many nouns as possible that start with a Japanese *hiragana* letter ('a', 'ka', and 'sa', each for 20 s). They were also instructed to pronounce the syllables 'a', 'i', 'u', 'e' and 'o' repeatedly during the pre-task and post-task periods. The total number of generated words was defined as task performance during the NIRS measurement. NIRS measurements were conducted using a two-channel system (NIRO-200, Hamamatsu Photonics, Japan) to detect changes in the concentration of oxygenated hemoglobin ([oxyHb]) in primal venous blood in the cerebral cortex. Two pairs of emission probes located in F7 and F8 (BA45 and BA47) and a detection probe located in Fp1 and Fp2 (BA10) were attached bilaterally to the subjects' foreheads. During data analysis, the difference between activation and baseline levels was defined as the size of activation (Δ [oxyHb]).

2.5. Statistical analyses

The presence of Hardy–Weinberg equilibrium was examined by the χ^2 test for goodness of fit using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan). The genetic case–control analysis and meta-analysis were performed using the Comprehensive Meta-Analysis software (Version 2.0, BIOSTAT, Englewood, NJ, USA). Cochran's χ^2 based Q statistical test was performed in order to assess possible heterogeneity among studies. The fixed-effect model described by Mantel–Haenszel was applied in the absence of heterogeneity ($p>0.05$). The significance of the pooled odds ratio (OR) was assessed using a z-test.

Statistical analyses of demographic variables and the NIRS analysis were performed using PASW Statistics 18.0 software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls or between genotypes were analyzed using a χ^2 test for categorical variables and the Mann–Whitney U-test for continuous variables. To control for confounding factors, the effects of the diagnosis, genotype and their interaction on prefrontal function were analyzed by two-way analysis of covariance (ANCOVA) with age, sex, years of education and performance on the VFT-letter task as covariates. The ANCOVAs on the NIRS data were conducted separately for the left and right hemisphere data. Pearson's correlation coefficients were used to assess relationships between the activation of the PFC and PANSS scores. The significance level for all statistical tests was set at two-tailed $p<0.05$.

3. Results

3.1. Association between variants of the SIGMAR1 gene and schizophrenia by meta-analysis

Among our subjects, the frequency of the Pro allele was higher in schizophrenia patients (34.5%) than in controls (32.6%) (Table 2). The direction of the difference in allele frequency between patients and controls is consistent with the initial study (Ishiguro et al., 1998), however, the difference is not statistically significant [$z=0.93$, $p=0.35$, OR (95% confidence interval) = 1.09 (0.91–1.30)]. Our study size of 478 cases and 631 controls had insufficient power to detect as small an effect as an OR of 1.12, as described in the previous GWAS report (O'Donovan et al., 2008). Thus, we performed a meta-analysis to increase the power to detect such a small effect. We selected five studies using the Schizophrenia Research Forum and

Table 2
Comparison of allele frequencies of the *SIGMAR1* polymorphism in a Japanese population.

Gln2Pro	SCZ, Number of allele (%)			CON, Number of allele (%)			Statistics for each study		
(rs1800866)	Pro	Gln	Sum	Pro	Gln	Sum	*p* value (*z*)	OR (95% CI)	Weight (fixed)
Present study	330 (34.5)	626 (65.5)	956	412 (32.6)	850 (67.4)	1262	0.35 (0.93)	1.09 (0.91–1.30)	40.3
Ishiguro et al.	202 (32.8)	414 (67.2)	616	242 (27.9)	624 (72.1)	866	0.045 (2.01)	1.26 (1.01–1.58)	25.3
Ohmori et al.	81 (31.4)	177 (68.6)	258	75 (26.8)	205 (73.2)	280	0.24 (1.18)	1.25 (0.86–1.82)	9.2
Uchida et al.	127 (31.9)	271 (68.1)	398	134 (32.5)	278 (67.5)	412	0.85 (−0.19)	0.97 (0.72–1.31)	14.7
Satoh et al.	58 (29.0)	142 (71.0)	200	62 (29.8)	146 (70.2)	208	0.86 (−0.18)	0.96 (0.63–1.47)	7.0
Takizawa et al.	27 (33.8)	53 (66.3)	80	34 (28.3)	86 (71.7)	120	0.42 (0.81)	1.29 (0.70–2.37)	3.4
Pool	825 (32.9)	1683 (67.1)	2508	959 (30.5)	2189 (69.5)	3148	0.047 (1.99)[a]	1.12 (1.00–1.26)	

SCZ: patients with schizophrenia, CON: healthy control. [a] heterogeneity across studies (I^2<0.01, Q=3.05, p=0.69). Significant *p*-values are shown in boldface and underlined.

PubMed (Ishiguro et al., 1998; Ohmori et al., 2000; Satoh et al., 2004; Takizawa et al., 2009a; Uchida et al., 2003). The five studies and the present study (six case–control studies) included a combined 1254 patients and 1574 controls. In each case–control study, the subjects were of Japanese ethnicity. The demographics of subjects in the combined studies are shown in Supplementary Table 1. A meta-analysis of Gln2Pro in all available data sets provided evidence for an association with schizophrenia [z=1.99, p=0.047, OR (95% confidence interval) = 1.12 (1.00–1.26)] and no evidence for heterogeneity across studies (Q=3.05, p=0.69) (Fig. 1, Table 2). The frequency of the Pro allele at Gln2Pro was higher in schizophrenia patients (32.9%) than in controls (30.5%).

3.2. The effect of the Gln2Pro polymorphism on prefrontal function as measured by NIRS

We examined the effects of *SIGMAR1* genotype, schizophrenia diagnosis and their interaction on frontal lobe function during verbal fluency in patients with schizophrenia and in controls (Table 3). Two-way ANCOVA revealed significant effects of schizophrenia diagnosis on bilateral prefrontal function (right: $F_{1,335}$=16.85, p=5.09×10^{-5}, η^2=0.048, left: $F_{1,335}$=4.34, p=0.038, η^2=0.013) and of genotype on right prefrontal function ($F_{1,335}$=6.24, p=0.013, η^2=0.018). Patients with schizophrenia showed a lower bilateral activation of the PFC during the VFT-letter task when compared with controls. Among both patients and controls, at-risk Pro carriers had a lower activation of the right PFC than subjects with the Gln/Gln genotype (Fig. 2). No significant effect of genotype on left prefrontal function or of genotype-diagnosis interaction on bilateral prefrontal function was found, although activation of the left PFC was marginally associated with the Gln2Pro genotype (p=0.075). As reported previously (Ikezawa et al., 2009), there was no correlation between the bilateral activation of the PFC during the VFT-letter task and any PANSS scores (positive, negative, cognitive, excitement or depression/anxiety syndrome scores) of the patients (p>0.08).

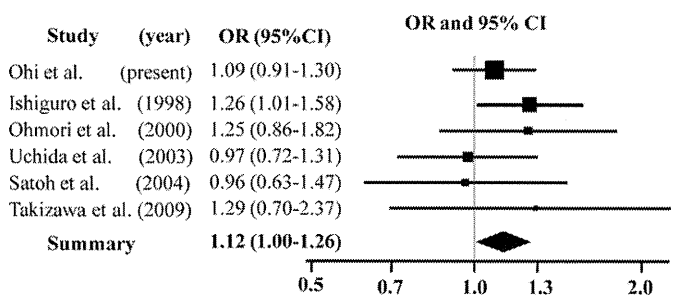

Fig. 1. Forest plot illustrating Gln2Pro polymorphism in the *SIGMAR1* gene based on all combined samples. Solid squares and horizontal lines indicate the weighted odds ratios and 95% confidence intervals. The overall result is shown by the diamond. The result of the meta-analysis shown here is under the fixed-effects model.

4. Discussion

In this study, we first provided evidence for an association between the Gln2Pro polymorphism in the *SIGMAR1* gene and schizophrenia in a Japanese population. The Pro allele frequency in Gln2Pro was higher in patients with schizophrenia (32.9%) than in controls (30.5%). Second, we used NIRS to examine whether the Gln2Pro genotype was associated with activation of the PFC during verbal fluency. We provided evidence that the at-risk Pro carriers had a lower activation of the right PFC than subjects with the Gln/Gln genotype.

We performed a meta-analysis of the association between Gln2Pro and schizophrenia in the overall combined populations of previous studies and the present study (Ishiguro et al., 1998; Ohmori et al., 2000; Satoh et al., 2004; Takizawa et al., 2009a; Uchida et al., 2003). All six independent cohorts included in the meta-analysis included subjects with Japanese ethnicity. Japan is relatively isolated at the eastern extreme of Asia. This isolation may be advantageous in investigating a complex genetic disorder such as schizophrenia because these groups are highly homogeneous, reducing the risk of spurious associations due to population stratification. Our meta-analysis of Japanese populations indicated a significant association between Gln2Pro and schizophrenia without heterogeneity among studies. Although the previous and present meta-analyses reported similar frequencies of the Pro allele in patients and controls (patients: 32% vs. controls: 29%), a previous meta-analysis of three independent cohorts reported no association between Gln2Pro and schizophrenia. This discrepancy may result from a type II error due to the small sample size of the earlier analysis (779 patients and 636 controls); in the present study, the sample included 1254 patients and 1574 controls. As expected and described in the previous GWAS report (O'Donovan et al., 2008), the OR observed in this analysis was quite small (1.12). This suggests that the majority of susceptibility-risk alleles for schizophrenia come from common variants with small effects.

We also used two-channel NIRS to demonstrate that the Gln2Pro genotype in the *SIGMAR1* gene was significantly associated with prefrontal function during a verbal fluency task. Among both diagnosed schizophrenics and health controls, Pro carriers had a lower activation of the right PFC during verbal fluency than those with the Gln/Gln genotype. These findings were independent of between-group differences in age, sex, years of education and task performance. Using 52-channel NIRS, Takizawa et al. (2009a,b) found that the bilateral activation of the PFC during the verbal fluency task was significantly lower in Pro carriers than in individuals with the Gln/Gln genotype among patients with schizophrenia, but not among healthy controls (Takizawa et al., 2009a). The sample size of this study (127 patients and 216 controls) was more than three times the sample size of a previous study that successfully detected a significant genotype effect of *SIGMAR1*; this previous study included only 40 patients and 60 controls (Takizawa et al., 2009a). The differences in site of activation between our study and the previous study by Takizawa et al. (2009a,b) might be explained by the differences in sample size or NIRS equipment (multi-channel vs. two-channel).

The transcriptional activity of the haplotype with Gln in *SIGMAR1* gene was significantly increased than that of the haplotype with Pro

Table 3
Impact of genetic variants in the *SIGMAR1* gene on activation of the prefrontal cortex during VFT-letter task.

Gln2Pro (rs1800866)	Schizophrenia ($N=127$)		Control ($N=216$)		p values ($F_{1,335}$ values)		
	Pro carrier ($N=70$)	Gln/Gln ($N=57$)	Pro carrier ($N=115$)	Gln/Gln ($N=101$)	Diagnosis effect	Genotype effect	Interaction
Activation of the rt. PFC	0.52 ± 1.05	0.90 ± 1.43	0.95 ± 1.09	1.24 ± 1.07	5.09×10^{-5} (16.85)	0.013 (6.24)	0.59 (0.29)
Activation of the lt. PFC	0.55 ± 1.10	0.99 ± 1.47	0.98 ± 1.22	1.05 ± 1.16	0.038 (4.34)	0.075 (3.18)	0.12 (2.39)

Means ± SD are shown. To control for confounding factors, the effect of diagnosis, the *SIGMAR1* genotype and their interaction on activation of prefrontal cortex was analyzed by two-way analysis of covariance with age, sex, years of education and performance score on the VFT-letter as covariates. Significant p-values are shown in boldface and underlined.

(Miyatake et al., 2004), suggesting that sigma-1 receptor signaling in subjects with the Gln/Gln genotype might be more active than that in Pro carriers. Because haloperidol acts as an antagonist for sigma-1 receptor (Cobos et al., 2008), haloperidol could attenuate the effect of the polymorphism on the sigma-1 receptor signaling. Patients with the Gln/Gln genotype ($N=5$, 500.0 ± 326.0 mg/day) tended to have taken more CPZeq of haloperidol than Pro carriers ($N=10$, 398.8 ± 338.2 mg/day) in patients with taking haloperidol, although it was not statistically significant ($p=0.46$). Thus, higher sigma-1 receptor signaling in patients with the Gln/Gln genotype might be reduced by haloperidol treatment.

NIRS, a neuroimaging method, is increasingly used in the investigation of frontal cortex dysfunction in schizophrenia. Compared with other neuroimaging methods such as functional magnetic resonance imaging and positron emission tomography, NIRS measurement is restraint free, easy to use, and portable, has a low running cost and is noninvasive. Using NIRS, three studies have suggested that Gln2Pro in *SIGMAR1*, Val158Met in catechol-O-methyltransferase (*COMT*) and genotypes based on a threshold of greater than 35 CAG repeats in the TATA box-binding protein (*TBP*) gene were associated with prefrontal hemodynamic response (Ohi et al., 2009; Takizawa et al., 2009a,b). The activation of the PFC during the verbal fluency task was significantly lower in carriers of the Pro allele in the *SIGMAR1* gene (when compared to individuals with the Gln/Gln genotype) and in individuals with the Val/Val genotype in the *COMT* gene (when compared to carriers of the Met allele) (Takizawa et al., 2009a,b). In addition, we have reported evidence that genotypes with greater than 35 CAG repeats in the *TBP* gene, which were more common among patients with schizophrenia, were significantly associated with PFC hypoactivation during the tower of Hanoi, a test of executive function. If combined imaging and genetics research demands a larger and wider variety of samples in the near future, NIRS has great potential as a neuroimaging modality to detect cortical function with ease and speed. Further research is needed to investigate whether these genotype effects can be replicated in different cohorts with larger sample sizes.

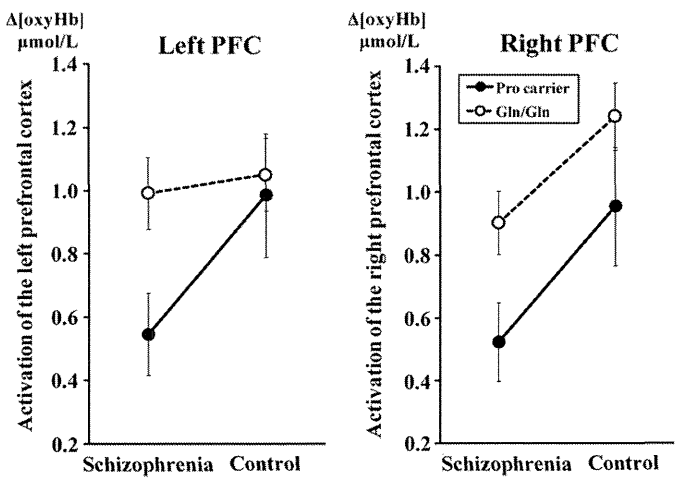

Fig. 2. Effect of the risk Gln2Pro genotype and diagnosis on activation of the bilateral PFC as measured by NIRS during the verbal fluency task. Carriers of the Pro allele in the *SIGMAR1* gene, which was more common among patients with schizophrenia, showed lower activation of the right PFC compared to individuals with the Gln/Gln genotype in both patients with schizophrenia and healthy controls (schizophrenia patients: Pro carriers $N=70$, Gln/Gln $N=57$; controls: Pro carriers $N=115$, Gln/Gln $N=101$). Closed circles represent Pro carriers. Open circles represent individuals with the Gln/Gln genotype. Bars represent the standard error. PFC: prefrontal cortex.

Several studies have suggested that sigma-1 receptor ligands are useful in the treatment of schizophrenia. In animal studies, sigma receptor antagonists such as BMY-14802 and panamesine (EMD57445) improved methamphetamine-induced behavioral sensitization in rats (Ujike et al., 1992a,b) and sigma receptor antagonists such as BMY-14802, haloperidol and NE-100 improved phencyclidine (PCP)-induced behavioral abnormalities (Ogawa et al., 1994; Okuyama et al., 1995; Takahashi et al., 2001). On the other hand, recent studies suggest that donepezil, a sigma-1 receptor agonist, plays a role in memory processing and that fluvoxamine, also a sigma-1 agonist, can improve PCP-induced cognitive deficits in mice (Hashimoto et al., 2007a; Kunitachi et al., 2009). In clinical trials, putative selective antagonists of sigma receptors such as eliprodil (SL 82.0715) and panamesine showed antipsychotic effects in schizophrenia (Frieboes et al., 1997; Huber et al., 1999; Modell et al., 1996; Muller et al., 1999), while sigma-1 receptor agonists (fluvoxamine) improved cognitive impairments and extra-pyramidal symptoms seen in schizophrenia (Furuse and Hashimoto, 2010a, b; Iyo et al., 2008). Due to the differential effects of sigma-1 agonists and antagonists, stabilization of the sigma-1 receptor may be useful in the treatment of schizophrenia. Despite the importance of prefrontal dysfunctions in schizophrenia, no drug has been approved for the treatment of this aspect of schizophrenia. We investigated the effect of rs1800866 using *in silico* analysis (Polyphen2 database; http://genetics.bwh.harvard.edu/pph2) (Adzhubei et al., 2010), which predicts whether non-synonymous SNP affects protein structure and function. However, this mutation was not predicted to affect protein structure and function with a score of 0.001 (sensitivity: 1.00; specificity: 0.06) on HumVar in this analysis. Further research will be required to clarify the effects of *SIGMAR1* functions on the pathophysiology of schizophrenia.

There were several limitations to this study. We evaluated both patients with schizophrenia and controls using SCID in the NIRS analysis, while we evaluated participants using unstructured clinical interview in the genetic association analysis. Because we did not use SCID to evaluate these subjects in the genetic association analysis, it might not be representative of the typical patients with schizophrenia and healthy subjects, despite the confirmations of the diagnosis by at least two trained psychiatrists. In the NIRS results, the main effect was significant both for diagnosis and genotype, but their interaction was not. This may indicate that both diagnosis of schizophrenia and Pro genotype reduce the PFC activity, but these findings are independent phenomena and do not mutually interact in the brain. On the other hand, a study by Takizawa et al. has found a significant interaction of diagnosis and Gln2Pro genotype (Takizawa et al., 2009a). Their results may be more straightforward than the present study because the genotype effect was seen only in the patients group. We could not exclude the possibility that many antipsychotics including haloperidol might interact with the polymorphism effect. The number of the subjects in the present study

was more than 3 timesthat of Takizawa et al. (Takizawa et al., 2009a); however, Takizawa et al. used 52-channel NIRS thatwas 26 times greater than the present study. We examined activation on PFC using two-channel NIRS because the brain activations during verbal fluency task with two 24-channel NIRS were prominent in frontal channels in schizophrenia (Suto et al., 2004). However, we might not have detected the most sensitive region affected by the Gln2Pro polymorphism due to narrow spatial coverage of the PFC.

In conclusion, the *SIGMAR1* polymorphism is associated with schizophrenia risk. Prefrontal function during verbal generation is modulated by variation in the *SIGMAR1* genotype. These findings may lead to a new target for antipsychotics.

Supplementary materials related to this article can be found online at doi:10.1016/j.pnpbp.2011.04.008.

Conflict of interest

All authors declare that they have no conflict of interest.

Acknowledgments

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ARCHIVAL REPORTS

Loss of Function Studies in Mice and Genetic Association Link Receptor Protein Tyrosine Phosphatase α to Schizophrenia

Nagahide Takahashi, Karin Sandager Nielsen, Branko Aleksic, Steffen Petersen, Masashi Ikeda, Itaru Kushima, Nathalie Vacaresse, Hiroshi Ujike, Nakao Iwata, Véronique Dubreuil, Naheed Mirza, Takeshi Sakurai, Norio Ozaki, Joseph D. Buxbaum, and Jan Sap

Background: Solid evidence links schizophrenia (SZ) susceptibility to neurodevelopmental processes involving tyrosine phosphorylation-mediated signaling. Mouse studies implicate the *Ptpra* gene, encoding protein tyrosine phosphatase RPTPα, in the control of radial neuronal migration, cortical cytoarchitecture, and oligodendrocyte differentiation. The human gene encoding RPTPα, *PTPRA*, maps to a chromosomal region (20p13) associated with susceptibility to psychotic illness.

Methods: We characterized neurobehavioral parameters, as well as gene expression in the central nervous system, of mice with a null mutation in the *Ptpra* gene. We searched for genetic association between polymorphisms in *PTPRA* and schizophrenia risk (two independent cohorts, 1420 cases and 1377 controls), and we monitored *PTPRA* expression in prefrontal dorsolateral cortex of SZ patients (35 cases, 2 control groups of 35 cases).

Results: We found that $Ptpra^{-/-}$ mice reproduce neurobehavioral endophenotypes of human SZ: sensitization to methamphetamine-induced hyperactivity, defective sensorimotor gating, and defective habituation to a startle response. *Ptpra* loss of function also leads to reduced expression of multiple myelination genes, mimicking the hypomyelination-associated changes in gene expression observed in postmortem patient brains. We further report that a polymorphism at the *PTPRA* locus is genetically associated with SZ, and that *PTPRA* mRNA levels are reduced in postmortem dorsolateral prefrontal cortex of subjects with SZ.

Conclusions: The implication of this well-studied signaling protein in SZ risk and endophenotype manifestation provides novel entry points into the etiopathology of this disease.

Key Words: Mouse model, myelination, *PTPRA*, RPTPα, schizophrenia, tyrosine phosphatase

Schizophrenia (SZ; OMIM database entry #181500) is diagnosed by the joint appearance of positive (hallucinations, delusions), negative (disturbed affective and social functioning), and cognitive symptoms. Initial hypotheses about the pathophysiologic mechanism derive from pharmacologic observations: blocking D2 receptors can alleviate positive symptoms, and *N*-methyl-D-aspartate receptor (NMDA-R) antagonists can mimic disease symptoms, which provides support for the dopaminergic and glutamatergic hypotheses. Imaging and postmortem analyses reveal that SZ is accompanied by neuropathologic abnormalities, including decreased myelin content, atypical neuronal cytoarchitecture, and altered laminar organization, suggesting abnormalities in neural development (1). Gene expression studies indicate abnormalities in myelination (2).

Schizophrenia has a significant genetic basis (3). Nonaffected kin can display quantifiable neurobehavioral abnormalities, perhaps reflecting manifestation of a subset of genetic predispositions. The identification of SZ-associated genes is starting to provide insights into disease etiology by implicating molecular signaling pathways. One of the first and most reproducible instances of genetic association with SZ is *neuregulin 1* (*NRG1*), the product of which, signaling via epidermal growth factor (EGF) receptor-like tyrosine kinases, modulates oligodendrocyte development, neuronal migration and differentiation, and glutamatergic and gamma-aminobutyric acid–ergic neurotransmission (4–8). Two other genes in the NRG1 pathway, *ERBB4* encoding a tyrosine kinase receptor for NRG1 and *PTPRZ1* encoding an ERBB4-associated protein tyrosine phosphatase in the oligodendrocyte lineage, are also genetically associated with SZ (9–11). NRG1 signaling may be functionally linked to NMDA-R modulation (11,12), perhaps via phosphorylation of the latter by the Src-family tyrosine kinases Fyn (13) and c-Src (14). Among other predisposition genes for SZ are cell adhesion molecules such as CHL1 and NCAM (15–21), the signaling activities of which also rely on Src-family kinases (SFKs).

Receptor protein tyrosine phosphatase RPTPα (encoded by human *PTPRA* and mouse *Ptpra*) is a physically associated signaling subunit of CHL1 and NCAM, through its well-documented role in regulating Fyn (22–24). RPTPα can also modulate SFKs downstream of EGF-receptor (*ERBB1*) activation (25). *Ptpra* is abundantly ex-

From the Conte Center for the Neuroscience of Mental Disorders (NT, TS, JDB) and the Departments of Psychiatry (NT, TS, JDB), Pharmacology and Systems Therapeutics (TS), Neuroscience (JDB), Genetics and Genomic Sciences (JDB), and Black Family Stem Cell Institute (TS), Mount Sinai School of Medicine, New York, New York; NeuroSearch A/S (KSN, SP, NM), Ballerup, Denmark; Department of Psychiatry (BA, IK, NO), Nagoya University Graduate School of Medicine, Aichi, Japan; Biotechnology Research and Innovation Centre and Institute of Biomedical Sciences (NV, JS), University of Copenhagen, Copenhagen, Denmark; Department of Neuropsychiatry (HU), Graduate School of Medicine and Dentistry, Okayama University, Okayama, and Department of Psychiatry (MI, NI), Fujita Health University School of Medicine, Aichi, Japan; and University Paris Diderot (VD, JS), Sorbonne Paris Cité, Epigenetics and Cell Fate, UMR 7216 CNRS, Paris, France.

Authors NT, KSN, and BA contributed equally to this work.

Address correspondence to Jan Sap, Ph.D., University of Paris Diderot, Sorbonne Paris Cité, Epigenetics and Cell Fate, UMR 7216 CNRS, Bâtiment Lamarck, Case 7042, 35 rue Hélène Brion, F-75205 Paris Cedex 13, France. E-mail: jan.sap@univ-paris-diderot.fr

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pressed in the developing central nervous system and remains highly expressed in the adult (26,27). In mice, loss of *Ptpra* function is associated with neurodevelopmental defects in peripheral myelination (28), oligodendrocyte differentiation and myelin basic protein (MBP) expression (29), radial cortical migration (27), misorientation of apical dendrites of deep layer pyramidal neurons (24), reduced NMDA-R phosphorylation, and impairments in synaptic plasticity and short-term memory (27,30,31). Many of these effects reflect a function of RPTPα in regulating SFKs (24,25,29,31–33). Interestingly, *PTPRA* maps to a chromosomal region (20p13) that has been linked to SZ (34,35).

Given the multifold involvement of RPTPα in neurodevelopmental and signaling pathways associated with SZ, we set out to explore whether loss of *Ptpra* function in mice engendered neurobehavioral abnormalities or gene expression signatures relevant to SZ. Positive findings led us to pursue association between polymorphisms at the *PTPRA* locus and disease risk and changes in *PTPRA* expression in dorsolateral prefrontal cortex of patients.

Methods and Materials

Full details of all procedures can be found in Supplement 1.

Mice

Generation of RPTPα-deficient ($Ptpra^{-/-}$) mice has been described previously (33). The allele was backcrossed 10 times with C57Bl/6J mice. Control wildtype (WT) mice were generated by intercross of $Ptpra^{+/-}$ heterozygotes.

Mouse Motor and Neurobehavioral Testing

Spontaneous exploratory locomotor activity and drug-induced hyperactivity were generally assessed as in Butini *et al.* (36), and prepulse inhibition and acute startle responses as in Andreasen *et al.* (37).

Gene Expression Analysis

RNA was extracted from mouse whole brain and human dorsolateral prefrontal cortex and subject to quantitative polymerase chain reaction (qPCR) analysis.

Genetic Association

This was performed essentially as in Ikeda *et al.* (38), followed by inclusion of a second independent cohort.

Results

$Ptpra^{-/-}$ Mice Display Enhanced Psychostimulant-Induced Hyperactivity, Deficient Sensorimotor Gating, and Failure to Habituate to a Startle Response

Dissection of multifactorial diseases is helped by the identification of genetically based quantitative nonapparent "endophenotypes" that are proximal consequences of genetic predisposition but precede or are not necessarily accompanied by manifestation of the disease itself. This reductionist approach is particularly relevant to the dissection of psychiatric disease and to its animal modeling (39).

RPTPα participates in several processes implicated in pharmacologic and neurodevelopmental descriptions of SZ, and $Ptpra^{-/-}$ mice manifest neuropathologic abnormalities reminiscent of those reported in patients. To determine whether loss of function (LOF) of mouse *Ptpra* results in behavioral and neuropsychological abnormalities associated with SZ, we focused on three models: locomotor response to psychostimulants, prepulse inhibition (PPI) as a measure of sensorimotor gating, and the water-maze test for spatial memory.

The studies were performed on a previously described *Ptpra* null allele (27). We first assessed sensorimotor capabilities to exclude the possibility of compounding effects (Table S1 in Supplement 1). Latency to fall off a beam or from an accelerating rotarod revealed no obvious abnormality in general sensorimotor capability of $Ptpra^{-/-}$ mice [beam walk: $F(1,33) = 0$, $p = 1$ and $F(1,33) = .298$, $p = .589$ respectively]. Spontaneous exploratory locomotor activity was also unaffected by *Ptpra* allelic status [$F(1,33) = 1.983$, $p = .169$). We concluded that *Ptpra* LOF does not engender sensorimotor abnormalities that would affect the subsequent analyses.

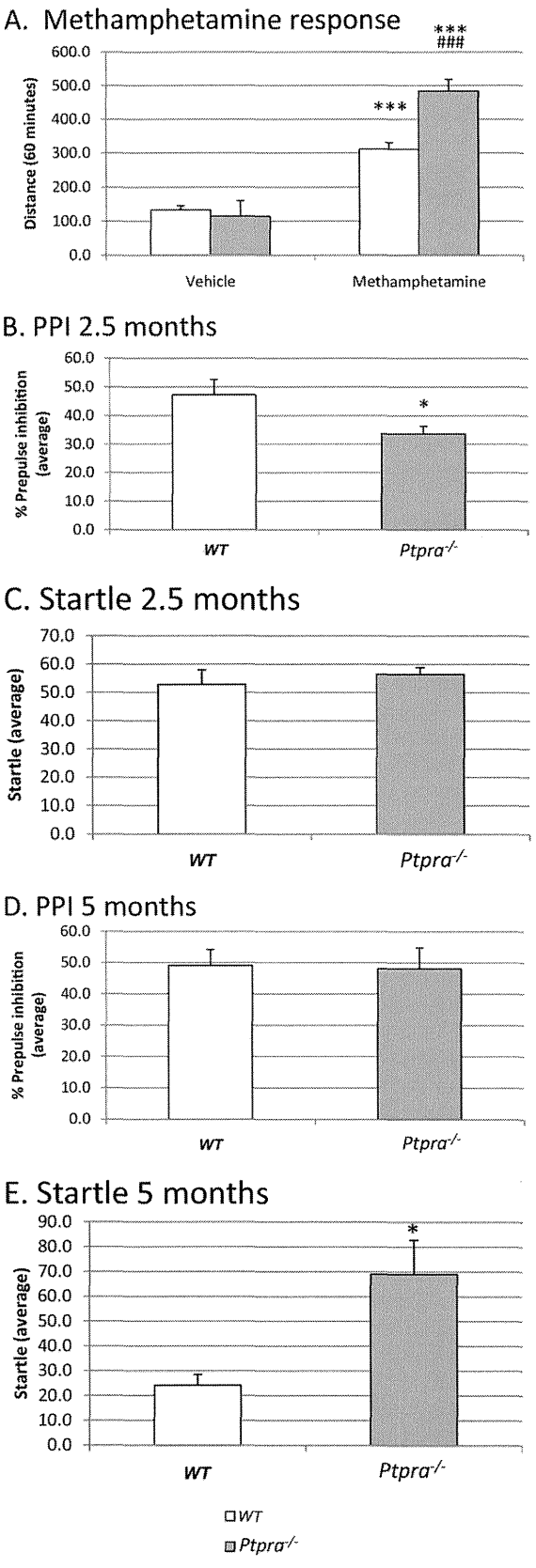
We subsequently asked whether *Ptpra* LOF altered the locomotor response to the psychostimulant methamphetamine (MAMPH), a pharmacologic model inspired by the dopaminergic hypothesis of SZ (40). We found locomotor activity after acute MAMPH administration (2 mg/kg) to be significantly higher in $Ptpra^{-/-}$ mice than in controls [main effect of genotype $F(1,31) = 5.753$, $p = .023$; drug treatment: $F(1,31) = 72.386$, $p < .001$; genotype × drug treatment: $F(1,31) = 8.797$, $p = .006$; post hoc multiple comparisons: MAMPH (WT) vs. MAMPH ($Ptpra^{-/-}$): $p < .001$; Figure 1A].

Given the functional association of RPTPα with NMDA-R (27,30,31), we also investigated the effect of administration of the noncompetitive NMDA-R antagonist MK-801, known for its ability to induce psychotic symptoms in healthy humans. However, at the dose used (.2 mg/kg), MK-801 did not significantly increase locomotor activity in WT mice (not shown).

We next assessed PPI of the startle response, a suggested SZ endophenotype. PPI denotes attenuation of a startle motor response to a sensory (acoustic) stimulus when the latter is immediately (< 500 msec) preceded by a milder stimulus. Used as an operational measure of sensorimotor gating, PPI is impaired in SZ individuals and their unaffected relatives, and antipsychotics can reverse impairment of PPI in experimental models (41). It constitutes a preattentive process akin to a reflex response. We tested the mice on two occasions (2.5 months apart) to determine 1) whether any PPI abnormality persisted and 2) whether normal habituation to the startle response occurred, because impaired habituation is a hallmark of SZ (42). Such a longitudinal design is rarely applied to knockout mice, partly because of difficulties in the use of test batteries in rodents (43,44).

At the initial age of analysis (2.5 months), we observed no difference in startle response between $Ptpra^{-/-}$ and WT mice [Figure 1C; $F(1,14) = .332$, $p = .576$]. In contrast, $Ptpra^{-/-}$ mice manifested a significant reduction in PPI [Figure 1B; $F(1,14) = 6.006$, $p = .032$). This genotype-dependent difference in PPI disappeared at a more advanced age [5 months; $F(1,13) = .034$, $p = .857$; Figure 1D], suggesting a critical time period for manifestation of this abnormal phenotype. Strikingly, however, at the more advanced age, the typical habituation (reduced response) to the acoustic startle stimulus alone observed in WT animals [startle at 2.5 months compared with 5 months: $F(1,14) = 11.797$, $p = .014$] did not occur in $Ptpra^{-/-}$ mice [startle at 2.5 months compared with 5 months: $F(1,13) = .013$, $p = .914$), leading to a significant difference in acoustic startle response during this retesting at 5 months (Figure 1E).

Finally, we subjected $Ptpra^{-/-}$ mice to a water-maze test, a hippocampal-dependent model of spatial memory. Impaired hippocampal-based function in SZ is well documented (45). Detailed analysis revealed no genotype differences in place finding (swim distance and latency to target; Figure 2A) nor in the probe test (recall of spatial position of the platform; Figure 2B) or reversal learning (Figure 2C).



Loss of *Ptpra* Function Leads to Reduced Central Nervous System Levels of Myelin Markers and SZ-Associated genes

Increased MAMPH sensitivity, impaired PPI, and failure to habituate to a startle response are commonly accepted indicators for modeling SZ-associated states in mice. To assess whether the relevance of *Ptpra*$^{-/-}$ mice as a model for SZ-associated abnormalities extends beyond neuropsychological parameters, we began assessing SZ-associated gene expression markers. Imaging analysis, postmortem brain studies, genetic association studies, and gene expression studies reveal that abnormal oligodendroglial function and myelination are commonly associated with SZ (2,46–49). One of the major targets of RPTPα, the Src family kinase Fyn, plays important roles in myelination (50–53). A transient defect in peripheral myelination has been documented in the strain of *Ptpra*$^{-/-}$ mice studied here (28); an independently generated *Ptpra*$^{-/-}$ strain was recently reported to display impaired oligodendrocyte differentiation in vitro and reduced MBP immunostaining in vivo (29). We therefore investigated the expression of myelin related genes in the brains of our strain of *Ptpra*$^{-/-}$ mice.

Figure 1. Methamphetamine (MAMPH) sensitivity, prepulse inhibition (PPI), and startle response in wildtype (WT) and *Ptpra*$^{-/-}$ mice. **(A)** Influence of genotype on locomotor response to MAMPH challenge. Methamphetamine resulted in pronounced hyperactivity in both genotypes ($p < .001$ vs. vehicle [WT] and vehicle [*Ptpra*$^{-/-}$], respectively). However, the locomotor response to MAMPH was exaggerated in *Ptpra*$^{-/-}$ compared with WT mice ($p < .001$, MAMPH [WT] vs. MAMPH [*Ptpra*$^{-/-}$]). The study was run as a within-subject design in which each individual mouse served as its own control by injecting them with vehicle, MAMPH (2 mg/kg), or MK801 (.2 mg/kg, data not shown, see text) in a semirandomized order ensuring representation of all treatment groups on each test day over 3 days. Compounds were dosed intraperitoneally immediately before test start, $n = 7$ or 8 (*Ptpra*$^{-/-}$) and 8 or 9 (WT). The animals were 3.5 months old at testing. Data are represented as mean distance traveled (± SEM) over 60 min. Statistical evaluation was performed by applying two-way analysis of variance (ANOVA) with genotype and drug as factors followed by Fishers Least Significant Difference test for multiple comparisons. ***$p < .001$ vs. vehicle (*Ptpra*$^{-/-}$) and vehicle (WT), respectively. ###$p < .001$ vs. WT-methamphetamine. **(B)** Effect of genotype on PPI of the acoustic startle response at age 2.5 months. *Ptpra* gene disruption leads to reduced prepulse inhibition of the acoustic startle response compared with WT mice at age 2.5 months ($p < .05$). Data from four prepulse intensities (pp 4, pp 8, pp 16, and pp 24) are collapsed and expressed as mean ± SEM, $n = 7$ (*Ptpra*$^{-/-}$) and 8 (WT). Statistical evaluation was performed by applying two-way ANOVA with genotype and sex as factors. *$p < .05$ vs. WT. **(C)** Effect of genotype on acoustic startle response at age 2.5 months. No effect of *Ptpra* gene disruption is seen on the startle response to a 120-dB noise burst at age 2.5 months compared with WT mice ($p > .05$). Data are expressed as mean ± SEM, $n = 7$ (*Ptpra*$^{-/-}$) and 8 (WT). Statistical evaluation was performed by applying two-way ANOVA with genotype and sex as factors. **(D)** Effect of genotype on prepulse inhibition of the acoustic startle response at age 5 months. At 5 months, the reduction of PPI noted at 2.5 months was no longer evident in *Ptpra*$^{-/-}$ mice ($p > .05$ vs. WT), indicating a critical time period for manifestation of this phenotype. Data from four prepulse intensities (pp 4, pp 8, pp 16, and pp 24) are collapsed and expressed as mean ± SEM, $n = 7$ for both genotypes. Statistical evaluation was performed by applying two-way ANOVA with genotype and sex as factors. **(E)** Effect of genotype on acoustic startle response at age 5 months. At 5 months, *Ptpra*$^{-/-}$ mice displayed an increased startle response to a 120-dB noise burst compared with WT mice ($p = .013$). This difference is due to a significantly reduced startle response in WT mice at age 5 months compared with 2.5 months ($p = .014$). This habituated response to a startle inducing stimulus is not evident in *Ptpra*$^{-/-}$ mice, because the startle response at 5 months of age is similar to that at 2.5 months ($p = .914$). Data are expressed as mean ± SEM, $n = 7$ (*Ptpra*$^{-/-}$ and WT). Intergroup comparisons were performed by applying two-way ANOVA with genotype and sex as factors. Intragroup comparisons were performed by applying one-way repeated-measure ANOVA with age and genotype as factors. *$p < .05$ versus WT.