Case Reports in Neurology
Case Rep Neurol 2010;2:52–56
DOI: 10.1159/000314406
Published online: May 12, 2010

ISSN 1662–680X
www.karger.com/crn

Major Depressive Disorder Complicated with Spinocerebellar Ataxia: Report of 2 Cases

Nagahisa Okamoto[a] Masafumi Ogawa[b] Yoshiko Murata[b]
Kota Sakamoto[a] Tatsuya Nagai[a] Maki Yamada[a]
Teruhiko Higuchi[a]

Departments of [a]Psychiatry and [b]Neurology, National Center Hospital of Neurology and Psychiatry, Kodaira, Tokyo, Japan

Key Words
Major depressive disorder · Spinocerebellar ataxia · Spinocerebellar degeneration · Selective serotonin reuptake inhibitor · Treatment

Abstract
Background: It is known that patients with spinocerebellar ataxia (SCA) tend to exhibit depressive symptoms. But the pathology of depressive symptoms complicated with SCA, including the reaction to the stress resulting from decreased motor function and central dysfunction due to neurodegeneration, is controversial and remains to be elucidated. To our knowledge, there have been hardly any reports on treatment methods of major depressive disorder (MDD) complicated with SCA.
Case Reports: We report 2 cases in which selective serotonin reuptake inhibitors (SSRIs) were effective against MDD complicated with SCA. Interestingly, one of the patients developed the symptoms of spinocerebellar degeneration (SCD) during the course of the MDD, and the other patient developed the symptoms of MDD during the course of SCA, but complete remission of the MDD occurred in both cases. In our cases, the depressive symptoms may have been caused mainly by an abnormality of reversible neural transmission including serotonin transmission due to central dysfunction, and there is the unlikely possibility that the depressive symptoms are reactive to the stress due to decreased motor function, because the depressive symptoms decreased with SSRIs.
Conclusion: Although cerebellar degeneration is irreversible in SCA patients, our cases suggest that MDD complicated with SCA may be reversible and treatable using antidepressants such as SSRIs with few adverse events. Therefore, it is important for neurologists to detect MDD complicated with SCA early and consult a psychiatrist in order to improve quality of life of SCA patients.

Nagahisa Okamoto
Department of Psychiatry, National Center Hospital of Neurology and Psychiatry
4-1-1, Ogawahigashi
Kodaira, Tokyo 187-8551 (Japan)
Tel. +81 423 412 711, Fax +81 423 461 705, E-Mail okamoton@ncnp.go.jp

Case Reports in Neurology | Case Rep Neurol 2010;2:52–56 | DOI: 10.1159/000314406 | Published online: May 12, 2010 | © 2010 S. Karger AG, Basel | ISSN 1662–680X | www.karger.com/crn

Introduction

It is known that patients with spinocerebellar ataxia (SCA) tend to exhibit depressive symptoms. But there have been hardly any reports regarding treatment of major depressive disorder (MDD) complicated with SCA. The side effects of conventional antidepressants such as tricyclic antidepressants are attributed to their nonspecific interaction with cholinergic, histaminergic, serotonergic, and dopaminergic receptors in the central nervous system, and they often cause various adverse events [1]. Therefore, antidepressants that do not have this nonspecific interaction, such as the selective serotonin reuptake inhibitors (SSRIs), have recently been preferred in the treatment of MDD because of their tolerability. Especially in patients with MDD complicated with neurodegenerative disease, it seems to be difficult to use antidepressants with a nonspecific interaction because of the patients' central vulnerability and their neurologic or autonomic symptoms. SCA consists of a heterogeneous group of neurodegenerative disorders. SSRIs seem to be valuable because they cause few adverse events due to their specific antidepressant action. We report 2 cases of MDD complicated with SCA who successfully responded to SSRIs.

Case Reports

Case 1

The patient is a 53-year-old woman. Her mother was diagnosed with probable SCA at 52 years of age and with MDD at 60 years of age. Her mother died at 72 years of age. Her mother's sister had been diagnosed with probable SCA.

At 49 years of age, the patient developed depressive symptoms, including a severely depressed mood in the morning, loss of interest, insomnia, fatigue, and somatic anxiety. She was diagnosed with MDD at a psychiatric clinic and treated with fluvoxamine (150 mg/day) and milnacipran (125 mg/day). But the depressive symptoms worsened, and anorexia, weight loss (6 kg in 3 months), and suicidal ideation ensued. Therefore, at 50 years of age, she was referred to our hospital. No abnormal findings were observed on serum examinations, including of thyroid function. Her symptoms met all of the diagnostic criteria of DSM-IV for MDD, and her total score on the 17-item Hamilton Rating Scale for Depression (HDSR) was 32. Brain magnetic resonance imaging (MRI) showed diffuse atrophy of the cerebellar cortex (fig. 1), but there were no clear cerebellar manifestations. She was treated with clomipramine (150 mg/day), sulpiride (300 mg/day), and amoxapine (150 mg/day), but treatment was ineffective. At 51 years of age, the depressive symptoms completely ceased within 3 months after starting paroxetine (40 mg/day). She then was able to perform housework without any problems.

At 52 years of age, she developed lightheadedness on standing, and midodrine (4 mg/day) was started. However, the lightheadedness became severer, and she gradually began to experience slurred speech and dysphasia. At 54 years of age, she developed a wide-based gait and often fell while walking. In addition to predominantly truncal cerebellar ataxia, she was found to have autonomic nervous system manifestations (urinary frequency, orthostatic hypotension), pyramidal tract signs (hyperactive lower limb tendon reflexes), impaired ocular pursuit movements, limited sursumversion, dysarthria, and dysphasia. Head-up tilt test was positive. She was diagnosed with probable SCA. She did not wish to undergo genetic diagnosis. Her score on the Mini-Mental State Examination (MMSE) was 22, and her full-scale IQ on the Wechsler Adult Intelligence Scale (WAIS)-III was 63, revealing a mild cognitive disorder. Though the neurological symptoms gradually progressed, MDD was in remission for 3 years after the start of paroxetine.

Case 2

The patient is a 37-year-old male. His mother was diagnosed with probable SCA at 40 years of age, and she died at 58 years of age. Genetic testing revealed that four of his relatives were positive for SCA3.

At 31 years of age, fine movements became difficult. He gradually began to experience lightheadedness on standing and diplopia. His gait became wide-based. At 34 years of age, he became aware of slurred speech and dysphasia, so he visited our hospital. Cerebellar ataxia of his limbs and trunk, autonomic nervous system signs (orthostatic hypotension), pyramidal tract signs (hyperactive tendon reflexes in his limbs), impaired ocular pursuit movements, dysarthria, and dysphasia were noted. Head-up tilt test was positive and he had an erectile disturbance. MMSE score was 27, but full-scale IQ on the WAIS-Revised was 66, revealing mild cognitive dysfunction. Brain MRI showed diffuse cerebellar atrophy (fig. 1). Analysis of the CAG repeats in the SCA3 locus revealed an expanded allele with 69 repeats, and he was diagnosed with SCA3. The symptoms progressed, and at 36 years of age he needed a wheelchair.

At 37 years of age, he developed a severe depressive mood in the morning associated with suicidal ideation and lost his appetite and hardly ate anything. His movements became extremely slow, and all he could do was lie in bed all day. Blood studies, including of thyroid function, showed no abnormalities. The patient fulfilled all of the diagnostic criteria of the DSM-IV for MDD and his total score on the HDSR-17 was 30. He was treated with paroxetine (20 mg/day), and moderate improvement in the depressive symptoms was observed. But acute urinary retention developed, and paroxetine was switched to sertraline (75 mg/day). Subsequently, the urinary retention resolved and the MDD remitted. Though the neurological symptoms gradually progressed, MDD was in remission for 1 year after the start of sertraline.

Discussion

SCA consists of a heterogeneous group of disorders that differ in the extent of the neuropathologica involvement of the cerebellum, brainstem, and basal ganglia. Patients with degenerative cerebellar diseases tend to exhibit depressive symptoms. A study [2] of patients with degenerative cerebellar diseases showed a high prevalence of all mood disorders (68%) including MDD (35.5%). Another study [3] showed that the depressive symptoms were most common in SCA3 patients (60%), as opposed to 23–27% of patients with other types of SCA.

However, the pathology of depressive symptoms associated with SCA is controversial and remains to be elucidated. A direct correlation between Beck Depression Inventory scores and motor incapacitation was found in SCA3 patients [4]. Therefore, it was concluded that depressive symptoms rather seemed to be reactive than primarily related to the disease process itself.

Yet, an association with central dysfunction due to neurodegeneration and depressive symptoms has also been highlighted. It was suggested that the greater depressive complaints in SCA3 may be due to dysfunction of frontal-subcortical circuits in the basal ganglia, structures that are uniquely involved in SCA3 as opposed to the other subtypes [3]. Furthermore, in recent years, there has been emerging evidence suggesting that the cerebellum is involved in various psychiatric disorders [5], and the cerebellar degeneration may be associated with depressive symptoms.

In our cases, depressive symptoms may have been caused mainly by an abnormality of reversible neural transmission including serotonin transmission due to central dysfunction, and there is the unlikely possibility that the depressive symptoms are reactive to the stress due to decreased motor function, because the depressive symptoms remitted with SSRIs.

There have been hardly any reports on the treatment of MDD complicated with SCA, and the only reports that exist are of a case in which electroconvulsive therapy was effective against the catatonic stupor of psychotic depression [6] and a case in which ECT was effective against organic catatonia [7].

Case Reports in Neurology
Case Rep Neurol 2010;2:52–56
DOI: 10.1159/000314406
Published online: May 12, 2010

ISSN 1662–680X
www.karger.com/crn

The symptoms of SCA developed during the course of MDD in case 1, and the symptoms of MDD developed during the course of SCA in case 2. However, complete remission of MDD was achieved with SSRIs in both cases.

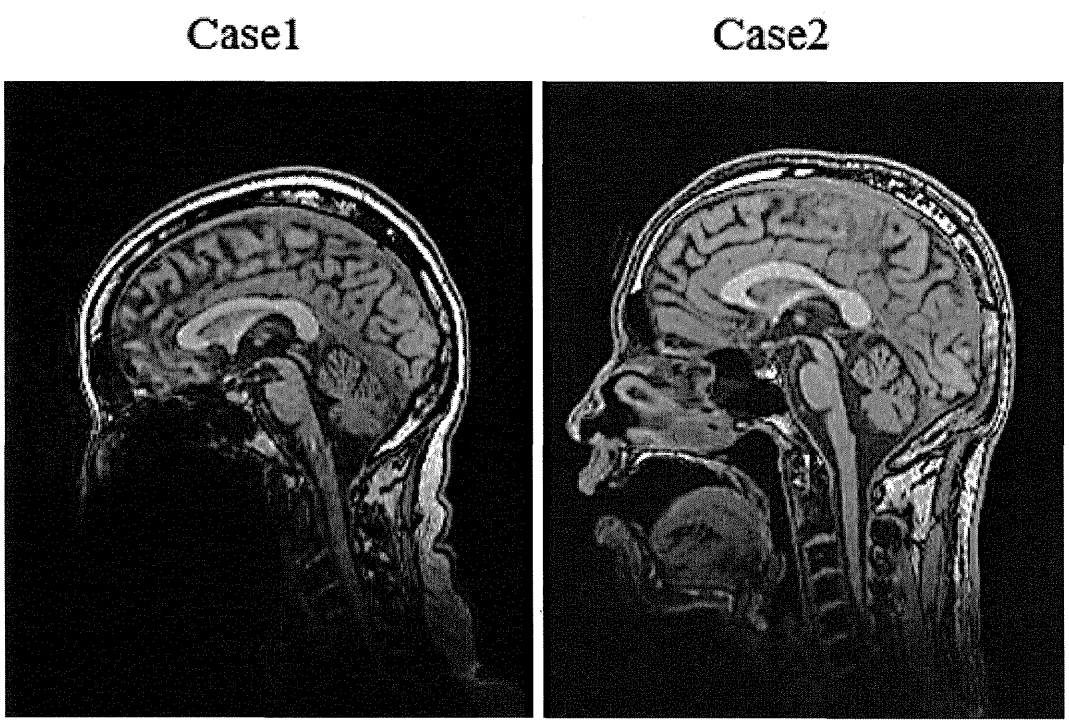
Conclusion

Although cerebellar degeneration is irreversible in SCA patients, our cases suggest that MDD complicated with SCA may be reversible and treatable using antidepressants with small adverse events such as SSRIs. Therefore, it is important for neurologists to detect MDD complicated with SCA early and consult a psychiatrist in order to improve quality of life of SCA patients.

Patients' Consent

Written consent for publication in print and electronically was obtained from the patients.

Fig. 1. The cerebellar cortex can be seen to be atrophic on T1-weighted magnetic resonance images obtained in case 1 and case 2.



References

1 Andrews JM, Nemeroff CB: Contemporary management of depression. Am J Med 1994;97:24S–32S.

2 Leroi I, O'Hearn E, Marsh L, Lyketsos CG, Rosenblatt A, Ross CA, et al: Psychopathology in patients with degenerative cerebellar diseases: a comparison to Huntington's disease. Am J Psychiatry 2002;159:1306–1314.

3 McMurtray AM, Clark DG, Flood MK, Perlman S, Mendez MF: Depressive and memory symptoms as presenting features of spinocerebellar ataxia. J Neuropsychiatry Clin Neurosci 2006;18:420–422.

4 Cecchin CR, Pires AP, Rieder CR, Monte TL, Silveira I, Carvalho T, et al: Depressive symptoms in Machado-Joseph disease (SCA3) patients and their relatives. Community Genet 2007;10:19–26.

5 Baldaçara L, Borgio JG, Lacerda AL, Jackowski AP: Cerebellum and psychiatric disorders. Rev Bras Psiquiatr 2008;30:281–289.

6 Suzuki K, Itou K, Takano T, Harada N, Awata S, Matsuoka H: Catatonic stupor superimposed on hereditary spinocerebellar degeneration resolved with electroconvulsive therapy. Prog Neuropsychopharmacol Biol Psychiatry 2006;30:1179–1181.

7 Folkerts HW, Stadtland C, Reker T: ECT for organic catatonia due to hereditary cerebellar ataxia. J ECT 1998;14:53–55.

Contribution: all authors participated in the management of the patient. N. Okamoto wrote the case report.

Genome-Wide Association Study of Schizophrenia in a Japanese Population

Masashi Ikeda, Branko Aleksic, Yoko Kinoshita, Tomo Okochi, Kunihiro Kawashima, Itaru Kushima, Yoshihito Ito, Yukako Nakamura, Taro Kishi, Takenori Okumura, Yasuhisa Fukuo, Hywel J. Williams, Marian L. Hamshere, Dobril Ivanov, Toshiya Inada, Michio Suzuki, Ryota Hashimoto, Hiroshi Ujike, Masatoshi Takeda, Nick Craddock, Kozo Kaibuchi, Michael J. Owen, Norio Ozaki, Michael C. O'Donovan, and Nakao Iwata

Background: Genome-wide association studies have detected a small number of weak but strongly supported schizophrenia risk alleles. Moreover, a substantial polygenic component to the disorder consisting of a large number of such alleles has been reported by the International Schizophrenia Consortium.

Method: We report a Japanese genome-wide association study of schizophrenia comprising 575 cases and 564 controls. We attempted to replicate 97 markers, representing a nonredundant panel of markers derived mainly from the top 150 findings, in up to three data sets totaling 1990 cases and 5389 controls. We then attempted to replicate the observation of a polygenic component to the disorder in the Japanese and to determine whether this overlaps that seen in UK populations.

Results: Single-locus analysis did not reveal genome-wide support for any locus in the genome-wide association study sample (best $p = 6.2 \times 10^{-6}$) or in the complete data set in which the best supported locus was *SULT6B1* (rs11895771: $p = 3.7 \times 10^{-5}$ in the meta-analysis). Of loci previously supported by genome-wide association studies, we obtained in the Japanese support for *NOTCH4* (rs2071287: $p_{meta} = 5.1 \times 10^{-5}$). Using the approach reported by the International Schizophrenia Consortium, we replicated the observation of a polygenic component to schizophrenia within the Japanese population ($p = .005$). Our trans Japan–UK analysis of schizophrenia also revealed a significant correlation (best $p = 7.0 \times 10^{-5}$) in the polygenic component across populations.

Conclusions: These results indicate a shared polygenic risk of schizophrenia between Japanese and Caucasian samples, although we did not detect unequivocal evidence for a novel susceptibility gene for schizophrenia.

Key Words: Genome-wide association study, *NOTCH4*, polygenic component, schizophrenia, *SULT6B1*

Epidemiologic studies show that genetic factors account for more than 80% of the population variance in susceptibility for schizophrenia; however, as with virtually all other relatively common disorders, it has historically proven difficult to identify the specific genetic variants involved (1).

The application of genome-wide association technology to large case–control samples of mainly European ancestry has recently implicated a number of risk loci for which the evidence is strong. These include loci defined by single nucleotide polymorphisms (SNPs) in which the effects are weak (odds ratios [ORs] 1.1–1.25) among which the strongest supported loci are *zinc finger protein 804 A* (*ZNF804A*) (2–5), a broad region including the major histocompatibility complex (MHC) on chromosome 6p21.3–22.1 (6–8), *neurogranin* (*NRGN*), and *transcription factor 4* (*TCF4*) (8).

Although the robust support for a number of recently implicated loci represents something of a break from the past inconsistencies, little of the genetic variance of schizophrenia can be explained by the loci identified thus far. One explanation for this is that much of the risk is conferred by common but weak genetic effects that require larger samples. Another explanation is that most of the risk cannot be readily detected by genome-wide association studies (GWAS), the missing genetic component being conferred by mutations that exert substantial individual effects that are rare or even unique to individual pedigrees.

Although the relative contributions of these classes of variant awaits empiric resolution, the GWAS of the International Schizophrenia Consortium (ISC) provided strong support for a substantial polygenic contribution (at least 30%) to the population risk of schizophrenia, much of which is conferred by common alleles with small effect sizes (6,9,10). The basic principle of their analysis was that in the presence of a substantial common polygenic component, although most of the individual genetic effects will not be

From the Department of Psychiatry (MI, BA, YK, ToO, KuK, IK, YI, TK, TaO, YF, NI), School of Medicine, Fujita Health University, Toyoake, Aichi, Japan; Medical Research Council (MI, HJW, MLH, DI, NC, MJO, MCO), Centre for Neuropsychiatric Genetics and Genomics, Department of Psychological Medicine and Neurology, School of Medicine, Cardiff University, Cardiff, United Kingdom; Department of Psychiatry (BA, IK, YI, YN, NO), Graduate School of Medicine, Nagoya University, Nagoya, and Japan Science and Technology Agency (BA, YK, ToO, KuK, IK, YI, YN, TK, TaO, YF, MS, RH, MT, NO, NI), the Core Research for Evolutional Science and Technology, Kawaguchi, Japan; Biostatistics and Bioinformatics Unit (MLH, DI), School of Medicine, Cardiff University, Cardiff, United Kingdom; Seiwa Hospital (TI), Institute of Neuropsychiatry, Tokyo; Department of Neuropsychiatry (MS), Graduate School of Medicine, University of Toyama, Toyama; Department of Psychiatry (RH, MT), Graduate School of Medicine, Osaka University, Osaka; Molecular Research Center for Children's Mental Development (RH, MT), United Graduate School of Child Development, Osaka University, Kanazawa University, and Hamamatsu University School of Medicine, Osaka; Department of Neurospsychiatry (HU), Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Science, Okayama; and Department of Cell Pharmacology (KoK), Graduate School of Medicine, Nagoya University, Nagoya, Japan.

Authors MI and BA contributed equally to this work.

Address correspondence to Nakao Iwata, M.D., Ph.D., Department of Psychiatry, School of Medicine, Fujita Health University, Toyoake, Aichi, 470-1192; Japan. E-mail: nakao@fujita-hu.ac.jp.

Received May 20, 2010; revised Jul 9, 2010; accepted Jul 9, 2010.

0006-3223/$36.00
doi:10.1016/j.biopsych.2010.07.010

BIOL PSYCHIATRY 2011;69:472–478

detectable in current sample sizes, the sum of many such effects across multiple SNPs might differ between cases and controls. After discounting the influence of various potential sources of bias, the authors concluded that the findings were best explained by the existence of an important polygenic component to the disorder comprising a large number of common alleles, although some contribution from low-frequency alleles was not excluded or deemed unlikely (6).

There were two additional striking findings in the ISC article (6). The first was that those alleles selected as "risk" alleles for schizophrenia were also enriched in people with bipolar disorder, supporting the hypothesis of shared genetic susceptibility between these disorders (11,12). The second was that sets of "risk" alleles defined from white individuals of European origin were better at predicting affected status in other white European subjects than they were in African Americans, although an attenuated effect was seen in an African American sample. This may be attributable to differences in allele frequencies and linkage disequilibrium between Europeans and African Americans, although genetic heterogeneity remains a possibility. In this article describing a study that sought novel susceptibility variants, we report the first GWAS for schizophrenia in a Japanese sample. Although the Japanese population is considered relatively homogeneous (13), GWAS studies in other populations strongly suggest that our study of 575 cases and 564 controls is underpowered to detect any findings at genome-wide levels of significance. Thus, we attempted to enhance power by following up the top 150 of the most strongly supported SNPs from the GWAS in an independent sample of 1511 cases and 1517 controls drawn from the Japanese population as well as 479 cases and 2938 controls from the United Kingdom (2). We also sought to examine whether the Japanese population shares with Europeans a polygenic component for schizophrenia and bipolar disorder using schizophrenia and bipolar case–control samples from the United Kingdom that have been previously subjected to GWAS (2,14). Because it is unlikely that stratification effects would bias the allele distributions en masse in samples ascertained in Japan in the same direction as in a European sample, confirmation of a shared polygenic effect argues strongly against the idea that residual uncontrolled stratification is responsible for the effect. Moreover, because rare alleles of large effect are expected to reflect an ongoing process of new mutation (to compensate for their removal by selection), the existence of transcontinental effects also argue against the idea that rare alleles alone can drive this effect, it being unlikely that relatively new variants would be carried on the same ancestral haplotypes in both populations.

Methods and Materials

Participants

We selected 575 patients with schizophrenia (43.5 ± 14.8 years) and 564 healthy controls (44.0 ± 14.4 years) for genome-wide association analysis (our screening GWAS: [JPN_GWAS]). All subjects were unrelated, living in the Tokai area of the mainland of Japan, and self-identified as Japanese. The details of the sample and copy number variation analysis of this GWAS data set have been reported previously (15), and see also Supplement 1.

For follow-up studies, we used an independent Japanese sample comprising 1511 cases (aged 45.9 ± 14.0 years) and 1517 controls (aged 46.0 ± 14.6 years) diagnosed and ascertained in the same way as the GWAS data set. These samples were recruited from three areas on the Japanese mainland, comprising the Kansai and Chugoku areas in addition to the Tokai area. To enhance the sample in the replication analysis, data were added from 934 Japanese controls genotyped by Illumina550 (Illumina, San Diego, California) as part of the Japanese Single Nucleotide Polymorphisms (JSNP) project (http://snp.ims.u-tokyo.ac.jp/index.html). If SNP data were available in the JSNP sample, we merged the two sample sets to form a final Japanese replication sample (we refer this as "Rep_JPN") comprising 1511 cases and 2451 controls (SNPs genotyped in both samples can be seen in Table S1 in Supplement 2).

We additionally included data from a UK schizophrenia GWAS data set of 479 cases and 2938 controls genotyped using the Affymetrix 500K array (Santa Clara, California), details of which have been reported before (2,14).

For the polygenic component analysis, we also included the Wellcome Trust Case-Control Consortium (WTCCC) bipolar disorder data set of 1868 cases and 2938 shared controls, details of which are reported elsewhere (2,14).

After complete description of the study to the subjects, written informed consent was obtained. This study was approved by the ethics committees of each university participating in this project.

GWAS and Quality Control

Genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 5.0 according to the manufacturer's protocol. After applying several quality control (QC) criteria (e.g., call rate ≥ 95%, autosomal chromosomes, Hardy–Weinberg equilibrium (HWE) ≥ .0001 and minor allele frequency [MAF] ≥ 5%; Supplement 1), the final GWAS consisted of 1108 samples (560 cases and 548 controls) and 297,645 SNPs (MAF ≥ 5%).

Q-Q plots were generated on the basis of allele-wise analysis of SNPs that passed QC (Supplement 1), and our observed value of λ is consistent with those generally reported in well-matched samples ($\lambda = 1.065$ and $\lambda_{1000} = 1.117$).

Follow-Up Genotyping

Follow-up genotyping in our independent Japanese case–control sample was performed by Sequenom (San Diego, California) using the Sequenom iPLEX Gold System. Markers that could not be assayed on this platform were genotyped using a TaqMan assay (Applied Biosystems, Foster City, California).

Candidate SNPs were selected for replication as follows. First, the top 200 SNPs were identified (corresponding to $p \sim < 5 \times 10^{-4}$). Highly correlated markers based on $r^2 > .9$ to a more significant marker within 100 kb (r^2 was based on HapMap information [release Number 24, October 2008] and our own GWAS from controls) were then removed. From this list, we included the following: 1) SNPs with $p < 5 \times 10^{-5}$ ($n = 15$ after 11 redundant SNPs removed. Total number = 26. Of these, two SNPs failed for primer design. 2) Under the premise that in GWAS analysis, power favors more common alleles and that the enrichment for true associations is greater in this category of alleles (6), SNPs with MAF ≥ 10% surpassing a more relaxed threshold ($P < \sim 3.5 \times 10^{-4}$) were selected, corresponding to the top 150 SNPs ($n = 76$ after 12 low MAF SNPs and 36 redundant SNPs removed. This resulted in a total of 124. Of these, 5 SNPs failed primer design. We additionally included 13 SNPs that ranked from 151st to 200th on the grounds that they could be included in the Sequenom panels of markers without compromising the design of the higher-priority SNPs. Consequently, 97 SNPs were genotyped in the replication sample, of which 5 did not pass QC on the basis of genotype call rate (> .95) and HWE ($p > .001$). All genotype calls were confirmed by visual inspection of cluster plots.

SNP-Based Association Analysis

Consistent with most other GWAS, our study is based upon allele-wise association analysis which assumes an additive model.

Genomic control adjusted p values were also calculated based upon median chi-square statistics. This was performed using PLINK v1.07 (16).

Combined analysis across data sets (Meta_JPN: JPN_GWAS + Rep_JPN, Meta_ALL: JPN_GWAS + Rep_JPN + UK schizophrenia) were conducted using the Cochran–Mantel Haenszel (CMH) approach conditioned by sample as implemented in PLINK v. 1.07.

Polygenic Component Analysis

Discovery (for selecting "score alleles" based on association statistics) and targeting (for calculation of polygenic score) samples are summarized in Table S2 in Supplement 1. Briefly, we examined five discovery and target pairs:

1. Japanese: A set of 280 cases and 274 controls were selected for discovery, and the results were tested in an additional set of 280 cases and 274 controls. The discovery/target samples were selected at random (on the basis of random number generation) from the Japanese GWAS data set. This procedure was repeated 1000 times to ensure the results of this analysis were representative of random divisions of the data set.

2, 3. Each of the UK schizophrenia (479 schizophrenia and 2938 controls) (2) and bipolar (1868 cases and 2938 controls) (14) samples were used separately as a discovery data set to generate lists of "risk" alleles that were tested in the full Japanese GWAS sample.

4, 5. The full Japanese GWAS sample was used as a discovery data set to generate lists of "risk" alleles that were tested in the UK schizophrenia and bipolar data sets.

For the UK data sets, we used the QC criteria applied in the primary manuscripts (2,14) in which SNPs that deviated from HWE ($p < 1 \times 10^{-5}$ in cases or .001 in control) and had a low call rate (< 97%) were excluded. Note that the criteria for HWE exclusion in the UK data set is slightly different from that in the Japanese GWAS. The precise choice of HWE filter is arbitrary, but we note that both data sets criteria are on the more stringent side of customary practice.

Following the ISC (6), we reduced the set of SNPs by removing SNPs that are in linkage disequilibrium (LD) using the same criteria applied by the ISC (r^2 threshold at .25, window size 200 SNPs). In the tests of the split Japanese data set, we used LD-pruned SNPs selected on the basis of the metrics in the full set of Japanese controls. For all comparisons between Japanese and European data sets, we pruned SNPs sequentially first on the basis of the LD metrics in the discovery data set and second on those in the target data set. Polygenic score was calculated by weighting scores for "risk" alleles by the logOR observed in the discovery data set according to the method used by the ISC (6).

Nominally associated alleles were selected on the basis of the genomic-control adjusted p value in the allele-wise association analysis from the discovery samples at the following liberal significance thresholds (P_T) ($P_T < .5$, $P_T < .4$, $P_T < .3$, $P_T < .2$ and $P_T < .1$). The polygenic score was calculated using PLINK v. 1.07. Nagaelkerke's pseudo R^2 (a measure of variance explained by a particular factor) was calculated by logistic regression analysis using *R* (http://www.r-project.org) with covariation for "nonmissing SNPs" according to the ISC study (6).

Results

Single Marker Association Analysis

A summary plot of the GWAS (MAF ≥ 5%) is presented in Figure S1 in Supplement 1. We did not observe any associations at a widely used approximate benchmark for genome-wide significance ($p = 7.2 \times 10^{-8}$) (17). The strongest associations were observed at rs12218361, which maps to chromosome 10 at 126.06 Mb and is 3′ of *ornithine aminotransferase* (*OAT*, $p_{allele} = 6.2 \times 10^{-6}$, two-tailed), and rs11895771, which maps to chromosome 2 at 37.27 Mb within *sulfotransferase family, cytosolic, 6 B, member1* (*SULT6B1*, $p_{allele} = 8.0 \times 10^{-6}$, two-tailed). The most significant 200 markers are given in Table S1 in Supplement 2.

We genotyped 97 LD-pruned SNPs mainly from the top 150 GWAS findings in an independent Japanese replication sample (1511 cases and 1517 controls). For 22 of these, it was possible to expand the control sample size using data from the Japanese population based on the public database (JSNP). Data for 81 SNPs were also available in the UK data set (Affymetrix 500 K chip) and were included in the association analysis. On the basis of the replication sample from Japanese (Rep_JPN) alone, rs9880957 showed the most significant association ($p = 2.8 \times 10^{-3}$, two-tailed, OR = 1.2), but the associated allele was not the same as in the GWAS. Additionally, we undertook set-based analysis (using PLINK) to investigate whether there was an excess of association signals for these top GWAS findings in the replication data set that surpassed nominal p thresholds (e.g., $p < .1$, .05, .01, .001) in the Rep_JPN and UK data sets (10,000 permutation without lambda correction for all SNPs that passed the p threshold). However, no significant enrichment was observed (data not shown). That finding is compatible with the polygenic analysis we describe subsequently and with the now widely accepted hypothesis that common alleles that might be detectable in principle by GWAS exert effects that are too weak to be substantially enriched for associations that surpassed the threshold we specified for follow-up.

In the CMH analysis of the complete Japanese sample (Meta_JPN: JPN_GWAS + Rep_JPN), the best p was found at rs1011131 in LOC392288 ($p = 1.2 \times 10^{-4}$, two-tailed), which is weaker than in the initial GWAS ($p = 2.5 \times 10^{-5}$, two-tailed). Further expanding the sample size by including UK samples (Meta-ALL: JPN_GWAS + Rep-JPN + UK schizophrenia) did not provide convincing support for any locus (Table S1 in Supplement 2). The strongest association signal in Meta_ALL was rs11895771 ($p = 3.7 \times 10^{-5}$, two-tailed) in *SULT6B1*, which had been ranked second in the screening GWAS (Table 1).

Excluding *ZNF804A* (the Japanese data for which were included in the paper by O'Donovan *et al.*) (2), we additionally tested regions containing schizophrenia candidate loci supported by genome-wide significant associations in previous GWAS data sets (6–8). Specifically, we focused on three regions: the MHC region (Chr6 25 ~ 33 Mb), *NRGN*, and *TCF4*. In this analysis, we first imputed ungenotyped SNPs in these regions (boundaries ± 1 Mb) for fine mapping (the imputation method is presented in Supplement 1). None of the specific SNPs at these loci that have been reported by others (6–8) as genome-wide significant were imputable in our Japanese GWAS sample (Figures S2–S4 in Supplement 1). However, interestingly, we did observe a strong, fairly well circumscribed association signal on chromosome 6 in the region of *NOTCH4* (Figure S2 in Supplement 1). Furthermore, genetic association within *NOTCH4* has been reported (18) in another Japanese study (non-overlapping with the present sample) at rs2071287 (Figure S2 in Supplement 1), which is in complete LD ($D' = 1$, $r^2 = .56$) with rs2071286, the best SNP tested in our GWAS data. Because that previously supported SNP (rs2071287) is also associated in our GWAS ($p = 2.1 \times 10^{-3}$), we then followed up this SNP in the Rep_JPN sample; rs2071287 was again significantly associated ($P_{allele} = .018$, two-tailed, Figure S5 in Supplement 1; note: we could not impute this SNP with high confidence in the UK schizophrenia

Table 1. Top Single Nucleotide Polymorphisms Based on GWAS and Meta-Analysis

CHR	SNP	BP	Closest Gene	Meta_ALL (JPN_GWAS+Rep_JPN+UK_SCZ)							Meta_JPN (JPN_GWAS+Rep_JPN)				JPN_GWAS		Rep_JPN		UK_SCZ	
				A1	MAF	A2	P_{CMH}	OR[a]	L95	U95	P_{CMH}	OR[a]	L95	U95	P_{allele}	OR[a]	P_{allele}	OR[a]	P_{allele}	OR[a]
2	rs11895771	37266439	*SULT6B1*	T	.49	G	3.7×10^{-5}	.84	.77	.91	4.1×10^{-4}	.84	.76	.92	8.0×10^{-6}	.64	.14	.92	.033	.84
7	rs1011131	19474460	*LOC392288*	G	.07	C	1.2×10^{-4}	1.30	1.14	1.48	1.2×10^{-4}	1.31	1.14	1.50	2.5×10^{-5}	1.78	.054	1.17	.63	1.14
14	rs1176970	40505514	*LOC644919*	G	.15	C	1.4×10^{-4}	1.22	1.10	1.35	3.0×10^{-4}	1.27	1.12	1.44	3.2×10^{-4}	1.58	.041	1.17	.14	1.14
1	rs4908274	103162502	*COL11A1*	A	.28	T	3.1×10^{-4}	1.20	1.09	1.32	3.1×10^{-4}	1.20	1.09	1.32	1.1×10^{-4}	1.45	.067	1.12	NA	NA
6	rs2294424	11860537	*C6orf105*	T	.41	C	5.0×10^{-4}	1.15	1.06	1.24	5.0×10^{-4}	1.17	1.28	1.07	1.2×10^{-4}	1.40	.081	1.1	.41	1.08
2	rs13010889	40617519		A	.15	C	.0011	.85	.77	.94	.0016	.85	.77	.94	8.7×10^{-5}	.67	.17	.92	.40	.84
2	rs17026152	40611159		A	.26	G	.0012	.85	.77	.94	.0012	.85	.77	.94	1.3×10^{-4}	.69	.15	.92	NA	NA
6	rs2787566	101985455	*GRIK2*	A	.04	G	.0014	1.34	1.12	1.61	.0014	1.39	1.1	1.7	2.8×10^{-4}	2.03	.15	1.19	.49	1.16
6	rs2071286	32287874	*NOTCH4*	T	.19	C	.0014[b]	.87	.79	.95	.0049[b]	.86	.78	.96	3.3×10^{-4}	.68	.23[b]	.93	.13	.87
8	rs17462248	29426926		G	.2	T	.0017	1.16	1.06	1.27	.020	1.14	1.0	1.3	2.1×10^{-4}	1.52	.60	1.04	.030	1.2

p values were calculated on the basis of the allele-wise test (two-tailed).
A1, minor allele based on whole sample; A2, major allele based on whole sample; BP, base position; CHR, chromosome(hg18); GWAS, genome-wide association study JPN_GWAS: our screening GWAS; L95, lower bound of 95% confidence interval for OR; MAF, minor allele frequency based on whole sample; NA, not analyzed; OR, odds ratio; SNP, single nucleotide polymorphism; U95, upper bound of 95% confidence interval for odds ratio, UK-SCZ: UK schizophrenia.
[a]OR was calculated on the basis of A1 in Meta-ALL as reference.
[b]Controls from Japanese SNPs (JSNP) were merged into the replication sample.

data set because of the high missing rate of 12%). Next we conducted a meta-analysis based on Meta_JPN (imputed data from JPN_GWAS was down-weighted using PROPER-INFO from SNPTEST by METAL: http://www.sph.umich.edu/csg/abecasis/metal/) and the sample of Tochigi (18). This provided fairly strong evidence for association ($P_{meta} = 5.1 \times 10^{-5}$, two-tailed, Figure S5 in Supplement 1).

Polygenic Component Analysis

p values and pseudo-R^2 statistics (Nagaelkerke's R^2) for the analysis based on the split Japanese sample are presented in Figure 1 and in Table S3 in Supplement 1. The polygenic scores in the target data were higher in the cases than the controls and, in most cases, significantly so. As in the ISC study, the evidence became stronger and the pseudo-R^2 improved at more liberal P_T values. The most significant correlation was found at $P_T < .5$ ($p = .005$). In this condition, the pseudo-R^2 was slightly lower ($R^2 = .021$) compared with the ISC study (6) in which $R^2 \leq .032$ were observed in the Caucasian samples (Figure 1), although we note that the ISC study used information from a greater number of SNPs, with the larger sample available to that group allowing the inclusion of SNPs with MAF as low as 2%.

The results of the analysis based on discovery in the UK schizophrenia data set and targeting the JPN_GWAS are shown in Figure 2 (Table S3 in Supplement 1). Again, as with the ISC data, the signal and predictive power improved at the more liberal thresholds, with only the most relaxed threshold (corresponding to the optimal threshold from the ISC study) attaining significance ($p = .029$). However, the analysis using the WTCCC bipolar sample for discovery and the Japanese as the target did not reveal significant support for shared risk across disorders (Figure 2 and Table S3 in Supplement 1).

Following are the results of the analyses based on discovery in the JPN_GWAS and testing in the UK schizophrenia and bipolar data sets. Alleles trained in this direction were highly significant, but weakly predictive, of schizophrenia status in the UK sample ($p_{min} = 7.0 \times 10^{-5}$) than those analyses based on training in the UK data sets. Again, no significant effect was observed for bipolar disorder. In the schizophrenia analysis, we observed no clear relationship

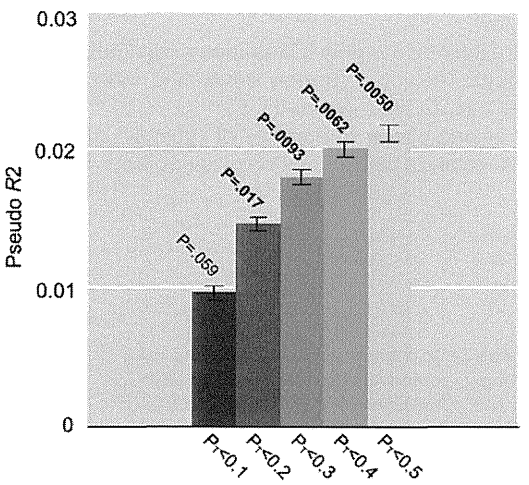


Figure 1. Polygenic component analysis for the pair within screening genome-wide association studies samples. $p_T = p$ threshold. Pseudo R^2 and *p* values represent the mean and median values, respectively, from 1000 random divisions of the data set. Error bars represent the 95% confidence intervals for R^2 from those repeat analyses. Bold numbers represent significant *p* values (< .05).

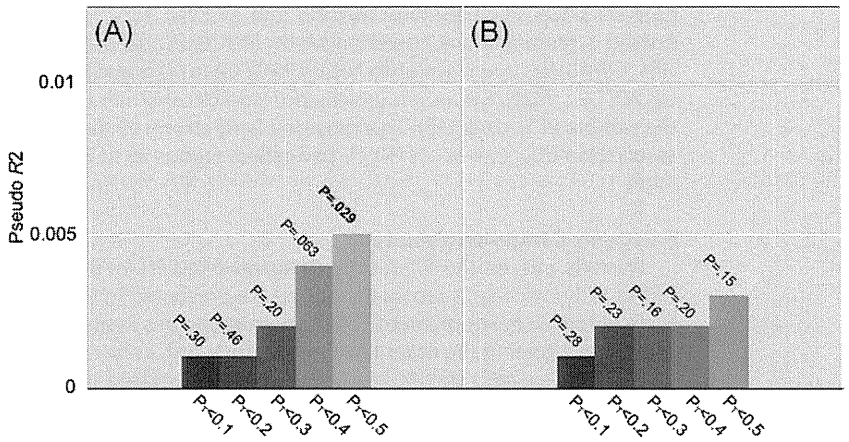


Figure 2. Polygenic component analysis for the pairs of Wellcome Trust Case-Control Consortium (WTCCC) data sets/screening genome-wide association studies (GWAS). **(A)** UK schizophrenia/screening GWAS discovery/target pair. **(B)** WTCCC bipolar/screening GWAS discovery/target pair. $p_T = p$ threshold. Bold numbers represent significant p values ($< .05$).

between the test allele significance threshold (P_T) and either the statistical support or the pseudo-R^2 (Figure 3 and Table S3 in Supplement 1).

Discussion

In this study, we did not detect unequivocal evidence for a novel susceptibility gene for schizophrenia, although our results do provide weak support for association between *SULT6B1* and schizophrenia, and our analyses of previously implicated regions and candidate genes provide support for the hypothesis that previous findings at the MHC region of chromosome 6 may point to *NOTCH4*. The absence of association at genome-wide levels of significance is not surprising given the relatively small size of our GWAS. Recent large-scale GWAS of schizophrenia suggest that the effect sizes of common risk alleles are small (ORs < 1.25). Power analysis suggests that our GWAS has only .18% power under an additive model to detect at $\alpha = 7.2 \times 10^{-8}$, a susceptibility variant with an allele frequency of .3 conferring an OR of 1.25. Clearly, with power like this, it would be extremely unlikely that any one locus would be detected at strong levels of support; however, in the presence of a thousand or more loci as has been suggested (6), the power to detect at least one of these would be considerably greater, albeit the subsequent power to replicate that specific locus would once again be low.

Despite the obvious power limitations, two findings are worthy of comment. The most strongly associated individual SNP was rs11895771 at *SULT6B1* (Meta-ALL $p = 3.7 \times 10^{-5}$). *SULT6B1* is a member of one of the subfamilies of cytosolic sulfotransferases (SULT) that catalyze the sulfonation of xenobiotics, hormones, and neurotransmitters, including 17β-estradiol and corticosterone (19), functions that are at least plausibly related to schizophrenia (20–22), and brain function (23–25) more widely.

The second locus of interest was *NOTCH4*. *NOTCH4* has been reported to be associated with schizophrenia in a small UK sample (26) (not overlapping with the present sample), but replication data from candidate gene studies have not been strongly supportive. However, a recent synthesis of GWASs as well as a large number of additional subjects reported a genome-wide significant association at rs3131296 (8), which is located within *NOTCH4* (Figure S2 in Supplement 1), although the extensive LD across the MHC region makes pinpointing the source of that signal to a specific gene impossible. It is therefore of interest in our evaluation of the MHC region that the signal clearly maximized to the *NOTCH4* region (Figure S2 in Supplement 1), lending support to the hypothesis that this may be the relevant susceptibility gene in the region. We are unable to evaluate the specific SNP (rs3131296) reported in the SGENE study for the Japanese population because of the failure of imputation. In the Japanese population, the MAF of rs3131296 differs considerably from that in Europeans (MAF = 10% and 2.3% for CEU and JPT populations, respectively, in HapMap Phase 3 data, 13% reported in SGENE), which means the ability of this marker to tag a common functional variant is likely to differ significantly between populations. Given the evidence for association observed in our study and the prior genetic evidence for *NOTCH4*, this locus warrants further detailed analysis in larger and more ethnically diverse samples.

This study provides the first independent (of the samples used by the ISC) replication of the polygenic score analysis reported by

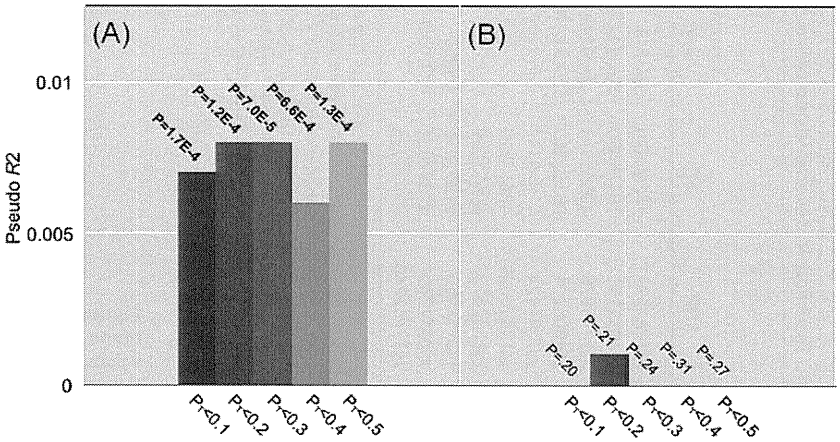


Figure 3. Polygenic component analysis for the pairs of the screening genome-wide association studies (GWAS)/ Wellcome Trust Case-Control Consortium (WTCCC) data sets. **(A)** Screening GWAS/UK schizophrenia discovery/ target pair. **(B)** Screening GWAS/ WTCCC bipolar discovery/target pair. $p_T = p$ threshold. Bold numbers represent significant p values ($< .05$).

the ISC (6). Although our sample is low powered (power is .6 for our full sample and .56 for half of the sample to detect at an alpha level of .5, a weak genetic effect [OR 1.1] conferred by an allele with a frequency of .3), the set of "risk" alleles (in quotation marks to emphasize that most are not likely to be true risk alleles) derived from half of the Japanese sample was significantly correlated with affection status in the other half of the samples. One possible important confounding factor to consider is an effect of population stratification. To check for this as a possible effect, we used 1) principal components analysis–adjusted (the first 10 principal components) discovery statistics for the selection of SNPs and 2) the first 10 principal component vectors as covariates in calculating the polygenic score in the target sample. However, the application of either or both of these did not lead to a material difference in the results (Table S4 in Supplement 1), indicating that stratification is not likely to explain our replication of the ISC findings.

Our Japan–UK analyses also suggests this effect is unlikely to be due to stratification (this was also convincingly argued in the ISC study) because the Japanese and UK schizophrenia samples are ascertained directionally for the same stratification biases and because the UK schizophrenia sample, but not the UK bipolar sample, would be unlikely to be stratified in that manner. Instead, those data point to a shared genetic component to schizophrenia susceptibility across major ethnic groups, as predicted by an effect driven by common "risk" alleles rather than rare alleles, although not excluding an effect of rare alleles, which are much more likely to reside on different haplotype backgrounds in different populations. However, there is also evidence for population differences in risk. Thus, the analyses restricted to the Japanese population showed much higher maximal estimates for R^2 (.021) compared with the analyses of schizophrenia between populations ($R^2 = .005 \sim .008$) and was more similar to the estimates of R^2 when the analyses were performed within European populations (6). The ISC also undertook one cross-population analysis, between Caucasian and African Americans. As in our study, R^2 was much lower between the ethnic groups (.004) than within the European populations. These results suggest that although at least some "risk" alleles are shared across populations, there are also differences in those "risk" alleles or at least in the extent to which they are tagged by markers at the density currently provided by the arrays we have studied. At a practical level, this means that failures to replicate findings across ethnic groups, even with respect to common alleles, should be treated with considerable caution.

One intriguing finding was our failure to find evidence that "risk" alleles for bipolar disorder in the European sample predict risk of schizophrenia in the Japanese sample (or vice versa). One likely explanation is that there is only a partial overlap between "risk" alleles for schizophrenia and bipolar disorder and that this, together with the additionally reduced R^2 because of ethnic differences, has affected our ability to demonstrate an effect. This interpretation is at least partially consistent with the ISC study in which the measures of R^2 that were observed in bipolar data sets were less than those observed in the schizophrenia data sets. A more interesting but speculative interpretation is that the Japanese sample represents a phenotypically purer form of schizophrenia than the European samples. These hypotheses require further evaluation in larger Japanese samples, exploration of aspects of the schizophrenia phenotype in the European samples, and transdiagnostic polygenic score analyses within Japanese samples.

This work was supported in part by research grants from the Japan Ministry of Education, Culture, Sports, Science and Technology; the Ministry of Health, Labor and Welfare; the Core Research for Evolutional Science and Technology; and the Health Sciences Foundation (Research on Health Sciences focusing on Drug Innovation). The UK research was supported by grants from the Medical Research Council and the Wellcome Trust.

The authors MCO and NI are joint last authors.

Dr. Ikeda reports receiving support from the Japan Society for the Promotion of Science postdoctoral fellowship for research abroad and is also supported by the Uehara Memorial Foundation and the Great Britain Sasakawa Foundation. The other authors report no biomedical financial interests or potential conflicts of interest.

Supplementary material cited in this article is available online.

1. O'Donovan MC, Craddock NJ, Owen MJ (2009): Genetics of psychosis; insights from views across the genome. *Hum Genet* 126:3–12.
2. O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V, *et al.* (2008): Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nat Genet* 40:1053–1055.
3. Steinberg S, Mors O, Borglum AD, Gustafsson O, Werge T, Mortensen PB, *et al.* (2010): Expanding the range of ZNF804A variants conferring risk of psychosis [published online ahead of print January 5]. *Mol Psychiatry.*
4. Riley B, Thiselton D, Maher BS, Bigdeli T, Wormley B, McMichael GO, *et al.* (2010): Replication of association between schizophrenia and ZNF804A in the Irish Case–Control Study of Schizophrenia sample. *Mol Psychiatry* 15:29–37.
5. Williams HJ, Norton N, Dwyer S, Moskvina V, Nikolov I, Carroll L, *et al.* (2010): Fine mapping of ZNF804A and genome-wide significant evidence for its involvement in schizophrenia and bipolar disorder [published online ahead of print April 6].
6. International Schizophrenia Consortium, Purcell, SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, *et al.* (2009): Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* 460: 748–752.
7. Shi J, Levinson DF, Duan J, Sanders AR, Zheng Y, Pe'er I, *et al.* (2009): Common variants on chromosome 6p22.1 are associated with schizophrenia. *Nature* 460:753–757.
8. Stefansson H, Ophoff RA, Steinberg S, Andreassen OA, Cichon S, Rujescu D, *et al.* (2009): Common variants conferring risk of schizophrenia. *Nature* 460:744–747.
9. Wray NR, Visscher PM (2009): Narrowing the boundaries of the genetic architecture of schizophrenia. *Schizophr Bull* 36:14–23.
10. Evans DM, Visscher PM, Wray NR (2009): Harnessing the information contained within genome-wide association studies to improve individual prediction of complex disease risk. *Hum Mol Genet* 18:3525–3531.
11. Craddock N, O'Donovan MC, Owen MJ (2009): Psychosis genetics: Modeling the relationship between schizophrenia, bipolar disorder, and mixed (or "schizoaffective") psychoses. *Schizophr Bull* 35:482–490.
12. Craddock N, O'Donovan MC, Owen MJ (2007): Symptom dimensions and the Kraepelinian dichotomy. *Br J Psychiatry* 190:361; author reply: 361–362.
13. Yamaguchi-Kabata Y, Nakazono K, Takahashi A, Saito S, Hosono N, Kubo M, *et al.* (2008): Japanese population structure, based on SNP genotypes from 7003 individuals compared to other ethnic groups: Effects on population-based association studies. *Am J Hum Genet* 83:445–456.
14. Wellcome Trust Case-Control Consortium (2007): Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 447: 661–678.
15. Ikeda M, Aleksic B, Kirov G, Kinoshita Y, Yamanouchi Y, Kitajima T, *et al.* (2009): Copy number variation in schizophrenia in the Japanese population. *Biol Psychiatry* 67:283–286.
16. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, *et al.* (2007): PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81:559–575.
17. Dudbridge F, Gusnanto A (2008): Estimation of significance thresholds for genomewide association scans. *Genet Epidemiol* 32:227–234.
18. Tochigi M, Zhang X, Ohashi J, Hibino H, Otowa T, Rogers M, *et al.* (2007): Association study between the TNXB locus and schizophrenia in a Japanese population. *Am J Med Genet B Neuropsychiatr Genet* 144B: 305–309.

19. Cao H, Agarwal SK, Burnside J (1999): Cloning and expression of a novel chicken sulfotransferase cDNA regulated by GH. *J Endocrinol* 160:491–500.
20. Eaton WW, Byrne M, Ewald H, Mors O, Chen CY, Agerbo E, Mortensen PB (2006): Association of schizophrenia and autoimmune diseases: Linkage of Danish national registers. *Am J Psychiatry* 163:521–528.
21. Baumgartner A, Pietzcker A, Gaebel W (2000): The hypothalamic-pituitary-thyroid axis in patients with schizophrenia. *Schizophr Res* 44:233–243.
22. Kulkarni J, de Castella A, Fitzgerald PB, Gurvich CT, Bailey M, Bartholomeusz C, Burger H (2008): Estrogen in severe mental illness: A potential new treatment approach. *Arch Gen Psychiatry* 65:955–960.
23. Cerqueira JJ, Pego JM, Taipa R, Bessa JM, Almeida OF, Sousa N (2005): Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviors. *J Neurosci* 25:7792–7800.
24. Enkel T, Koch M (2009): Chronic corticosterone treatment impairs trace conditioning in rats with a neonatal medial prefrontal cortex lesion. *Behav Brain Res* 203:173–179.
25. Gogos A, Nathan PJ, Guille V, Croft RJ, van den Buuse M (2006): Estrogen prevents 5-HT1A receptor-induced disruptions of prepulse inhibition in healthy women. *Neuropsychopharmacology* 31:885–889.
26. Wei J, Hemmings GP (2000): The NOTCH4 locus is associated with susceptibility to schizophrenia. *Nat Genet* 25:376–377.

OPEN ACCESS Freely available online

PLoS one

Reduced Rate of Neural Differentiation in the Dentate Gyrus of Adult Dysbindin Null (Sandy) Mouse

Naomi Nihonmatsu-Kikuchi[1,2], Ryota Hashimoto[3,4,5], Satoko Hattori[5], Shinsuke Matsuzaki[3,6,7], Takiko Shinozaki[1], Haruka Miura[1], Shigeru Ohota[2], Masaya Tohyama[3,6,7], Masatoshi Takeda[3,4], Yoshitaka Tatebayashi[1]*

1 Mood Disorders Research Team, Tokyo Institute of Psychiatry, Tokyo, Japan, **2** Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan, **3** The Osaka-Hamamatsu Joint Research Center for Child Mental Development, Graduate School of Medicine, Osaka University, Osaka, Japan, **4** Department of Psychiatry, Osaka University Graduate School of Medicine, Osaka, Japan, **5** Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan, **6** Department of Anatomy and Neuroscience, Graduate School of Medicine, Osaka University, Osaka, Japan, **7** Department of Child Development and Molecular Brain Science, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, Osaka, Japan

Abstract

Genetic variations in the gene encoding dysbindin has consistently been associated with schizophrenia and bipolar disorder, although little is known about the neural functions carried out by dysbindin. To gain some insight into this area, we took advantage of the readily available dysbindin-null mouse sandy (sdy−/−) and studied hippocampal neurogenesis using thymidine analogue bromodeoxuridine (BrdU). No significant differences were found in the proliferation (4 hours) or survival (1, 4 and 8 weeks after the last BrdU injection) of progenitors in the subgranular regions of the dentate gyrus between sdy−/− and sdy+/+ (control) mice. However, 4 weeks after the last BrdU injection, a significant reduction was observed in the ratio of neuronal differentiation in sdy−/− when compared to that of sdy+/+ (sdy+/+ =87.0±5.3% *vs.* sdy−/− =71.3±8.3%, $p=0.01$). These findings suggest that dysbindin plays a role during differentiation process in the adult hippocampal neurogenesis and that its deficit may negatively affect neurogenesis-related functions such as cognition and mood.

Citation: Nihonmatsu-Kikuchi N, Hashimoto R, Hattori S, Matsuzaki S, Shinozaki T, et al. (2011) Reduced Rate of Neural Differentiation in the Dentate Gyrus of Adult Dysbindin Null (Sandy) Mouse. PLoS ONE 6(1): e15886. doi:10.1371/journal.pone.0015886

Editor: Takeo Yoshikawa, RIKEN Brain Science Institute, Japan

Received October 13, 2010; **Accepted** November 25, 2010; **Published** January 18, 2011

Funding: Y.T. was supported by a Grant-in-Aid for Scientific Research from the Japanese Society for the Promotion of Science (KAKENHI 20390317).http://www.mext.go.jp/a_menu/shinkou/hojyo/main5_a5.htm. R.H. was supported by the Japanese Ministry of Education, Culture, Sports, Science and Technology, CREST of JST, and by a Grant-in-Aid for Scientific Research on Priority Areas (Research on the Pathomechanisms of Brain Disorders) from the MEXT (18023045). http://www.jst.go.jp/kisoken/crest/. http://www.mext.go.jp/english/. S.M. was supported by a Grant-in-Aid for Young Scientists (B) from the Japanese Society for the Promotion of Science. http://www.jsps.go.jp/j-grantsinaid/index.html. M.T. was supported by Health and Labour Sciences Research Grants, Japan. http://www.mhlw.go.jp/bunya/kenkyuujigyou/hojokin.html. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: tatebayashi-ys@igakuken.or.jp

Introduction

The dysbindin-1 gene (dystrobrevin-binding protein 1) was originally identified as a gene associated with schizophrenia through its linkage to chromosome 6p [1]. Several subsequent studies have replicated the association between this locus and schizophrenia. In addition, two recent and independent reports have linked certain dysbindin-1 risk haplotypes with bipolar disorder [2,3]. Dysbindin-1 is widely distributed in the brain, and has been detected both pre- and post-synaptically [4]. A recent immunoelectron microscopy study further revealed that, in the hippocampus, dysbindin-1 is located in synaptic vesicles of axospinous terminals in the dentate gyrus inner molecular layer (DGiml) and CA1 stratum radiatum and in postsynaptic densities and microtubules of dentate hilus neurons and CA1 pyramidal cells [5].

To date, no amino acid sequence mutation in the dysbindin-1 protein that might contribute to the risk of major psychosis has been identified. Furthermore, several studies have implicated the involvement of many different alleles and haplotypes as susceptibility variants. These polymorphisms, however, may modulate dysbindin-1 expression levels since reduced dysbindin message and/or protein levels have been found in schizophrenic brains such as prefrontal cortex and hippocampal formation, brain areas commonly affected by the disorder [6,7,8]. In the hippocampus of schizophrenic patients, dysbindin-1 reductions occur in the synaptic terminal fields of glutamatergic neurons, especially those located in the DGiml [7].

Although its function in the brain is still not well understood, it may play a role in both glutamatergic and dopaminergic neurotransmission [9,10,11]. For example, knockdown of endogenous dysbindin with siRNA has been shown to reduce glutamate levels in cultured neurons, suggesting that a decrease in dysbindin levels has synaptic consequences [7,10]. Furthermore, altered dysbindin-1 expression may contribute to cognitive impairments prominent in schizophrenia, including deficits in attention, memory and executive function [12–14]. More recently, "sandy" (sdy−/−) mice, spontaneously occurring dysbindin null mice, have been shown to exhibit a number of behavioral abnormalities associated with reductions in forebrain dopamine transmission [15,16].

In the present study, we investigated the adult neurogenesis in the dentate gyrus (DG) of the hippocampus in syd−/− mice, in order to further understand the roles of dysbindin-1 in the pathogenesis of major psychosis. We found that sdy−/− mice exhibit significantly reduced rate of neuronal differentiation in the DG at 4 weeks after the progenitor proliferation, although all the other parameters of the neurogenesis examined remained unaltered in sdy−/− mice when compared to those in control mice (sdy+/+). This finding indicates that dysbindin plays a role during the differentiation process of the adult hippocampal neurogenesis.

Results

Dysbindin expression

A deletion within the homologous gene in mice accounts for the phenotype known as "sandy" (sdy−/−), which is characterized by albinism and bleeding disorders [17]. This deletion is from nucleotide 3701 of intron 5 to nucleotide 12377 of intron 7, and essentially results in the total loss of dysbindin. We confirmed this lack of dysbindin protein in the sdy−/− mouse hippocampus by Western blot analysis (Figure 1).

Progenitor proliferation

To evaluate progenitor cell proliferation in the DG as well as in the entire hippocampus, bromodeoxyuridine (BrdU) was injected intraperitoneally both in sdy−/− and control (sdy+/+) mice (4 months old female mice, n = 4) and the BrdU-labeled cells were counted in mouse hippocampus killed 4 h after the injection. No significant difference was observed between the two groups in terms of the number of BrdU-labeled cells in the DG (sdy+/+ mice, 573±26; sdy−/− mice, 663±87; $p>0.05$) (Figure 2, 4 h), suggesting that BrdU bioavailability was similar in both. The distribution of the dividing cells in the hippocampus of the sdy−/− mice was not obviously different from that seen in sdy+/+ mice. The cells were predominantly found in clusters at the border between the granular layer (GL) and the hilus (HL) (i.e., subgranular layer: SGL) of the DG (Figure S1).

Progenitor survival

The survival of BrdU-incorporated progenitor cells at 4 months old female mice was also examined in the DG 1 (n = 4), 4 (n = 8) and 8 w (n = 3) after the last BrdU injection. In the SGL, estimates of the total BrdU-positive populations were 1,153±144 (1 w), 625±417 (4 w), and 647±300 (8 w) in sdy−/− mice and 1,282±175 (1 w), 448±69 (4 w), and 652±95 (8 w) in sdy+/+ mice (Figure 2, 1 w, 4 w, 8 w), with no statistically significant differences between groups.

Progenitor differentiation

To further examine the effects of dysbindin depletion, differentiation was examined at 4 w after BrdU injection by concurrent immunolabelling for BrdU with neuronal (NeuN) or glial (NG2) markers. NG2 was preferred not only because it serves as a marker for oligodendrocyte progenitor cells (OPCs), but also because OPCs constitute the major dividing glial cell population of the adult CNS [18]. NG2-positive OPCs are distinct from neurons, mature oligodendrocytes, astrocytes, and microglia, and are distributed throughout the gray and white matter [19]. No significant difference was found between groups in either the number or the percentage of BrdU-positive cells that co-labeled for NG2 (Figure S2) in the DG (data not shown); this was also true for other areas of the hippocampus as well (CA1 – 3) (Figure 3).

We found that an equal number of BrdU-positive cells co-labeled with NeuN in the SGL in both the sdy−/− (329±27) and sdy+/+ (326±40) mice 4 w after the last BrdU injection. However, the percentage of BrdU-positive cells that co-labeled with NeuN was significantly less (−15%, $p<0.01$) in sdy−/− mice (71.3±8.3%) than in sdy+/+ mice (87.0±5.3%) (Figure 3), mostly due to the increased, albeit insignificant, number of BrdU-positive cells in the SGL of sdy−/− mice (Figure 2).

Figure 1. Lack of dysbindin-1 expression in the hippocampus of sdy−/− mice. Homogenates from the hippocampus region of control mice (Ctrl) and sdy−/− mice (Dys KO) were subjected to Western blotting. Primary antibody: a monoclonal mouse dysbindin antibody (1:1000); secondary antibody: an anti-mouse HRP-linked antibody (1:2000).
doi:10.1371/journal.pone.0015886.g001

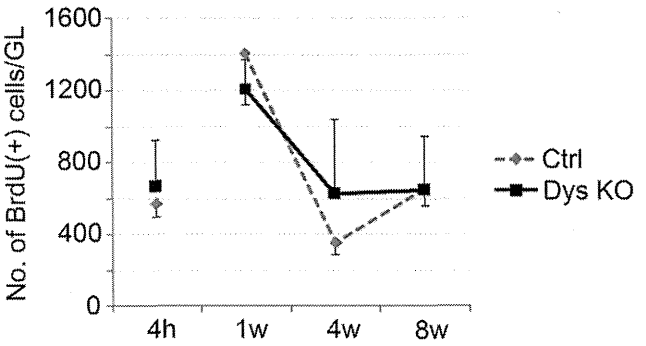


Figure 2. The survival of newborn cells in the SGL of sdy−/− mice. The number of thymidine analogue bromodeoxuridine (BrdU) - incorporated cells was not significantly different in the granular cell layer (GL) between sdy+/+ (Ctrl) and sdy−/− (Dys KO) mice at any time point (4 h, 1 w, 4 w, and 8 w). The data are shown as the mean ± s.d.
doi:10.1371/journal.pone.0015886.g002

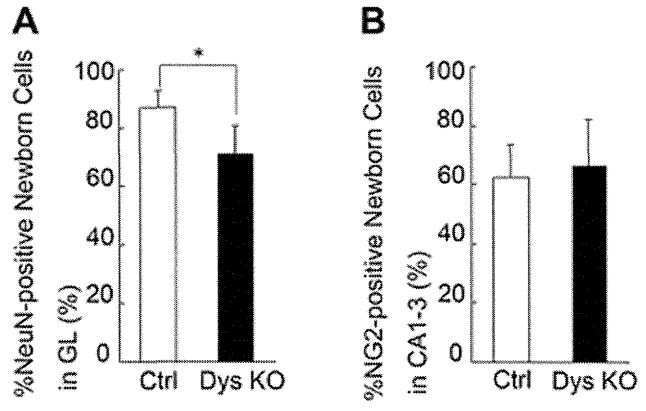


Figure 3. Reduced rate of neuronal differentiation of newborn cells in sdy−/− mice. The rate of NeuN-positive newborn cells in the granular cell layer (GL) of sdy−/− (Dys KO) mice was significantly lower compared to that of sdy+/+ mice (Ctrl) (*$p = 0.01$), as opposed to that of NG2-positive cells in other areas of the hippocampus (CA1 – 3) at 4 weeks after thymidine analogue bromodeoxuridine (BrdU) injection. The data are shown as the mean ± s.d.
doi:10.1371/journal.pone.0015886.g003

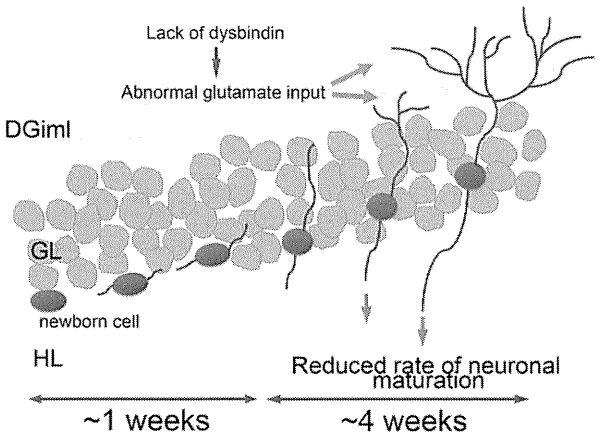


Figure 4. Effect of lack of dysbindin-1 expression on the adult hippocampal neurogenesis. Newborn cells (blue) in the dentate gyrus of sdy−/− (Dys KO) may receive abnormal glutamate inputs within the inner molecular layer (DGiml, orange) due to the lack of dysbindin-1 expression during the critical periods for neuronal differentiation (during the first 3~4 w from the birth), resulting in the reduced rate of neuronal differentiation. GL and HL are granule cell layer and hilus, respectively.
doi:10.1371/journal.pone.0015886.g004

Discussion

Neurogenesis in adult mammalian brains actively occurs at the subventricular zone of the lateral ventricles and at the SGL of DG in the hippocampus. In the present study, we investigated the nature of hippocampal neurogenesis in spontaneously occurring dysbindin null (sdy−/−) mice. We found that, although the numbers of new neurons generated in the SGL of sdy−/− mice are almost equal to those of sdy+/+ mice, the rates of neuronal differentiation stemming from newly generated cells are significantly lower in sdy−/− mice.

Newborn cells in the SGL initially receive GABAergic signalling from interneurons within the DG [20]. GABA initially induced the depolarization of immature newborn cells within the first 2–3 w after birth, depending on the gradient of Cl^- levels (i.e., intracellular Cl^- levels are higher than extracellular ones) [21–24]. These cells then gradually migrate into the granular layer and mature as a result of the shift from depolarization to hyperpolarization. Signaling through the NMDA receptor plays a cell autonomous role in the survival of neuronally differentiating newborn cells during the first 3~4 w after the birth, which coincides with the formation of dendritic spines and functional glutamatergic inputs (Figure 4) [20,25–28].

Although glutamate levels in the entire hippocampal formation of sdy−/− mice remain unaltered compared with those of sdy+/+ mice [15], the expression levels of dysbindin-1 reduced in the DGiml of schizophrenia cases while those of the vesicular glutamate transporter (VGluT-1), a glutamate terminal marker, increased [7], indicating the abnormal glutamate transmission in the DGiml in schizophrenia patients (Figure 4). Moreover, sdy−/− mice exhibited impaired learning in the Morris water maze and T-maze, as well as long-term memory retention deficits in the Barnes circular maze test [15,16,29], which is dependent upon a correctly hippocampal functioning and, in part, on the neurogenesis occurring therein [28]. Collectively, our finding that the rate of neuronal differentiation of 4 w-old newborn cells was lower in the DG of sdy−/− mice may explain not only the behavioral abnormalities observed in the sdy−/− mice [15,16,29] but also, at least in part, the cognitive, memory or IQ abnormalities associated with genetic variations in human [12–14,30–33] and with histopathological abnormalities found in the postmortem brains of schizophrenia patients [5,7,8]. Thus, further study of dysbindin-1 genotypes in relation both to specific schizophrenia subtypes and to cognitive endophenotypes are warranted, as does an in-depth investigation of the role of dysbindin in glutamate neurotransmission and in other neuronal functions in the brain.

Materials and Methods

Animals

The experiments were largely done on material from a previously published study [15]. "Sandy" (sdy−/−) mice were raised at The Jackson Laboratory, Bar Harbor, ME. Sandy mice have an autosomal recessive coat color mutation that arose spontaneously in the inbred DBA/2J strain. Both sdy−/− mice and wild-type mice derived from heterozygote crossings were used in all experiments. All animals were housed in humidity- and temperature-controlled rooms with a 12-hour (hr) light cycle with free access to food and water. All protocols were approved by the Animal Care and Use Committee of the Tokyo Institute of Psychiatry.

Western blotting

Following deep anesthetization with sodium pentobarbital, the hippocampus was dissected from each mouse. Hippocampal tissues were homogenated in TNE buffer (20 mM tris-HCl, pH 7.5, 150 mM NaCl, and 1 mM EDTA) containing 1% NP-40 and RIPA buffer and protease inhibitor cocktail (Roche, Sydney, Australia) and centrifuged at 15,000× g for 10 min. Lysates were boiled with SDS sample buffer (0.125 M Tris-HCl, pH 6.8, 10% 2-mercaptoethanol, 4% SDS, 10% sucrose, and 0.004% bromopheonl blue) for 5 min and subjected to SDS–PAGE. Proteins were separated on SDS–PAGE and electrotransferred onto Immobilon-P Transfer Membranes (MILLIPORE, Billerica, USA). Membranes were incubated in PBS containing 5% skim milk and 0.05% Tween 20 for 1 h and blotted with primary antibodies at 4°C overnight. An anti-dysbindin monoclonal antibody (1:1000) was used as a primary antibody. Mouse monoclonal anti-dysbindin antibody was produced using GST-fused human dysbindin as antigen, as previously described [34,35]. The membranes were incubated with an anti-mouse HRP-linked secondary antibody (1:2000, Cell Signalling Technology) for 1 h. Proteins were detected with an ECL kit (Amersham Biosciences, Buckinghamshire, UK) and were then exposed to X-ray films, according to the manufacturer's protocol.

Bromodeoxyuridine administration

Bromodeoxyuridine (BrdU; Sigma, St. Louis, MO) was dissolved in phosphate buffer saline (PBS), pH 7.4, at a concentration of 10 mg/ml and filter sterilized. Mice were divided into four treatment groups (Group-I ~ Group-IV). In Group-I, mice received one injection of BrdU (50 μg/g of body weight, i.p.) and were killed 4 h later. In Group-II to -IV, mice received daily injections of BrdU (50 μg/gm of body weight, i.p.) for 3 consecutive days. The mice were then killed 1 (Group-II), 4 (Group-III), or 8 (Group-IV) weeks (w) after the final injection.

Tissue processing

The animals were anesthetized and transcardially perfused with PBS followed by 4% paraformaldehyde in PBS. Brains were removed, post-fixed for 1 day at 4°C, and then cryoprotected

overnight in 20% sucrose in PBS. Coronal sections were cut with a freezing microtome through the hippocampus at 40 μm. Four 1-in-5 series were collected, plated onto glass slides, and stored at −30°C until histochemical analysis.

BrdU detection

To allow for the detection of BrdU-labeled cells (see below), sections were pretreated for 30 min in 2N HCl at 37°C to denature DNA. The sections were then incubated for 10 min in 100 mM sodium borate, pH 8.5, to neutralize the residual acid.

Immunofluorescence

Sections were incubated for 30 min in PBS containing 5% goat serum and 0.4% Triton X-100. Using the same buffer solution, the sections were incubated overnight at 4°C in primary antibodies (monoclonal rat anti-BrdU (Harlan Sera-Lab); polyclonal rabbit anti-NG2 (Chemicon, Temecula, CA); monoclonal mouse anti-neuron-specific nuclear protein (NeuN; Chemicon)), followed by 2 h at room temperature in corresponding fluorochrome-conjugated goat secondary antibodies (anti-mouse FITC, anti-mouse rhodamine Red-X (RRX), anti-rabbit Cy5, anti-rabbit FITC, anti-rabbit RRX, and/or anti-rat FITC; all from Jackson ImmunoResearch). Each of the above steps was followed by four 5-min rinses in PBS. The sections were mounted onto gelatin-coated slides, dried, and coverslipped with ProLong antifade medium (Molecular Probes, Eugene, OR).

Cell counting

Cell counts were performed by an observer who was unaware of the treatment status of the animals. For each animal, a complete series of 1-in-5 sections was analyzed with a light microscope at 600 x magnification. The number of immunoreactive cells was counted in the granular layer (GL) (4 h, 1 w, 4 w, and 8 w) and in the entire hippocampus (4 w). We defined the GL as the granular cell layer plus the areas ~10 μm deep in the subgranular zone and 5 μm deep in the molecular layer of the DG. The total number of BrdU-positive cells was estimated by multiplying the number of profiles by 5. For cell phenotyping, BrdU-positive cells were analyzed for colocalization with NeuN (in the GL) or NG2 (in the hippocampus). Data were analyzed by ANOVA followed by Scheffé's test for *post hoc* multiple comparisons (Figure 2 and 3). This was done for the two groups, normal controls (sdy+/+) and "Sandy" (sdy−/−) mice.

Supporting Information

Figure S1 BrdU-positive cells in dentate gyrus (DG) of sdy−/− mice. Sections were the DG of sdy−/− mice after 4 weeks from the last thymidine analogue bromodeoxuridine (BrdU) injections. BrdU-incorporating cells (green) were located mostly between the granular layer (GL) and the hilus (HL). Nuclei were visualized by using Topro-3 (Invitrogen, blue). Images were collected on high-resolution confocal microscopy (LSM510 excitator, Zeiss). Confocal *z* stacks were captured for each section (0.76–0.78 μm increments) using a 20x objective (C-Apochromat, Zeiss). Composite images were reconstructed using Imaris 5.0.3 software (Zeiss). Scale bars represent 100 μm.
(TIF)

Figure S2 NG2-positive oligodendrocyte progenitor cells (OPCs) in the hippocampus of sdy−/− mice. Sections were the hippocampus of sdy−/− mice after 4 weeks from the last thymidine analogue bromodeoxuridine (BrdU) injections. NG2 labeling is shown in red (arrow heads). Arrows indicate BrdU-positive (green), NG2-positive OPCs. Nuclei were visualized by using Topro-3 (Invitrogen, blue). Images were collected on high-resolution confocal microscopy (LSM510 excitator, Zeiss). Confocal *z* stacks were captured for each section (0.36 μm increments) using a 40x water immersion objective (C-Apochromat, Zeiss). Composite images were reconstructed using Imaris 5.0.3 software (Zeiss). Scale bars represent 50 μm.
(TIF)

Acknowledgments

We thank Dr. Tatsunori Hisatsune for his critical reading of this paper.

Author Contributions

Conceived and designed the experiments: RH SO M. Tohyama M. Takeda YT. Performed the experiments: SH SM TS HM. Analyzed the data: NNK SH SM HM YT. Contributed reagents/materials/analysis tools: RH SM M. Tohyama M. Takeda YT. Wrote the paper: NNK RH SM YT.

References

1. Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, et al. (2002) Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. Am J Hum Genet 71: 337–48.
2. Guo AY, Sun J, Riley BP, Thiselton DL, Kendler KS, et al. (2009) The dystrobrevin-binding protein 1 gene: features and networks. Mol Psychiatry 14: 18–29.
3. Breen G, Prata D, Osborne S, Munro J, Sinclair M, et al. (2006) Association of the dysbindin gene with bipolar affective disorder. Am J Psychiatry 163: 1636–8.
4. Benson MA, Newey SE, Martin-Rendon E, Hawkes R, Blake DJ (2001) Dysbindin, a novel coiled-coil-containing protein that interacts with the dystrobrevins in muscle and brain. J Biol Chem 276: 24232–41.
5. Talbot K, Cho DS, Ong WY, Benson MA, Han LY, et al. (2006) Dysbindin-1 is a synaptic and microtubular protein that binds brain snapin. Hum Mol Genet 15: 3041–54.
6. Bray NJ, Preece A, Williams NM, Moskvina V, Buckland PR, et al. (2005) Haplotypes at the dystrobrevin binding protein 1 (DTNBP1) gene locus mediate risk for schizophrenia through reduced DTNBP1 expression. Hum Mol Genet 14: 1947–54.
7. Talbot K, Eidem WL, Tinsley CL, Benson MA, Thompson EW, et al. (2004) Dysbindin-1 is reduced in intrinsic, glutamatergic terminals of the hippocampal formation in schizophrenia. J Clin Invest 113: 1353–63.
8. Weickert CS, Straub RE, McClintock BW, Matsumoto M, Hashimoto R, et al. (2004) Human dysbindin (DTNBP1) gene expression in normal brain and in schizophrenic prefrontal cortex and midbrain. Arch Gen Psychiatry 61: 544–55.
9. Numakawa T, Yagasaki Y, Ishimoto T, Okada T, Suzuki T, et al. (2004) Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia. Hum Mol Genet 13: 2699–708.
10. Kumamoto N, Matsuzaki S, Inoue K, Hattori T, Shimizu S, et al. (2006) Hyperactivation of midbrain dopaminergic system in schizophrenia could be attributed to the down-regulation of dysbindin. Biochem Biophys Res Commun 345: 904–9.
11. Murotani T, Ishizuka T, Hattori S, Hashimoto R, Matsuzaki S, et al. (2007) High dopamine turnover in the brains of Sandy mice. Neurosci Lett 421: 47–51.
12. Fanous AH, van den Oord EJ, Riley BP, Aggen SH, Neale MC, et al. (2005) Relationship between a high-risk haplotype in the DTNBP1 (dysbindin) gene and clinical features of schizophrenia. Am J Psychiatry 162: 1824–1832.
13. Gornick MC, Addington AM, Sporn A, Gogtay N, Greenstein D, et al. (2005) Dysbindin (DTNBP1, 6p22.3) is associated with childhood-onset psychosis and endophenotypes measured by the Premorbid Adjustment Scale (PAS). J Autism Dev Disord 35: 831–8.
14. Straub RE, Egan MF, Hashimoto R, Masumoto M, Weickert CS, et al. (2003) The schizophrenia susceptibility gene dysbindin (DTNBP1, 6p22.): analysis of haplotypes, intermediate phenotypes and alternative transcripts. Biological Psychiatry 53(suppl.): 167S–168S.
15. Hattori S, Murotani T, Matsuzaki S, Ishizuka T, Kumamoto N, et al. (2008) Behavioral abnormalities and dopamine reductions in sdy mutant mice with a deletion in Dtnbp1, a susceptibility gene for schizophrenia. Biochem Biophys Res Commun 373: 298–302.

16. Cox MM, Tucker AM, Tang J, Talbot K, Richer DC, et al. (2009) Neurobehavioral abnormalities in the dysbindin-1 mutant, sandy, on a C57BL/6J genetic background. Genes Brain Behav 8: 390–7.
17. Li W, Zhang Q, Oiso N, Novak EK, Gautam R, et al. (2003) Hermansky-Pudlak syndrome type 7 (HPS-7) results from mutant dysbindin, a member of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). Nat Genet 35: 84–9.
18. Dawson MR, Polito A, Levine JM, Reynolds R (2003) NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. Mol Cell Neurosci 24: 476–88.
19. Nishiyama A, Komitova M, Suzuki R, Zhu X (2009) Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. Nat Rev Neurosci 10: 9–22.
20. Zhao C, Deng W, Gage FH (2008) Mechanisms and functional implications of adult neurogenesis. Cell 132: 645–60.
21. Wang DD, Krueger DD, Bordey A (2003) GABA depolarizes neuronal progenitors of the postnatal subventricular zone via GABAA receptor activation. J Physiol 550: 785–800.
22. Overstreet Wadiche L, Bromberg DA, Bensen AL, Westbrook GL (2005) GABAergic signaling to newborn neurons in dentate gyrus. J Neurophysiol 94: 4528–32.
23. Tozuka Y, Fukuda S, Namba T, Seki T, Hisatsune T (2005) GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. Neuron 15: 803–15.
24. Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, et al. (2006) GABA regulates synaptic integration of newly generated neurons in the adult brain. Nature 439: 589–93.
25. Ge S, Pradhan DA, Ming GL, Song H (2007) GABA sets the tempo for activity-dependent adult neurogenesis. Trends Neurosci 30: 1–8.
26. Deng W, Aimone JB, Gage FG (2010) New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat Rev Neurosci 11: 339–50.
27. Esposito MS, Piatti VC, Laplagne DA, Morgenstern NA, Ferrari CC, et al. (2005) Neuronal differentiation in the adult hippocampus recapitulates embryonic development. J Neurosci 25: 10074–86.
28. Tashiro A, Sandler VM, Toni N, Zhao C, Gage FH (2006) NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. Nature 442: 929–33.
29. Takao K, Toyama K, Nakanishi K, Hattori S, Takamura H, et al. (2008) Impaired long-term memory retention and working memory in sdy mutant mice with a deletion in Dtnbp1, a susceptibility gene for schizophrenia. Mol Brain 1: 11.
30. Fallgatter AJ, Herrmann MJ, Hohoff C, Ehlis AC, Jarczok TA, et al. (2006) DTNBP1 (dysbindin) gene variants modulate prefrontal brain function in healthy individuals. Neuropsychopharmacology 31: 2002–10.
31. Burdick KE, Lencz T, Funke B, Finn CT, Szeszko PR, et al. (2006) Genetic variation in DTNBP1 influences general cognitive ability. Hum Mol Genet 15: 1563–8.
32. Hashimoto R, Noguchi H, Hori H, Ohi K, Yasuda Y, et al. (2009) Association between the dysbindin gene (DTNBP1) and cognitive functions in Japanese subjects. Psychiatry Clin Neurosci 63: 550–6.
33. Hashimoto R, Noguchi H, Hori H, Nakabayashi T, Suzuki T, et al. (2010) A genetic variation in the dysbindin gene (DTNBP1) is associated with memory performance in healthy controls. World J Biol Psychiatry 11: 431–8.
34. Kubota K, Kumamoto N, Matsuzaki S, Hashimoto R, Hattori T, et al. (2009) Dysbindin engages in c-Jun N-terminal kinase activity and cytoskeletal organization. Biochem Biophys Res Commun 379: 191–5.
35. Okuda H, Kuwahara R, Matsuzaki S, Miyata S, Kumamoto N, et al. (2010) Dysbindin regulates the transcriptional level of myristoylated alanine-rich protein kinase C substrate via the interaction with NF-YB in mice brain. PLoS One 5: e8773.

Psychiatry and Clinical Neurosciences 2010; **64**: 592–607 doi:10.1111/j.1440-1819.2010.02148.x

Review Article

Apolipoprotein E and central nervous system disorders: Reviews of clinical findings

Masatoshi Takeda, MD, PhD,[1]* Rocío Martínez, PG Chem,[2] Takashi Kudo, MD, PhD,[1] Toshihisa Tanaka, MD, PhD,[1] Masayasu Okochi, MD, PhD,[1] Shinji Tagami, MD, PhD,[1] Takashi Morihara, MD, PhD,[1] Ryota Hashimoto, MD, PhD[1] and Ramón Cacabelos, MD, PhD[2]

[1]*Department of Psychiatry, Osaka University Graduate School of Medicine, Osaka, Japan; and* [2]*EuroEspes Biomedical Research Center, and Camilo José Cela University, Corunna, Spain*

Dementia is a major health problem in developed countries with over 25 million people affected worldwide and probably over 75 million people at risk during the next 20 years. Alzheimer's disease (AD) is the most frequent cause of dementia (50–70%), followed by vascular dementia (30–40%), and mixed dementia (15–20%). AD pathogenesis is still to be elucidated but it is believed to be the complex interaction between genetic and environmental factors in later life. Three causative genes for familial AD have been identified: amyloid precursor protein, presenilin-1, and presenilin-2. There are 150 genes involved with increased neuronal vulnerability to premature death in the AD brain. Among these susceptibility genes, the apolipoprotein E (ApoE) gene is the most prevalent as a risk for AD pathogenic process in which complex interactions between genetic and environmental factors are involved, leading to a cascade of pathogenic events converging in final pathways to premature neuronal death. Some of these mechanisms are common to several neurodegenerative disorders that differ depending upon the genes affected and the involvement of environmental conditions.

ApoE is a key lipoprotein in lipid and cholesterol metabolism and it is also the major risk gene for AD and many other central nervous system disorders. The pathogenic role of ApoE-4 is still to be clarified; however, diverse evidence suggests that ApoE may play pleiotropic functions in dementia and central nervous system disorders.

Key words: Alzheimer's disease, apolipoprotein E, dementia, genetic risk, neurodegeneration.

THE NUMBER OF dementia patients has increased significantly due to extended life spans. Dementia occurs in 6–15% of the elderly, causing a social problem in Japan and in other countries.[1] At present, clinicians are expected to attend 2 million dementia patients with behavioral and psychological problems of dementia, which causes a serious burden to the patients as well as to the caregivers.[2] Pharmacological treatment, mainly by anti-psychotics, works with some patients,[3–5] but is not always useful. Some opinions recommend refraining from prescribing anti-psychotics for dementia with behavioral and psychological problems of dementia,[6] and Chinese herbal medicine is recommended by some opinion leaders.[7–9] Behavioral therapy,[10] aromatherapy[11] and animal-assisted therapy[12] are sometimes useful. Different strategies are recommended by the clinical settings either at home[13] or in institutions,[14,15] targeting each type of dementia, including Alzheimer's disease (AD),[2] frontotemporal dementia,[16] diffuse Lewy body disease,[17] and vascular dementia.[18]

Considering the limited effectiveness of these interventions, researchers are now more interested in implementing early diagnosis,[19] disease-modifying therapeutics,[20,21] and even prevention of dementia.[22]

Correspondence: Masatoshi Takeda, MD, PhD, Department of Psychiatry, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita City, Osaka 565-0871, Japan. Email: mtakeda@psy.med.osaka-u.ac.jp

Received 5 July 2010; revised 25 August 2010; accepted 1 September 2010.

The concept of mild cognitive impairment (MCI) and subjective cognitive impairment (SCI) have been proposed to stimulate the understanding of the earlier stage, or prodromal stage of dementia,[1] in which the interaction in the aging brain of genetic factors and lifestyle, influencing environmental factors, is the major issue to be elucidated.[23] Apolipoprotein E (ApoE) is recognized as the most powerful genetic risk factor for dementia, and it is also a key player in lipid metabolism. We believe that the clinical findings related to ApoE should be taken into consideration, in order to reconcile gene and environmental interactions in the pathogenesis of dementia, including AD. The pathogenic role of ApoE-4 is still to be clarified. The biological functions of ApoE will be reviewed with regard to dementia and other central nervous system (CNS) disorders.

Apolipoprotein E gene

The three major isoforms of human ApoE(19q13.2) (ApoE-2, ApoE-3, ApoE-4) are coded by the ε2, ε3, and ε4 alleles. Differences in the amino acid sequence at sites A (residue 112) and B (residue 158) of the ApoE molecule distinguish the ApoE-2 (Cys/Cys), ApoE-3 (Cys/Arg), and ApoE-4 (Arg/Arg) isoforms.[24,25]ApoE-3 is the most frequent isoform (wild-type), and ApoE-4 differs from ApoE-3 in a Cys-to-Arg change at position 112 (ApoE-4/Cys112Arg). ApoE-2 (Arg158Cys) is the most common isoform of the four different mutations at the E2 position with isoelectric focusing. The other three ApoE-2 isoforms are E2(Lys146Gln), E2(Arg145Cys), and E2(Arg136Ser).[26] The ApoE gene encodes a 299-amino acid polypeptide (Mw 34 200). This gene is in close proximity with the APOC1, APOC2 and GPI genes in the same region of 19q.[27] Sequence haplotype variation in 5.5 kb of genomic DNA encompassing the whole ApoE locus and adjoining flanking regions revealed the existence of 22 diallelic sites defining 31 distinct haplotypes. Sequence analysis suggested that haplotypes defining the ApoE-3 and ApoE-2 alleles were derived from the ancestral ApoE-4 and that the ApoE-3 group of haplotypes had increased in frequency, relative to ApoE-4, over the past 200 000 years. Substantial heterogeneity is present in the three classes of sequence haplotypes, with interpopulation differences in the sequence variation underlying the protein isoforms, probably explaining conflicting results when interpreting phenotypic associations with variation in the common protein isoforms.[28,29]

The ApoE alleles show a peculiar distribution throughout the world.[30] The ApoE-3 allele is the most frequent in all human societies, especially in populations with a long-established agricultural economy, such as those of the Mediterranean basin, where the allele frequency is 0.849–0.898. ApoE-4 is the ancestral allele, with a frequency that still remains higher in Pygmies (0.407), Khoi San (0.370), Papuans (0.368), Lapps (0.310), some Native Americans (0.280), Australian Aborigines (0.260), and Aborigines of Malaysia (0.240) where an economy of foraging still exists, or food supply is scarce or sporadically available. The frequency of the ApoE-2 allele fluctuates with no apparent trend (0.145–0.02) and is absent in Native Americans and very low (<1%) in southern Europeans.[30–32]

Biological function of ApoE

The best known effect of ApoE is the regulation of lipid metabolism; however, in addition to its role in the transport of cholesterol and the metabolism of lipoprotein particles,[33] ApoE may be involved in many other physiological and pathological processes, including immunoregulation, nerve regeneration, activation of lipolytic enzymes (hepatic lipase, lipoprotein lipase, lecithin : cholesterol acyltransferase), ligand for several cell receptors, neuronal homeostasis, and tissue repair.[29]

ApoE is essential for the normal catabolism of triglyceride-rich lipoprotein constituents. The interaction of ApoE and the low-density lipoprotein (LDL) receptor controls the removal of ApoE-rich lipoproteins (very low-density lipoprotein [VLDL], chylomicron remnants, intermediate density proteins) and determines the homeostasis of cholesterol and triglycerides.[31] Some studies indicate that ApoE polymorphism variation may explain 14–17% of the genetic variability of plasma cholesterol concentrations.[31,34,35]

The three ApoE isoforms have different affinities for the LDL receptor, ApoE-3 and ApoE-4, showing similar affinities and ApoE-2 exhibiting a defective binding activity. ApoE plays a critical role in lipoprotein metabolism and plasma lipid homeostasis through its high-affinity binding to the LDL-receptor family. In solution, ApoE is an oligomeric protein, and the C-terminal domain causes ApoE's aggregation. The aggregation property presents a major difficulty for structural determination of this protein. Using protein engineering techniques, Fan *et al.* identified a monomeric, biologically active ApoE

C-terminal domain mutant. This mutant replaces five bulky hydrophobic residues in the region of residues 253–289 with either smaller hydrophobic or polar/charged residues (F257A, W264R, V269A, L279Q, V287E). These residues are critical for aggregation but may not be important for maintaining the structure, stability, and lipid-binding activity of this ApoE domain, suggesting that ApoE may use different epitopes for its aggregation property, helical structure/stability, and lipid-binding activity.[36]

ApoE-2-containing remnants and VLDL particles are slowly removed from the plasma and induce an upregulation of the liver LDL receptor and subsequent low concentration of plasma cholesterol. VLDL-ApoE-4 particles are removed from plasma faster than VLDL-ApoE-3 particles, inducing a down-regulation of the LDL receptor, and thus the VLDL-ApoE-4 phenotype is associated with higher concentration of circulating cholesterol.[31]

The ApoE genotype is an important determinant of plasma and CSF ApoE and lipid levels. The ApoE-2 allele is associated with high concentrations of ApoE and the ApoE-4 allele with lower ApoE levels.[31] Some authors have found association between ApoE-2 and ApoE-4 and high levels of plasma triglycerides as well as an association of ApoE-3 and low levels of triglycerides in the general population.[35]

It has also been suggested that individuals carrying the ApoE-4 genotype had a significantly greater increase in triglycerides accompanied by an increase in bodyweight, suggesting that obese individuals with an ApoE-4 allele might be at increased risk for developing hypertriglyceridemia and atherosclerosis; however, recent studies in the AD population clearly indicate that: (i) cholesterol levels are markedly increased in ApoE-4/4 carriers; (ii) triglyceride levels are the lowest in ApoE-4/4; and (iii) ApoE-4/4 carriers show a high atherogenic activity. These differences probably reflect the influence of endogenous factors interacting with ApoE to induce an alteration in lipid metabolism in patients with AD.[37–39]

The ApoE-4/4 and ApoE-3/4 genotypes have also been associated with high systolic blood pressure levels.[40] It has been suggested that the ApoE-2 allele may exert a protective effect on coronary atherosclerosis,[41,42] and that the ApoE-4 allele increases the risk of myocardial infarction and atherosclerosis.[31,42] After studying the association of ApoE with birthweight, Garces *et al.* suggested that the interaction of the ApoE genotype and birthweight may be an important determinant of atherosclerosis.[43]

Sullivan *et al.* studied the pattern of ApoE expression in the CNS. Immunocytochemistry on brain sections from three human ApoE targeted replacement mouse lines, wild-type mice, African green monkeys, and humans, and showed a predominantly glial pattern of ApoE expression. The levels of human ApoE protein in the hippocampus and frontal cortex were similar between targeted replacement mice and non-demented human tissue. Within a given brain region, the levels of ApoE were very similar amongst all three isoforms, which contrasts sharply with plasma, where ApoE2 levels are 16-fold higher than ApoE3 and ApoE4 levels. Across brain regions, cerebellar ApoE levels were significantly higher than cerebral ApoE levels.[44] In the human brain, ApoE-4 dose correlates inversely with dendritic spine density in the hippocampus.[45] ApoE is expressed at high levels in hepatocytes, macrophages, fibroblasts and astrocytes. Neurons also express ApoE at lower levels than astrocytes in response to various physiological and pathological conditions, including excitotoxic stress. Neuronal expression of ApoE is regulated by a diffusible factor or factors released from astrocytes, and this regulation depends on the activity of the extracellular signal-regulated kinase (Erk) pathways in neurons.[46] For many years, alterations in ApoE and defects in the ApoE gene have been associated with dysfunctions in lipid metabolism, cardiovascular disease, and atherosclerosis. An enormous number of studies, however, clearly documented the role of ApoE-4 as a risk factor for AD.

ApoE in Alzheimer's disease (AD)

In 1993 Allen Roses and co-workers found a clear association between ApoE genotypes and AD, demonstrating that the frequency of the ApoE-4 allele was significantly higher in late-onset Alzheimer's disease (LOAD).[47] Since then, many studies have confirmed this, reporting an increased frequency of the ApoE-4 allele in AD and the association of the ApoE-4 allele with LOAD and sporadic forms of AD. A protective effect of ApoE-2 for LOAD has also been proposed[48] and confirmed.[49] There is also a significant lowering of age at onset for subjects with ApoE-4/4 as compared to other ApoE genotypes.[50] ApoE-4 promotes arteriosclerosis and is less frequent in centenarians than in controls, and ApoE-2, which was associated with type III and type IV hyperlipemia, is more frequent in people with higher longevity rates.[51] The risk for AD increases from 20% to 90% and mean age at