3. Results

3.1. FMR1 allele frequency in the general population

For the *FMR1* (CGG)n allelic expansion, we analyzed 946 normal Japanese samples (576 males and 370 females). A total of 1161 alleles, (513 male and 324 female samples), were considered appropriate for amplification of the CGG repeat region of the *FMR1* gene. We could not amplify the repeat region from 155 alleles, (63 males and 46 females). Results indicated there were no carriers with an allele for full mutation or premutation. As seen in Table 1 and Fig. 1, all of the detected alleles were within the normal range (≤50 CGGs). The number of CGG repeats ranged from 8 to 50, with a modal number of 27 (35.75%), a second peak at 26 (19.29%), and a minor peak at 34 (5.25%). Jointly, 26–28 repeats were found in 844 alleles (72.61%). Intermediate size was defined as 40–50 repeats, and a total of 6 alleles were found within this range (5 males and 1 female). The overall normal allele frequency was 99.48% (1155/1161) and the intermediate allele prevalence was 0.52% (6/1161), i.e., 1:194 X chromosomes. Allele distribution observed in this study was significantly different from previous analyses that used the CLUMP software to examine Caucasian [4] (730.53, $p = 0.000010$), Mexican [22] (563.14, $p = 0.000010$) and other Japanese [16] (505.23, $p = 0.000010$) populations.

3.2. Analysis in autistic patients

Among the 109 patients (116 alleles), no expanded or intermediate alleles with more than 50 repeats were found (Table 2). All affected children had normal alleles that ranged from 16 to 36 with the first peak at 26 (38.79%). As compared to the general population, there were no significant differences noted among the *FMR1* allele frequencies. We also analyzed the patient's parents (106 mothers and 106 fathers, total 318 alleles), and found no premutation allele carriers (data not shown).

4. Discussion

In this study, we used the DNA samples from collected by the Pharma SNP consortium (PSC), a DNA Bank. The samples were kept relatively longer than the usual DNA testing. The amplification of the CGG repeat region is sometimes difficult if the sample is not fresh. Only the limited DNA samples were available and we could not amplify the repeat region from 155 alleles.

In previously reported studies, the CGG repeat allele frequencies differed ethnically [16,22] from the data reported by Arinami et al. for the normal Japanese population. The data reported by Arinami et al. also differed from our current results. The modal repeat numbers reported by Arinami et al. were 28 (40.5%), 29 (30.8%) and 35 (7.8%) while our numbers were 27 (35.8%), 26 (19.3) and 28 (17.6). The reason for this difference might

Table 1
Distribution of *FMR1* allele in normal Japanese population.

CGG	Female	Male	%	CGG	Female	Male	%
<Normal range>							
8	0	1	0.09	30	5	4	0.78
9	0	0		31	5	3	0.69
10	0	0		32	7	5	1.03
11	0	0		33	37	21	5.00
12	0	0		34	29	32	5.25
13	0	1	0.09	35	8	4	1.03
14	0	0		36	6	3	0.78
15	0	0		37	5	4	0.69
16	1	0	0.09	38	1	0	0.09
17	3	0	0.26	39	0	1	0.09
18	1	6	0.60	*<Intermediate>*			
19	1	1	0.17	40	0	1	0.09
20	11	3	1.21	41	0	1	0.09
21	16	12	2.33	42	0	0	
22	9	8	1.55	43	0	0	
23	5	1	0.52	44	0	0	
24	5	2	0.60	45	0	1	0.09
25	8	4	1.03	46	0	1	0.09
26	145	79	19.29	47	1	0	0.09
27	227	189	35.75	48	0	0	
28	100	104	17.57	49	0	0	
29	12	20	2.76	50	0	1	0.09
				Total	648	513	100

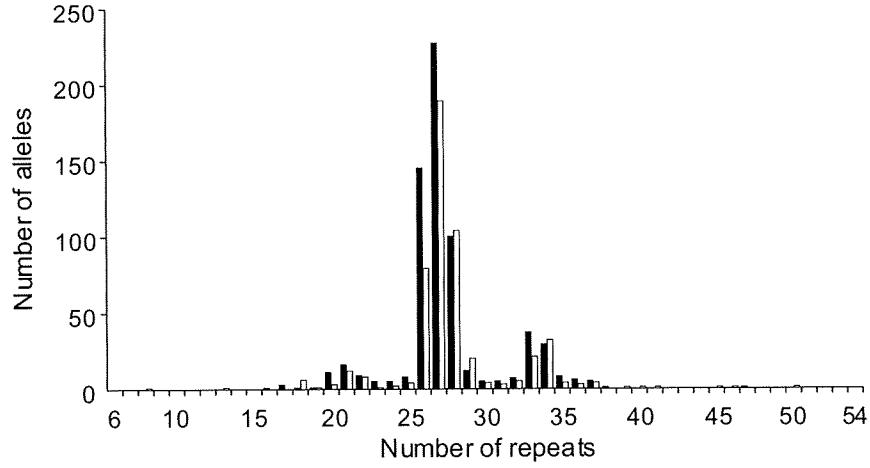

Fig. 1. Allele frequencies for CGG repeat in *FMR1* in 1161 normal Japanese population. Male and female were indicated by black and white column, respectively. See also Table 1.

Table 2
FMR1 allele frequencies in autistic patients and normal controls.

No. of *FMR1* CGG repeats		Autism ($n = 116$ alleles)		Controls ($n = 1161$ alleles)	
		Male ($n = 102$)	Female ($n = 14$)	Male ($n = 513$)	Female ($n = 648$)
6–39	(Normal)	102	14	508 (99%)	647 (99%)
40–54	(Intermediate)	0	0	5 (0.97%)	1 (0.15%)
55–200	(Premutation)	0	0	0	0
⩾200	(Full mutation)	0	0	0	0

be related to the different automated sequencer methodology and the PCR slippage. However, the difference was not simply associated with just the size but also the pattern, and thus the sample differences could have influenced the data.

In a previous screening of the mental retardation (MR) population in Japan, the prevalence of FXS ranged from 0.8% [15] to 2.4% [17] in males with MR. This result is slightly lower than that which has been reported for the Caucasian MR population rates, where it accounted for 2.6–8.7% among male patients with MR [29]. In contrast, the frequency of FXS in southern Taiwan [23] was 1.9% in the male MR populations, which is closer to that reported in Japan. These findings suggest that there is a difference in the prevalence between other Asian populations. However, since it is unlikely that all of the fragile X patients have been completely accounted for in Japan, it is important that a wide screening for FXS in the MR population be undertaken. Therefore, in order to more accurately study the prevalence of FXS, the frequency of the intermediate and premutation alleles within the normal population needs to be determined [5].

This is the first study that has focused on the prevalence of FXS by analyzing the intermediate and premutation alleles in the Japanese population. After screening 1161 X chromosomes from non-retarded healthy individuals, we found no carriers with the premutation allele. While the lack of any premutation allele has been confirmed in other reports in Japan [16,17], the number of collected samples in those studies was fewer than we collected in this study. Here, we estimated the frequencies of the intermediate allele (which ranged from 40 to 50 repeats) in normal Japanese subjects to be 1 in 103 males and 1 in 324 females. These frequencies were lower than the intermediate allele frequencies reported in the previous studies [10–13]. However, based on our findings, the prevalence of FXS subjects in Japan can be estimated to be 1 in about 10,000, which is lower than the predicted prevalence in Caucasian populations and in the subsets of Mediterranean and Pakistani populations (~1 in 4,000 males) [24,25]. Even so, it needs to be pointed out that the sample numbers used in our statistical analyses were relatively small and therefore, further analyses with sample numbers greater than 10,000 will need to be carried out in order to conclusively demonstrate the FXS prevalence in Japan. The structure of the CGG repeat may different in Japanese and the precise sequence study will be necessary.

We also performed an analysis that examined the length of the CGG repeats in Japanese autistic patients as a possible candidate locus for autism. The higher prevalence of autism that is seen in males versus females

suggests the possible involvement of the X chromosome. While some earlier studies have reported little or no association between FXS and autism [26,27], others have found a high association [28]. In this study, we did not find any significant difference in the distribution of the *FMR1* alleles nor did we find any premutation or intermediate alleles in any of the autism samples.

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A 糖代謝異常症1(肝型糖原病) Meet the Expert

1 病態と概念(図1)

糖原病はグリコーゲンの分解，合成にかかわる酵素群の遺伝的異常により起こる疾患である．グリコーゲン代謝はグリコーゲンからglucose-1-phosphateに至るステップをglycogenolysis(proximal glycogen degradation)，glucose-6-phosphate(G-6-P)以降をglycolysis(distal glycogen degradation)と分けている．一般に前者における酵素欠損ではグリコーゲンの蓄積が著明であり，後者ではグリコーゲンの蓄積が軽微である．

糖原病0型はグリコーゲン合成の障害であり，肝臓にはグリコーゲンの蓄積はなく，本章で報告されている他の3例とは病態が異なっている．肝型糖原病における問題は解糖過程の障害に直接起因する低血糖，グリコーゲンの臓器蓄積のみではなく，二次的に引き起こされる代謝の変化によっても症状の多様性がもたらされる．低血糖の程度にもよるが，低血糖によるinsulin/glucagonの低下により遊離脂肪酸が増加する．また，I型では産生されたG-6-Pがグルコースへ変換されないため蓄積し，解糖が進むため，乳酸が増加する．同時に，マロニルCoAも増加することでβ酸化が障害され，ケトン体の産生は低下する．高乳酸が持続する場合は腎からの尿酸排泄障害が起こり，高尿酸血症も呈する．I型とIII型では絶食で前者は高乳酸血症であるのに反し，後者ではケトーシスを起こすのが異なる点である．

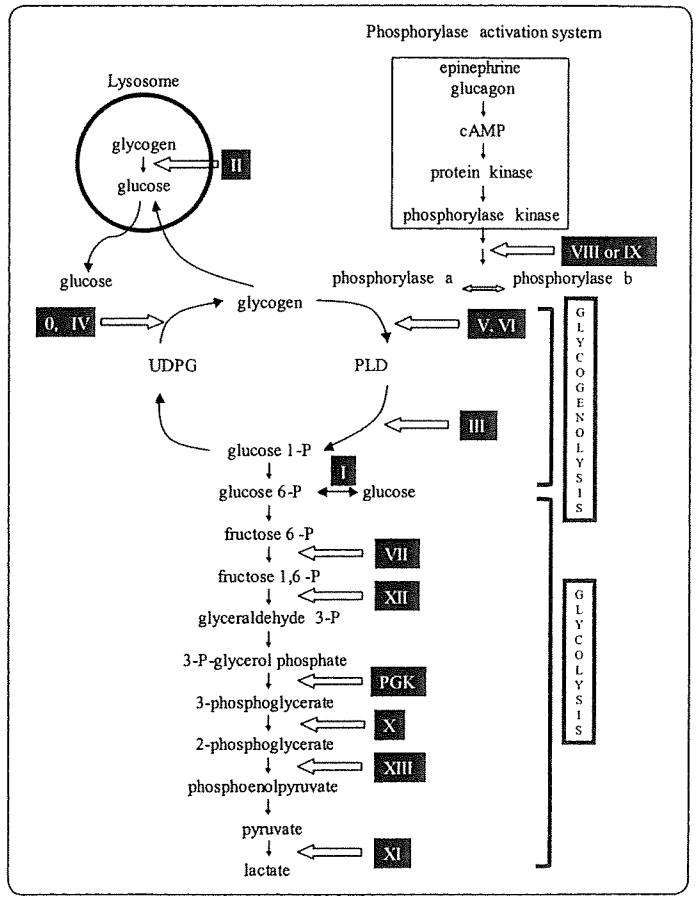

図1 グリコーゲン代謝
文献1より改変．

糖原病は肝型を中心としておおむね発見された順にギリシャ数字が病型に割り当てられ，VII型までは問題なく使用されている．しかし，phosphorylase b kinase欠損症は酵素学的，分子遺伝学的に細分化が進み，従来VIa型，VIII型あるいはIX型と命名され混乱しているが，臓器発現と遺伝形式によって分類することが主流となってきている．本章では一応VIII型としておく．

2 糖原病の臨床

肝型糖原病は**表1**のように現在7種類が報告されているが，糖原病は臨床病型としてhepatic(肝型)，muscular(筋型)，generalized

(全身型)に分類できる．肝型で多くを占める病型はI型，III型，VIII型であり，この3型で約90%を占めている．本章の3症例(I型，III型，VIII型)に比較的共通してみられる症状は肝腫大(主訴としては腹部膨満：protruded abdomen)，低血糖に起因する症状(空腹時のirritability，inactivityなど)，低身長(short stature)，特有な顔貌(rounded doll face)などである．

このなかでI型は最も症状が重い．それは解糖と糖新生の両者から生じたG-6-Pをグルコースに変換できないからである．つまり，解糖，糖新生の両経路からのグルコース産生に障害があるため，低血糖症状が比較的重度であると考えられる．理論的にはdebranching enzyme，リソソームのα-glucosidaseにより若干のグルコースが供給されるが不十分である．III型，VIII型では前者はphosphorylaseでグリコーゲンが分解できること，また両者とも糖新生系は正常であるので，低血糖は比較的軽微である．年齢が進めば肝腫大も軽快してくる．

表1　肝型糖原病

病型	欠損酵素	Gene symbol	遺伝子部位	酵素診断可能組織
0型	Glycogen syntase			
肝型		*GYS2*	12p12.2	肝臓
筋型		*GYS1*	19q13.3	筋肉
I型 (von Gierke)				肝臓
Ia	Glucose-6-phosphatase	*G6PC*	17q21	
Ib	G6P translocase (T1)	*SLC37A4*	11q23.3	
Ic	Phosphotranslocase (T2)	*NPT4* (?)	NPT4 (?)	
Id	Glucose translocase (T3)	?	?	
III型 (Cori)		*AGL*	1p21	筋，肝臓，白血球，赤血球線維芽細胞
IIIa	Amylo-1,6-glucosidase			
IIIb				
IIIc				
IIId	α-1,4-glucantransferase			
IV型 (Andersen)	Branching enzyme	*GBE1*	3p12.3	赤血球，肝臓
乳児肝型				
乳児神経筋型				
成人型				
APBD				
VI型 (Hers)	Liver phosphorylase	*PYGL*	14q21-q22	肝臓，白血球？
Phosphorylase activation system	Phosphorylase kinase			
VIII(IXA):XLG type I/II*	α-subunit of PBK	*PHKA2*	Xp22.2-p22.1	赤血球，白血球，線維芽細胞
autosomal recessive	β-subunit of PBK	*PHKB*	16q12-q13	肝臓
IXB	γ or δ subunit of PBK	*PHKG2*	16p12.1-p11.2	肝臓
IXC	cardiac muscle PBK	?	?	?
IXD (adult form)	muslce PBK	*PHKA1*	Xq12-q13	筋
X (multisystem)	protron kinase?	?	?	?
Fanconi-Bickel症候群	Glucose transporter 2	*GLUT2* (*SLC2A2*)	3q26.2-q27	—

＊typeIIは血球での酵素診断はできない．

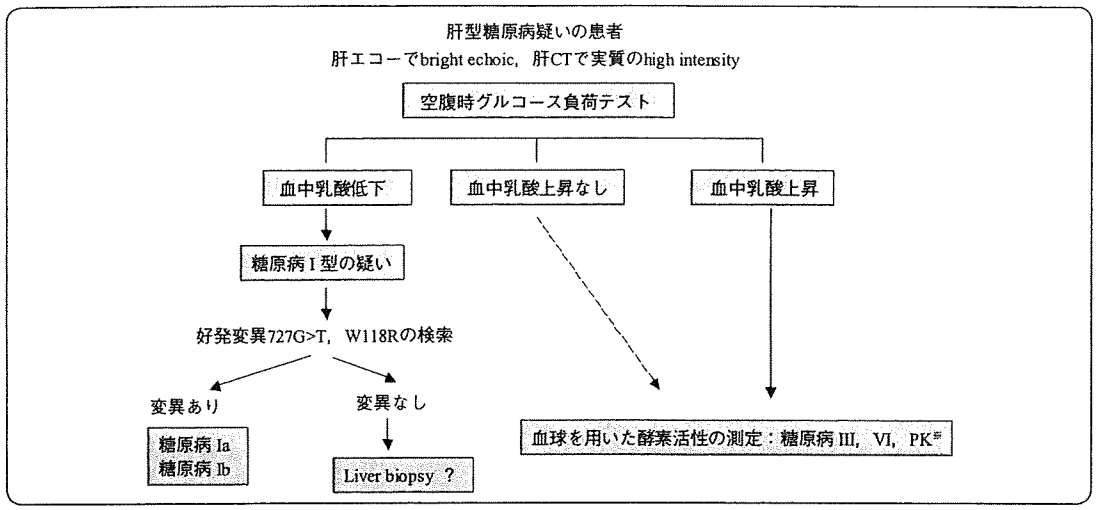

図2　肝型糖原病の鑑別診断

※ phosphorylase b kinase

糖原病0型は稀な糖原病であり肝腫大はなく，肝にはグリコーゲンの貯蔵が不十分であるため乳児幼児期に絶食で容易に低血糖が生じ，ケトーシスを伴う．血中の乳酸やアラニン値は低く，またグルコース負荷後インスリン値が正常でありながら高血糖や高乳酸の状態が続く場合は0型を疑うことができる．ケトン性低血糖症のなかにはおそらく本症が存在していると思われる．

3　診断と検査，どのように進めていくか

肝型糖原病の主要症状は本章で記載された4例のうち0型を除いて肝腫大，低血糖，ビリルビン上昇を伴わない肝機能障害，高乳酸血症(I型)，高脂血症，肝臓エコーでbright echo，肝臓CTではhigh intensity（ただし高脂血症の程度で肝臓にも脂肪の沈着が強い場合もある）が典型である．診断のアルゴリズムを**図2**に示すが，以前に比較すると肝生検の頻度は減少している．多くの肝型糖原病はI型，III型，VIII型であり血球を用いた酵素測定，遺伝子好発変異の検索でほとんど診断が可能であるからである．フェルナンデスの負荷試験は有用であるものの，乳幼児にはやや侵襲もあり，筆者らは**図2**のような手順で検査を進めている．

4　治療と予後のうえでの注意点

コーンスターチによる血糖の維持が基本的な治療になる．軽症肝型であるIII型，VIII型，VI型の予後は良好で，年齢が長じるに従って肝腫大は軽快し肝機能も改善してくる．しかし，III型でCK高値を合併している場合はIIIa型の可能性があり，将来的に筋力低下あるいは心筋症を起こしてくるので注意が必要である．I型は肝臓に線腫が発生し，肝硬変，癌化することがあること，また腎不全への移行についても注意が必要である．

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Pompe病の酵素補充療法（Myozyme）

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key words　Pompe disease, enzyme replacement therapy, lysosomal disease, acid alpha-glucosidase, limb-girdle muscular dystrophy, autophagy, glycogen storage disease type II

要　旨

Pompe病はacid alpha-glucosidaseが欠損し，リソソーム内のグリコーゲンの分解が障害される疾患で，乳児型は急速に心不全および呼吸不全が進行し，ほとんどが1歳までに死亡し，遅発型は肢帯筋優位のミオパチーの進行は緩徐ながら，呼吸不全が進行し死にいたる．この予後不良な疾患に対し酵素補充療法が実現し，乳児型では生命予後が改善し，遅発型でも有効性が報告されている．酵素補充療法は骨格筋に比しより心筋に効果的である．Pompe病では不可逆的な骨格筋構造の破壊が進行するため，良好な治療効果を得るためには早期診断および早期治療開始が重要である．acarboseを用いた末梢血における酵素診断方法が改良され，より迅速な診断も可能となっている．遅発型では診断までに長い期間が費やされる傾向がある．肢帯型筋ジストロフィー症や呼吸筋症状が前面にでるミオパチーでは，酵素診断による診断の見直しが検討されるべき症例が存在すると考えられる．

動　向

リソソーム酵素欠損症の中で，骨格筋が侵される唯一の疾患であるPompe病に対して，酵素補充療法が日本では2007年に認可され，現在50人以上がMyozymeによる治療を受けていると推測される．乳児型Pompe病では生命予後の改善は明らかであるが，遅発型への効果について今後さらに詳細が明らかになることが期待される．骨格筋構造破壊の進行以前に治療開始を実現するために，診断についていくつかの試みがなされ，迅速で侵襲の少ない末梢血を用いた酵素診断が改良されている．なお，現状では遅発型Pompe病の診断には長時間が費やされており，肢帯型筋ジストロフィー症と診断されている症例の中に遅発型Pompe病が混在している可能性があるため，診断の再検討が必要である．

A. 酵素補充療法のコンセプト

Pompe病（糖原病II型）はリソソーム酵素acid alpha-glucosidase（GAA, acid maltase）が欠損[1]し，さまざまな組織のリソソーム内にグリコーゲンが蓄積する常染色体劣性遺伝性のまれな疾患であり，発症頻度は1：40,000[2]と推定される．臨床的には特に心筋，骨格筋を侵し，

心筋症およびミオパチーを呈する．Pompe病に対し，CHO cellが産生するrecombinant human GAA（Myozyme，alglucosidase alpha，Genzyme）による酵素補充療法が開発され，臨床的な経験が蓄積されてきている．

Pompe病の酵素補充療法は，リソソーム酵素がreceptor-mediated endocytosisにより細胞に取り込まれる機構に基づいている[3]．rhGAAは酵素を細胞表面でcation-independent mannose 6-phosphate 受容体（CI-MPR）と結合するmannose-6-phosphate groupを含む前駆体である．受容体-酵素結合体は，細胞内に入り，エンドソームに運搬される．さらにlate endosome内の酸性のpHが受容体-酵素結合を離開させ，酵素は標的小器官であるリソソームに運搬される．内因性GAA前駆体と同様，rhGAAも中間体さらに成熟体に変換され[4] グリコーゲンを分解する．

B. 酵素補充療法の効果

1. 乳児型Pompe病

乳児型Pompe病は完全酵素欠損であり，乳児型は全身の著明な筋緊張低下，筋力低下および心肥大を呈し，多くは心不全および呼吸不全により1歳未満に死亡する[5-7]．

乳児型Pompe病における臨床試験[8] では，生後6カ月までに酵素補充療法（1回/2週間，20mg/kgまたは40mg/kg）を開始した乳児型Pompe病18例において，酵素補充療法の効果と安全性が評価されている．対象としてSpO_2 90%未満または，CO_2分圧が静脈で55mmHgまたは動脈で40mmHgを超える呼吸障害がある症例は除外されている．治療継続不能となるような重大な副反応の報告はない．

この臨床試験では，対照の未治療コホート群の生後18カ月の生存率は1.9%に対し，酵素補充療法を受けた群では全例が生存していた．同時点での人工呼吸器を必要としない生存率は66.7%であり，酵素補充療法により死亡リスクを99%減少，死亡または侵襲的人工呼吸管理のリスクを92%，死亡や非侵襲的人工換気を含めた人工呼吸管理のリスクを88%減少させた．酵素補充療法により生命予後は著しく改善されるだけでなく，心筋肥厚は全例で改善し，left ventricular mass index（LVMI）のZスコアは52週間で7.1から3.3に低下した．運動機能は治療開始後52週の時点で，18人中7人が歩行可能，3人が支持歩行可能，3人が座位可能だが，運動機能の改善が得られない症例は5人存在していた．

rhGAAに対するIgG抗体が88.9%に出現するが，臨床的効果との関連性は不明である．またCRIM（cross-reacting immunologic material）陰性例の集積は少ないためCRIMの有無と臨床効果の関連性は充分に明らかにされていないが，CRIM陰性例では高力価の抗体の産生や，治療効果が制限される可能性があるため，今後さらなる評価が必要となる．

上記以外にも，乳児型Pompe病への酵素補充療法の経験が蓄積されている[9,10]．治療開始が生後6カ月未満であっても呼吸障害が治療前に存在した例や治療開始が生後6カ月以上の症例を含む8例を対象とした報告[10] では，呼吸不全の進行や，感染症を契機に呼吸不全/心不全をきたして死亡した例は少なくない（52週の試験期間中に2名，その後生後23.5カ月から33.8カ月で4例）．

運動機能への効果についても，治療開始が生後6カ月以上の症例を含む臨床試験[8] や，rabbit milkから生成されたrhGAAにより治療された症例の骨格筋病理と臨床効果の関連[11] に基づき，良好な効果を得るためにはより早期の治療開始が必要であると考えられる．

2. 遅発型Pompe病

遅発型Pompe病は残存酵素活性を有し，乳児期以降に発症し，通常心筋は侵されず，重篤な心筋症は示さない．発症時期や進行はさまざまで，近位筋優位のミオパチーが緩徐に進行し，横隔膜罹患のために呼吸不全をきたす[12,13]．

90例を対象とした，二重盲検ランダム化プラセボコントロール試験（試験期間18カ月，試験開始時の平均年齢44歳）が終了し，6分間歩行距離の有意な延長（平均30mの延長）や，%予想努力性肺活量（%FVC）の1%上昇（プラセボ群では3%減少）が報告されている．遅発型への酵素補充療法の臨床効果の詳細が今後明らかにされることが期待される．

18例（平均年齢 30.8歳）を対象とした遅発型に対する臨床効果の報告[14]は，全例が車椅子を要し，1例を除いて人工呼吸管理を必要とする進行した症例における6カ月以上の酵素補充療法の結果で，呼吸機能の改善が10例にみられ，13人で運動機能が改善し，残りの5例では進行がみられず，治療開始前の水準を保った．

遅発型Pompe病の臨床効果の報告は，いまだ報告が少ないため，今後詳細が明らかにされる必要があるが，3例の8年間の治療効果の報告[15]も合わせると，遅発型Pompe病では酵素補充療法により運動機能障害，呼吸障害の進行が阻止され，QOLの改善が得られると考えられる．

C. Pompe病の病態（autophagic buildup）と早期治療開始の重要性

Pompe病はリソソーム酵素欠損症のうち唯一骨格筋を主要な罹患臓器とする疾患であるが，Pompe病の酵素補充療法に必要な酵素量はGaucher病の20倍である．Pompe病患者において酵素補充療法による骨格筋における蓄積したグリコーゲンのクリアランスは一般に低い[16]．ノックアウトマウスでは心筋に比し骨格筋からのグリコーゲンのクリアランスが低い[17,18]．組織により必要なGAA量が異なる可能性や，外因性の酵素がリソソームに運搬されるのに必要な蛋白（CI-MPRなど）の発現が組織により異なることが原因として提唱されている[18,19]．

GAA欠損によるPompe病の1次的な病態は，リソソーム内に分解されないグリコーゲンが蓄積することであるが，“autophagyの異常な蓄積”という2次的な病態が骨格筋の筋構築の破壊の進行に深く関与する．進行した骨格筋では，myofibrilが消失し，autophagyの蓄積がみられる[19,20]．

autophagyは，蛋白やミトコンドリアなどの小器官を分解するためにリソソームに運び，リサイクルすることにより細胞質の新生と分解の均衡を保つ細胞に保存された機構である[21]．macroautophagy（以下autophagy）では，二重膜構造のautophagosomeがリソソームやlate endosomeと融合し取り込んだ内容物を分解，リサイクルする．飢餓やその他のストレスがautophagyのinducerとして知られている．細胞に必要な機構であるautophagyがPompe病骨格筋線維ではKOマウスおよびhumanで過剰に蓄積し，筋線維の中心部に全長にわたってautophagic areaの形成がみられる．

autophagic buildupのプロセスはKOマウスでは，無症状である1カ月齢でautophagosomeの小胞が出現し，6カ月齢では，autophagic buildupは筋線維のstriationを分断する．24カ月齢では，筋線維は萎縮し，autophagic buildup areaはさらに拡大し，myofibrilと完全に置き換わる．同じく24カ月のマウスでは，autophagic areaには小胞構造は消失し，リソソーム，autophagosome膜の遺残のみが検出される．Pompe病では何らかのメカニズムによりautophagyが誘導され，さらにリソソームの機能不全からautophagic

areaが拡大し，骨格筋構造の破壊をもたらすと考えられる[22]．

骨格筋組織の破壊を進行させるPompe病の病態の研究が進み，骨格筋に対するよりよい治療効果を得るために，早期治療開始の重要性が強調され，診断方法についてもいくつかの検討が実施された．

D. Pompe病の酵素診断方法の改良と遅発型Pompe病の診断の問題点

1. 酵素診断方法の改良

Pompe病の診断は，線維芽細胞および筋組織におけるGAA活性測定によるものが最も信頼性が高い．筋病理では，症例または筋組織の部位によってはPompe病に特有な所見（vacuolar myopathy，リソソーム内のグリコーゲンの蓄積や酸フォスファターゼ活性の上昇）を示さない場合があること，また，遺伝子検索では，GAA遺伝子変異は200以上が報告されていることから，酵素活性測定が最も現実的な診断方法である．線維芽細胞の培養には時間を要すること，筋生検は侵襲的であるなどの欠点があり，酵素補充療法の導入に伴い，迅速で非侵襲的な診断方法の改良が求められた．分離末梢リンパ球は従来から酵素診断に用いられたが，GAAと同様酸性域に至適pHをもつGAAのホモロガスなアイソザイムmaltase-glucoamylase[23]が発現する白血球混入の可能性があるため，偽陰性となる可能性があった．acarboseをmaltase-glucoamylase活性を特異的に抑制する濃度(3～9μM)で測定系に加えることにより，リンパ球[24]のみならず白血球[25]，全血においても信頼性の高いGAA活性測定が可能となった．

現在，Pompe病診断として，末梢血を用いた方法を用いることにコンセンサスが得られ，その他の組織による活性測定またはDNA分析を併用すべきであるとされている[26]．

また，濾紙血を用いた活性測定方法[27]が確立され，新生児マススクリーニングの導入が検討されている．台湾では13万人以上の新生児を対象とした新生児スクリーニングが試みられ，新生児マススクリーニングされなかった場合の診断は生後3カ月～6カ月であることに対し，新生児スクリーニングをした場合には生後1カ月未満に診断が可能であったと報告している[28]．新生児スクリーニングの導入に際しては，スクリーニングでは乳児型と遅発型を明確に区別できない点，偽陽性の問題や，無症状の遅発型Pompe病の治療開始時期の問題，コストの問題などについて今後さらに検討が必要である[29]．

2. 遅発型Pompe病の診断の問題点

遅発型Pompe病の臨床症状は肢帯筋優位の筋力低下であり，軽度の骨格筋症状にもかかわらず呼吸不全が前景に立つ症例ではPompe病を疑う必要がある．症状発現年齢は1歳～71歳[12]と幅広く，症状進行の程度も多様である．さらに生検筋組織でも，特に光顕では正常に近い症例があるため，遅発型Pompe病の診断は容易ではない．

Müller-Felber[13]らによれば，遅発型Pompe病38例の症状発現から受診または診断までの期間はそれぞれ平均10.4年および7.1年であり，診断確定までに11/38例で他の診断がなされていた．

我々の28症例の後方視的分析[30]でも，症状出現から確定診断までの期間は，軽度の筋症状（走るのが遅い，転びやすいなど）出現からは平均11.7年（0～38年），日常生活に支障をきたす筋症状（階段昇降困難や呼吸不全）出現からは平均7年（0～26年）であり，遅発型Pompe病では診断までに長期間を費やしている現状が明らかとなった．診断確定前の臨床診断は筋ジストロフィー症が主体で，肢帯型が最も多かった．多発

性筋炎と診断された例もあった.

遅発型Pompe病の臨床症状は肢帯型筋ジストロフィー症（LGMD）と一部オーバーラップするため，臨床的にLGMDと診断される症例では，Pompe病を鑑別疾患としてとらえる必要がある．原因不明のLGMDや，筋力低下に比較して不釣合いに呼吸障害を強く認める例[31]では，酵素補充療法が可能となった現在，Pompe病の可能性を再検討すべきである．多発性筋炎と診断されているがステロイド無効例のなかにも遅発型Pompe病が含まれている可能性がある．筋生検を再度施行することなくリンパ球での酵素活性測定が可能であるため，我々は現在Pompe病の可能性がある症例について，依頼があれば酵素診断を行っている．

むすび

Pompe病に対して酵素補充療法が可能となり，乳児型では1歳を超えて生存することが可能となり，遅発型では呼吸機能，運動機能の改善または進行を防ぐ効果が報告されている．酵素補充療法では骨格筋症状の改善が得られない症例もあり，よりよい効果を得るために早期の治療開始が重要である．酵素補充療法の導入に伴い，迅速で侵襲のない酵素診断の方法も改良された．遅発型Pompe病の診断には，肢帯型筋ジストロフィー症と類似の症状を示す症例のなかにPompe病が含まれている可能性があることを常に念頭におくべきである．一方で，Pompe病の骨格筋組織破壊を引き起こす2次的な病態（過剰なautophagyの蓄積）のコントロールの方法についての検討も必要であると考えられる．他の疾患と同様，今後遺伝子治療や，シャペロン療法などの開発も期待される．

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Involvement of *SMARCA2*/BRM in the SWI/SNF chromatin-remodeling complex in schizophrenia

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Chromatin remodeling may play a role in the neurobiology of schizophrenia and the process, therefore, may be considered as a therapeutic target. The *SMARCA2* gene encodes BRM in the SWI/SNF chromatin-remodeling complex, and associations of single nucleotide polymorphisms (SNPs) to schizophrenia were found in two linkage disequilibrium blocks in the *SMARCA2* gene after screening of 11 883 SNPs (rs2296212; overall allelic $P = 5.8 \times 10^{-5}$) and subsequent screening of 22 genes involved in chromatin remodeling (rs3793490; overall allelic $P = 2.0 \times 10^{-6}$) in a Japanese population. A risk allele of a missense polymorphism (rs2296212) induced a lower nuclear localization efficiency of BRM, and risk alleles of intronic polymorphisms (rs3763627 and rs3793490) were associated with low *SMARCA2* expression levels in the postmortem prefrontal cortex. A significant correlation in the fold changes of gene expression from schizophrenic prefrontal cortex (from the Stanley Medical Research Institute online genomics database) was seen with suppression of *SMARCA2* in transfected human cells by specific siRNA, and of orthologous genes in the prefrontal cortex of *Smarca2* knockout mice. *Smarca2* knockout mice showed impaired social interaction and prepulse inhibition. Psychotogenic drugs lowered *Smarca2* expression while antipsychotic drugs increased it in the mouse brain. These findings support the existence of a role for BRM in the pathophysiology of schizophrenia.

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INTRODUCTION

Schizophrenia is a chronic, severe and disabling brain disorder that affects approximately 1% of the world's population. A large body of data consistently supports the involvement of complex genetic components causally linked to schizophrenia. Association studies including genome-wide scans have identified many risk alleles that have small effects (1–5). In addition to genetic studies, altered expression of many genes and proteins in schizophrenic brains has been documented (6) and epigenetic regulation in schizophrenia has also been studied (7). Among epigenetic mechanisms, the role of chromatin modification in psychiatric disorders and related fields has been reported for epilepsy (8), drug addiction (9,10), depression (11), autism (12), fear (13), learning and memory (13,14), social cognition (15) and stress-related behaviors (16,17). Histone modifications may contribute to the pathogenesis of prefrontal dysfunction in schizophrenia (18).

The *SMARCA2* gene encodes BRM, one of the earliest described chromatin remodeling multiprotein complexes in the yeast SWI/SNF complex (19–23), and highly conserved among eukaryotes (24). The SWI/SNF molecules are mutually exclusive within the complexes and harbor ATPase activity (25–27). This complex functions by destabilizing the interactions between DNA and histones in the nucleosome in an ATP-dependent reaction (28,29). Mammalian SWI/SNF complexes are present in biochemically diverse forms, indicating that they may have specialized nuclear functions (30). ATP-dependent remodeling complexes involved in chromatin opening or compaction are important in the regulation of transcriptional processes associated with development, cellular differentiation and oncogenesis (22,31–35).

BRM interacts with several transcription factors and other DNA-binding proteins and is involved in chromatin structural modification in the epigenetic regulation of gene expression (36). *SMARCA2* expression is induced to a high level during differentiation to neurons and astrocytes, suggesting an important role in neural cell differentiations (37). Because BRM potentially influences expression of many genes, it is hypothesized that functional changes to *SMARCA2*/BRM may contribute to gene expression changes reported to occur in schizophrenia.

RESULTS

SNPs in two linkage disequilibrium blocks within the SMARCA2 gene were associated with schizophrenia in the Japanese population

Initially, in 100 Japanese schizophrenic patients, 11 883 SNPs were screened for association with schizophrenia. These SNPs exist on the Illumina Human-1 BeadChip and are also deposited in a Japanese SNP (JSNP) database of 1480 Japanese control chromosomes (http://snp.ims.u-tokyo.ac.jp/index.html). The potential impact of population structure on this association study was evaluated by using the genome-wide χ^2 inflation factor, λ, as a genomic control (38,39). The estimated value of λ was 1.05, by which genome-wide association *P*-values were corrected. After correction for multiple testing of these 11 883 SNPs, no significant association was found between them and schizophrenia (Supplementary Material, Table S1).

In the replication 1 cohort, comprised of 576 Japanese schizophrenic patients and 576 Japanese control subjects, an attempt was made to replicate the association of the top 5 SNPs, as ranked by the association *P*-values, with schizophrenia. A potential association was found for only one, rs2296212 (one-sided allelic $P = 0.009$) (Supplementary Material, Table S1). Subsequent analysis in the replication 2 samples of 1344 Japanese schizophrenic patients and 1344 Japanese control subjects confirmed the association for this SNP (one-sided allelic $P = 0.04$). The *P*-value for the association was 5.8×10^{-5} when the initial genome-wide sample and the total replication $1 + 2$ samples were combined (Table 1).

Because the *SMARCA2* gene encodes BRM in SWI/SNF chromatin remodeling, SNPs within genes that encode proteins potentially involved in chromatin remodeling were re-evaluated for association with schizophrenia. *CREBBP, DNMT1, DNMT2, DNMT3A, DNMT3B, HAT1, HDAC2, HDAC3, HDAC4, HDAC7, HDAC9, HNMT3A, MYST1, MYST2, MYST4, SIN3A, SIN3B, SMARCA3, SMARCA4, SMARCA5* and *SMARCC1* were selected in addition to the *SMARCA2* genes, with a less stringent criteria of association used (*P*-values of less than 0.05). For this screen, SNPs from the same 100 schizophrenia patients were screened with an Illumina HumanHap370 BeadChip and compared with those in 1868 chromosomes of Japanese volunteers listed in the JSNP database [deposited October 2007, (http://snp.ims.u-tokyo.ac.jp/index.html)]. Although potentially significant association was observed in the *SMARCC1* and *DNMT3B* genes, this did not exist after correction for multiple testing (Supplementary Material, Table S2). An attempt was made to replicate the associations of rs13063042 and rs17079785 in *SMARCC1* and rs2424932 in *DNMT3B* with schizophrenia in the replication 1 sample. However, no significant results were obtained (Supplementary Material, Table S2).

The *SMARCA2* gene is located at chromosome 9p24.3 and spans 178 282 bp comprising 34 exons. The SNP of rs2296212 was non-synonymous, D1546E, in exon 33. Subsequent genotyping of 34 tag SNPs in the *SMARCA2* gene in the replication $1 + 2$ samples identified a significant association with schizophrenia for three SNPs [rs2066111 in intron 12 (allelic $P = 8.2 \times 10^{-5}$), rs3763627 in intron 12 (allelic $P = 1.2 \times 10^{-5}$) and rs3793490 in intron 19 (allelic $P = 3.0 \times 10^{-6}$), Fig. 1 and Table 1]. Resequencing of the coding region of *SMARCA2* in 24 Japanese patients with schizophrenia identified no non-synonymous mutations except for D1546E.

The distance between D1546E and rs3793490 was 95 351 bp and these two SNPs were not in linkage disequilibrium ($r^2 = 0$, $D' = 0$–0.16). The three intronic SNPs were in modest linkage disequilibrium with each other; the *r* squares were 0.59 between rs2066111 and rs3763627, 0.52 between rs206111 and rs3793490 and 0.85 between rs3763627 and rs3793490. Therefore, SNPs in two linkage disequilibrium blocks in the *SMARCA2* gene, one in the middle and one in the 3′ region of the gene, were found to be associated with schizophrenia in our Japanese population.

Table 1. Associations between SNPs in the SMARCA2 gene and schizophrenia in the Japanese populations

SNP ID	Population		Genotype count (frequency)			*P*	Allele count (frequency)		*P*	Odds ratio (95% CI)
rs2296212			CC	CG	GG		C	G		
	Affected (screening)	$n = 100$	59 (0.59)	32 (0.32)	9 (0.09)		150 (0.75)	50 (0.25)		1.90 (1.34–2.69)
	Controls (JSNP)[a]	$n = 750$					1276 (0.85)	224 (0.15)	0.0003	
	Affected (replication 1)	$n = 572$	370 (0.65)	168 (0.29)	34 (0.06)		908 (0.79)	236 (0.21)		1.29 (1.05–1.60)
	Controls (replication 1)	$n = 574$	398 (0.69)	160 (0.28)	16 (0.03)	0.02	956 (0.83)	192 (0.17)	0.009[b]	
	Affected (replication 2)	$n = 1343$	907 (0.68)	384 (0.29)	52 (0.04)		2198 (0.82)	488 (0.18)		1.14 (0.99–1.31)
	Controls (replication 2)	$n = 1338$	933 (0.70)	373 (0.28)	32 (0.02)	0.07	2239 (0.84)	437 (0.16)	0.04[b]	
	Affected (replication 1 + 2)	$n = 1915$	1277 (0.67)	552 (0.29)	86 (0.04)		3106 (0.81)	724 (0.19)		1.18 (1.05–1.33)
	Controls (replication 1 + 2)	$n = 1912$	1331 (0.70)	533 (0.28)	48 (0.03)	0.005	3195 (0.84)	629 (0.16)	0.0027[b]	
	Overall (screening+replicatinon)	$n = 2015$	1336 (0.66)	584 (0.29)	95 (0.05)		3256 (0.81)	74 (0.19)		1.25 (1.12–1.39)
	Overall (JSNP+replication)						4471 (0.84)	853 (0.16)	5.8×10^{-5}	
rs2066111			GG	AG	AA		G	A		
	Affected (replication 1 + 2)	$n = 1912$	974 (0.51)	776 (0.41)	162 (0.08)		2724 (0.71)	1100 (0.29)		1.22 (1.10–1.34)
	Controls (replication 1 + 2)	$n = 1907$	846 (0.44)	866 (0.45)	195 (0.10)	8.0×10^{-5}	2558 (0.67)	1256 (0.33)	8.2×10^{-5}	
rs3763627			AA	AT	TT		A	T		
	Affected (replication 1 + 2)	$n = 1905$	1116 (0.59)	690 (0.36)	99 (0.05)		2922 (0.77)	888 (0.23)		1.26 (1.14–1.40)
	Controls (replication 1 + 2)	$n = 1900$	1002 (0.53)	744 (0.39)	154 (0.08)	1.0×10^{-5}	2748 (0.72)	1052 (0.28)	1.2×10^{-5}	
rs3793490			GG	GT	TT		G	T		
	Affected (screening)	$n = 122$	9 (0.07)	37 (0.30)	76 (0.62)		55 (0.23)	189 (0.77)		1.29 (0.94–1.77)
	Controls (JSNP)	$n = 934$	83 (0.09)	344 (0.37)	507 (0.54)	0.13	510 (0.27)	1358 (0.73)	0.11	
	Affected (replication 1 + 2)	$n = 1895$	109 (0.06)	688 (0.36)	1098 (0.58)		906 (0.24)	2884 (0.76)		1.27 (1.15–1.41)
	Controls (replication 1 + 2)	$n = 1908$	162 (0.08)	767 (0.40)	979 (0.51)	4.0×10^{-6}	1091 (0.29)	2725 (0.71)	3.0×10^{-6}	
	Overall (screening+replicatinon)	$n = 2017$	118 (0.06)	725 (0.36)	1174 (0.58)		961 (0.24)	3073 (0.76)		1.25 (1.14–1.38)
	Overall (JSNP+replication)	$n = 2842$	245 (0.09)	1111 (0.39)	1486 (0.52)	2.0×10^{-6}	1601 (0.28)	4083 (0.72)	2.0×10^{-6}	

Allelic *P*-values were calculated by chi-square test (two-sided) and genotypic *P*-values were Cochran-Armitage test.
[a]http://snp.ims.u-tokyo.ac.jp/cgi-bin/SnpInfo.cgi?SNP_ID=IMS-JST050328.
[b]One-sided.

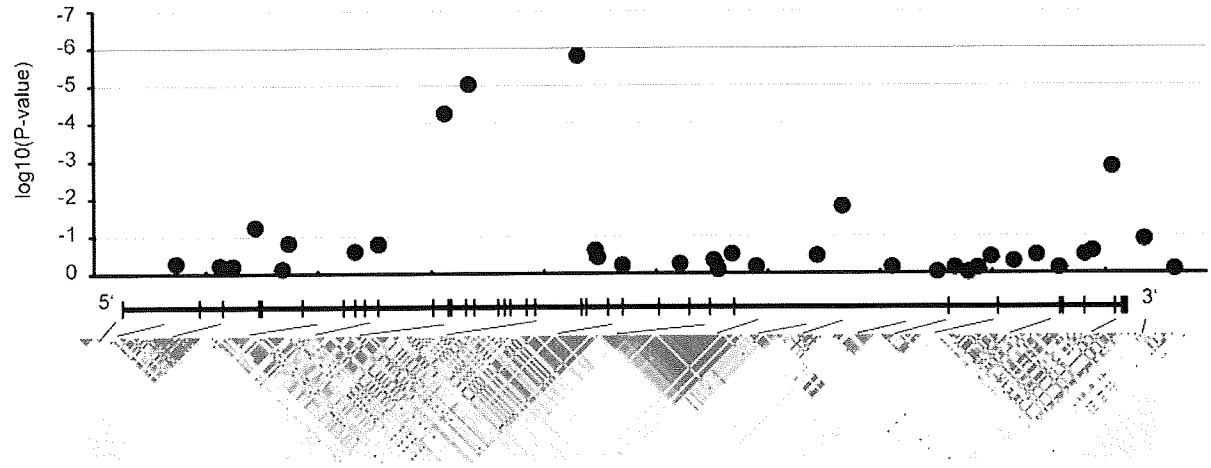

Figure 1. Association of tag SNPs in *SMARCA2* with schizophrenia in the replication samples. Linkage disequilibrium in the HapMap data is also shown with red indicating high linkage disequilibrium and white denoting low linkage disequilibrium. Exons are shown in the middle.

Risk alleles of intronic SNPs were associated with lower *SMARCA2* gene expression level in the postmortem prefrontal cortex

Transcription level in the postmortem prefrontal cortex, as measured by TaqMan real-time PCR, was not significantly different by diagnosis, ethnicity, age, sex, postmortem intervals (PMI) or pH of brain samples. Linear multiple regression analysis including sex, age, PMI, pH, ethnicity, diagnosis and genotypes of the SNPs present resulted in a significant associations of rs3763627 ($P = 0.005$) and rs3793490 ($P = 0.01$) with SMARCA2 expression level. The difference in *SMARCA2* expression level between the genotypes is shown in Figure 2. Alleles observed more frequently in the schizophrenia group were associated with a low expression level of *SMARCA2* in the prefrontal cortex. When Australian and Japanese schizophrenic subjects, or schizophrenia patients and controls were separately analyzed, there were no significant differences seen in *SMARCA2* expression level. *SMARCA2* expression level was not significantly different between the genotypes of rs2066111 and rs2296212.

The risk allele E1546 causes lower nuclear localization efficiency of BRM

BRM has several domains highly conserved among species, such as yeast (23), Drosophila (40) and mammals (27). Although D1546E is located downstream to the bromodomain, the polymorphism is in the highly conserved region among mammalian species, and glutamic acid (E) at the 1546 bp site in human is commonly found among mammalian species (Fig. 3A).

BRM is localized in the nucleus (19). In order to analyze any functional difference of BRM between the two allele types, localization of BMR was investigated by EGFP fusion protein (Fig. 3B) transfected into the human glioblastoma cell line T98G. The D1546 type of EGFP fused BRM (EGFP/BRM*D) localized to the nucleus; however, the E1546 type of EGFP fused BRM (EGFP/BRM*E) existed both in the cytoplasm and nucleus (Fig. 3C and D). Cells transfected with the two different alleles had a different morphology (Fig. 3C). This finding indicates lower nuclear localization efficiency of the E1546 isoform (BRM*E) in transfected cells, and it is hypothesized that the E1546 isoform has less functionality than the D1546 type (BRM*D).

Lower function of the E1546 form of BRM was supported by transcriptional changes seen in transfected cells

The functional capability of BRM*E was evaluated by comparisons of gene expression changes that were introduced by *SMARCA2**E, *SMARCA2**D and siRNA targeted towards SMARCA2. pDEST26 expression vectors were constructed with *SMARCA2**E or *SMARCA2**D and introduced into T98G cells. Transcription of *SMARCA2**E and *SMARCA2**D was 50-folds higher than that of control cells (Fig. 4A). The siRNA targeted against the *SMRACA2* gene was introduced into the same cell lines and translation level decreased to approximately 1/10 of that from control cells (Fig. 4A). Transcription levels of *SMARCA2* were comparable to the level of translation seen after the immunoblot analysis (Fig. 4A). After transfection of the pDEST26 with *SMARCA2*E*, the pDEST26 with *SMARCA2*D*, and siRNA in T98G cells, gene expression level was measured using Sentrix Human WG-6 BeadChips (Illumina, CA, USA). Expression changes introduced by *SMARCA2*E*, compared with that of *SMARCA2*D*, were significantly correlated with those of the siRNA treatment, compared with that of mock-treated cells (Fig. 4B, $P < 0.0001$), again supporting a lower functionality of BRM*E compared with BRM*D. When outliers defined as values exceeding two standard deviations from the mean in *SMARCA2**E against *SMARCA2**D, and from that in siRNA were excluded, the correlation was more significant ($P < 0.00001$).

Gene expression changes seen after suppression of SMARCA2 in transfected human cells were correlated with those found in the postmortem prefrontal schizophrenic brains

To evaluate the relationship between lower functioning of SMARCA2 and the gene expression profile seen in schizophrenia, gene expression changes after introduction of

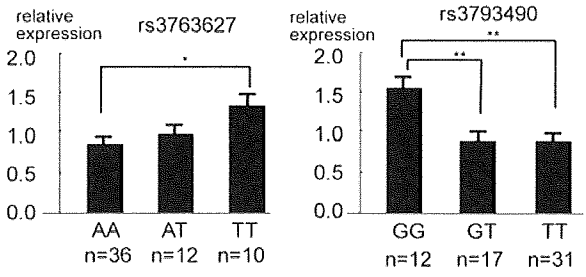

Figure 2. *SMARCA2* expression levels in the postmortem prefrontal region by genotype [rs3763627 (intron 12) and rs3793490 (intron 19)]. The symbols * and ** indicate $P < 0.05$ and $P < 0.01$, respectively, by Student's *t*-test. The A allele of rs3763627 ($P = 0.005$) and the T allele of rs3793490 ($P = 0.01$) were associated with lower expression level, when genotype was coded as 0, 1 or 2 depending on the number of copies of the risk allele present, and multiple a simple regression model was fitted. The vertical scores show average (SEM) of relative expression in each of the three genotype groups, compared with mean gene expression in the total samples.

siRNA to T98G cells were compared with those from the postmortem prefrontal cortex of schizophrenia patients. Data from all frontal cortex studies were utilized to determine the mean expression of each gene. Among 1051 genes with significant expression changes seen in schizophrenic patients from the SMRI database ($P < 0.05$, SMRIDB, https://www.stanleygenomics.org/), 445 genes could not be evaluated due to their low expression level in T98G cells. The fold change of expression of the remaining 606 genes from the SMRIDB was significantly correlated with expressional changes seen after siRNA treatment in T98G cells ($P < 0.0009$) (Fig. 5A). When outliers defined as mean values exceeding 10-folds in siRNA against mock were excluded, the correlation was still significant ($P < 0.003$).

Gene expression profiles from *Smarca2* knockout mice prefrontal cortex were correlated with those seen in the postmortem prefrontal schizophrenic brains

To evaluate the relationship between lower functionality of BRM and the gene expression profile of schizophrenia, gene expression profiles from the prefrontal cortex of *Smarca2* knockout mice (41) were compared with those from the postmortem prefrontal cortex of schizophrenic patients in the SMRIDB. Gene expression in the prefrontal cortex of three pairs of *Smarca2* −/− mice and littermate wild-type mice (*Smarca2* +/+) at 8 weeks of age were measured with MouseWG-6 BeadChips (Illumina, CA, USA). Expression level of 586 genes was significantly different between *Smarca2* −/− mice and *Smarca2* +/+ mice (*t*-test, $P < 0.05$). Fifty-two orthologous genes were found between these 586 genes and the 1051 genes with significant expression changes seen from schizophrenic patients compared with controls in the SMRIDB (Table 2). The fold change in expression of these 52 genes in *Smarca2* −/− mice compared with *Smarca2* +/+ mice was significantly correlated with the fold changes of orthologous genes in schizophrenia compared with controls in the SMRIDB ($P < 0.002$) (Fig. 5B). When outliers defined as mean values in *Smarca2* −/− mice exceeding 2-folds from the mean in *Smarca2* +/+ mice were excluded, the correlation was more significant ($P < 0.001$).

Confirmation of genes interacting with BRM by ChIP assay

Although transcriptional changes were observed in many genes by down- or up-regulation of the *SMACA2* gene in cultured T98G cells or in the *Smarca2* knockout prefrontal brain, they were direct or indirect consequences of interaction with the BRM-containing SWI/SNF chromatin remodeling factors. To confirm the association of endogenous BRM with the promoters of these transcriptionally influenced genes, ChIP assay was carried out using an antibody against BRM. DNA regions that interacted with BRM were collected and the sequence for −1163 to −1026 bp up-stream of the *HOMER1* gene confirmed by PCR. The *HOMER1* gene was selected because, among the genes in Table 2, it exhibited a more than 10-fold reduction in the expression in T98G cells after transfection by the siRNA targeted towards *SMARCA2*, and a more than 10-fold increase after transfection of the pDEST26 with *SMARCA2*E* or the pDEST26 with *SMARCA2*D*. These regions were also detected after ChIP assay using an antibody against MeCP2, which interacted with BRM (Fig. 6).

Impaired social interaction and prepulse inhibition in *Smarca2* knockout mice

To evaluate schizophrenia-related behaviors in *Smarca2* knockout mice, social interaction and prepulse inhibition (PPI) of the acoustic startle reflex was measured. Male and female data were combined for the analysis because no sex differences were observed. *Smarca2* −/− mice spent a significantly shorter time when making contact with an unfamiliar intruder mouse compared with mice of the other genotypes (Fig. 7A, $P = 0.03$). There was no significant difference in novelty seeking behavior between genotypes (data not shown). *Smarca2* −/− mice showed significant disturbance of PPI at 78 dB ($P = 0.02$) and a trend toward disturbance of PPI at 82 dB and 86 dB ($P = 0.07$) compared with the other genotypes (Fig. 7B).

Smarca2 gene expression in the mouse brains was decreased by psychotogenic drugs treatments and increased by antipsychotic drug treatments

The involvement of aberrant NMDA receptor signaling and a hyperdopaminergic state has been assumed in the pathophysiology of schizophrenia. *Smarca2* expression was evaluated in the mouse brain using the MK-801 non-competitive antagonist of NMDA receptors, the indirect dopamine receptor agonist methamphetamine and the antipsychotic drugs haloperidol and olanzapine. The expression of *Smarca2* was significantly decreased after the administration of MK-801 or methamphetamine and increased by administration of haloperidol and olanzapine (Fig. 7C).

DISCUSSION

SNPs in two linkage disequilibrium blocks in the *SMARCA2* gene were associated with schizophrenia in Japanese populations and the risk alleles are likely to confer a lower functioning of *SMARCA2*/BRM through altered gene

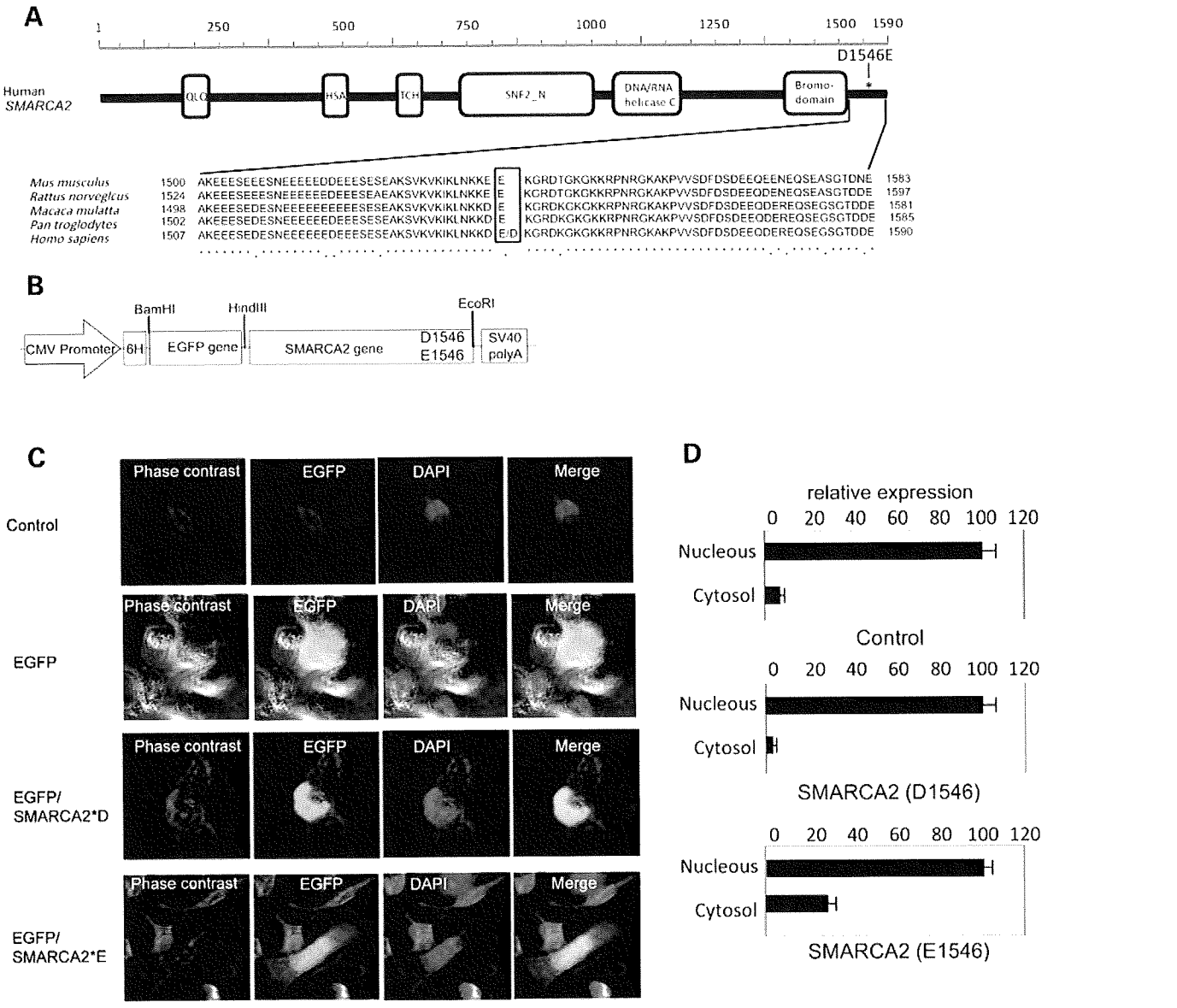

Figure 3. Nuclear localization efficiency of BRM between alleles of rs2296212 (D1546E). (**A**) Domains in BRM and evolutional comparisons of sequences around human D1546E. QLQ, Gln-Leu-Gln motif; HAS, Helicase/SANT-associated, DNA binding; TCH, associated with TFs and helicases; DNA/RNA helicase C; Bromodomain, an acetyl-lysine binding domain. Arrow indicates the position of the D1546E polymorphism. (**B**) Plasmid construction for the fusion proteins. CMV pro., cytomegalovirus promoter; 6H, 6 histidine; EGFP, enhanced green fluorescence protein; SMARCA2; SV40 poly A, SV40 polyadenylation signal. (**C**) Different cell morphology and intracellular localization of EGFP-SMARCA2 fusion protein between D1546 and E1546 forms in transfected T98G human glioblastoma cells. Control: a cell transfected with pDEST26 vector; EGFP: cells transfected with pDEST26 with EGFP; EGFP/SMARCA2*D: a cell transfected with pDEST26 EGFP-SMARCA2 (D1546); EGFP/SMARCA2*E: cells transfected with pDEST26 EGFP-SMARCA2 (E1546). One hundred cells were visualized for each sample. (**D**) Quantification of BRM expression in nucleus and cytosol of cells by western blot analysis. Protein expression level is shown as a mean (SEM) ratio of the protein expression level in the nucleus. Data from triplicate experiments were normalized to the expression of β-actin.

expression or intracellular localization. Although the study did not find significant differences in *SMARCA2* transcription levels in the postmortem prefrontal cortex between schizophrenic patients and controls (data not shown), the SMRI database showed a non-significant trend toward decreased *SMARCA2* transcription levels in schizophrenics compared with controls ($P = 0.07$). Additionally, a disruption of the *SMARCA2* gene in a patient with schizophrenia has been reported (42). BRM is involved in the modification of chromatin structures in epigenetic regulation of gene expression (36). Therefore, it was hypothesized that low functionality of *SMARCA2*/BRM is associated with schizophrenia through its pleiotropic effects on transcriptional regulation of many genes. This hypothesis is supported by gene expression profiles from the prefrontal cortex and behavioral observations in *Smarca2* knockout mice.

From an evolutionary perspective, the amino acid residue corresponding to human D1546E in *SMARCA2* (*Smarca2*) of dog, mouse, rat, horse, cow and chimpanzee is E. Therefore, E1546 is probably the ancestral type in humans (Fig. 3A).

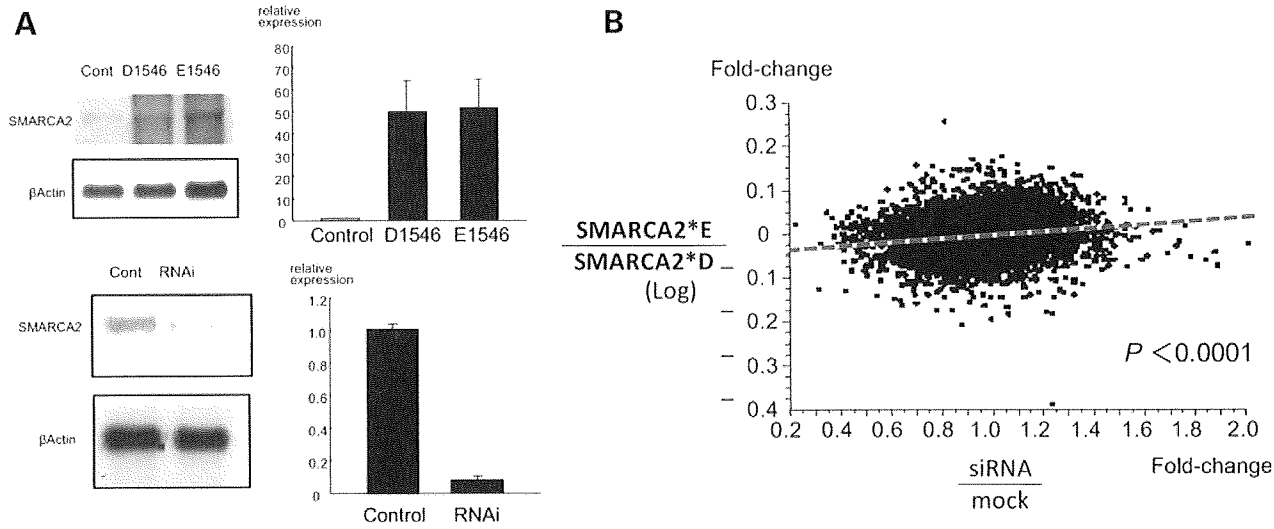

Figure 4. Evaluation of the E1546 and D1546 forms of BRM by gene expression profiles. (**A**) The expression levels of *SMARCA2* in T98G cells transfected by the pSMARCA2*E and *D constructs detected by northern blotting (upper-left) and real-time PCR (upper-right). For real-time PCR, the expression ratio is shown using the transcription level of a control as 1. The expression levels of *SMARCA2* gene knocked down with siRNAs by northern blotting (lower-left) and real-time PCR (lower-right). The data from triplicate experiments were normalized to the expression of *β-actin* or *GAPDH* gene. (**B**) Regression analysis between fold difference in expression in T98G cells transfected with pDEST26 with *SMARCA2*E*, cells transfected with pDEST26 with *SMARCA2*D* and cells transfected with siRNA compared with mock transfected cells. Gene expression after transfection was measured with Sentrix Human WG-6 BeadChips (Illumina).

Because the allele frequencies of D1546 were 0.85 in Japanese, 0.75 in Yoruba and 0.9 in CEPH families (according to the HapMap data), it might be assumed that D1546, the protective allele for schizophrenia, arose during the human evolutionary process and spread due to its positive selection pressure against schizophrenia.

The second finding of the present study is that BRM is a potential key protein in schizophrenia. Functional differences between alleles in humans are likely to be small, as indicated by the relatively small odds ratios of the risk alleles observed in Japanese populations (1.18 to 1.27). Therefore, the contribution of genetic variations in the *SMARCA2* gene region to the development of schizophrenia may be small. However, the present study suggests a greater role of the *SMARCA2* gene than the genetically determined role in the pathophysiology and amelioration of schizophrenia because psychotogenic drugs (well-established pharmacological models of schizophrenia) decreased *Smarca2* expression and an antipsychotic drug increased expression in the mouse brain. These findings support the hypothesis of BRM as being a potential key molecule involved in schizophrenia. The hypothesis is that various psychotogenic factors including genetic ones decrease BRM, which further affects expression of various other genes that then contribute to the development of schizophrenia.

BRM is involved in the epigenetic mechanisms of psychotogenic and antipsychotic drugs. As for the relationship of these drugs with epigenetic mechanisms, the influences of methamphetamine on DNA methyltransferase mRNA levels (43,44) and that of D2-like antagonists and MK-801 on the phosphorylation of histone H3 at serine 10 and the acetylation of H3-lysine 14 (45) have been reported. The present study also indicates the involvement of the SNF/SWI family protein in the epigenetic mechanisms through which psychotogenic and antipsychotic drugs act.

The statistical evidence in the present study should be considered cautiously given that genotyping was based upon different platforms performed in different laboratories for screening with the Illumina BeadChips. This could likely result in false positives. Therefore, importance was placed on real statistical support from the replication performed. However, the initial screening using 11 883 SNPs and 100 schizophrenia patients is far from a complete genome coverage and has an extremely low power to detect a true association. This may affect the credibility of the results. Confirmation of associations in populations other than Japanese is necessary.

There are many questions yet to be answered. The mechanisms of influences of psychotogenic and antipsychotic drugs on *SMARCA2* expression and the mechanisms of regulation of each gene listed in Table 2 involving BRM are unknown. An interaction between the promoter region of the *HOMER1* gene and BRM was confirmed using the ChIP assay. HOMER proteins provide constitutive forms of Homer (also known as CC-Homers) and immediate early gene products. Homer proteins interact with both group 1 metabotropic glutamate receptors (mGluRs) of mGluR1 and mGluR5 (46) and Shank-GKAP-PSD95-NMDA receptor complexes, as well as with proteins that regulate intracellular calcium signaling. *Homer1* knockout mice also show behavioral and neurochemical phenotypes relevant to schizophrenia (47,48).

The finding that psychotogenic drugs decreased Smarca2 expression can be interpreted as suggesting that changes in *SMARCA2* are consequences of schizophrenia rather than causes. Although genetic association in humans and altered behavior in Smarca2 knockout mice may support causation at least in part, the experiments from this study indicate the existence of factors that influence SMARCA2 expression

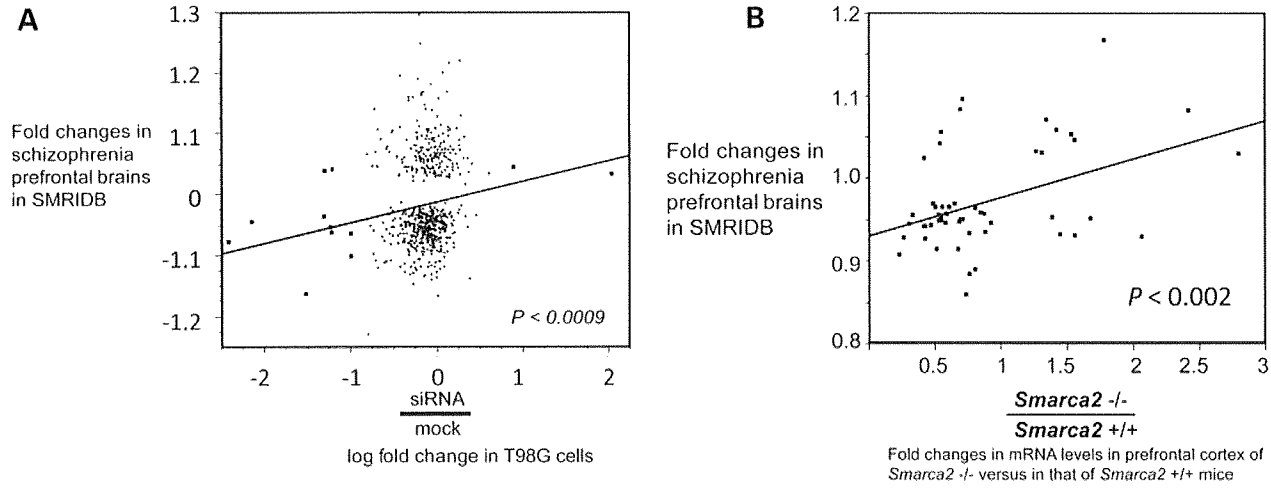

Figure 5. Correlation of transcriptional fold changes in the postmortem prefrontal cortex in SMRIDB with those in T98G cells after siRNA treatments (**A**) and with *Smarca2* knockout prefrontal mouse brains (**B**). Transcriptional fold changes by siRNA against mock in T98G cells were log-transformed (A). A simple regression analysis was carried out.

and may be related to schizophrenia. Further studies exploring such factors are warranted.

Frequent loss of BRM expression has been reported in lung cancers (49) and gastric cancer (50) and a lower average level of BRM expression in prostate cancers (51). Controversy concerning the incidence of cancer in schizophrenia exists; however, lower respiratory (52) and prostate cancers (53) in schizophrenia patients have been reported. A recent meta-analysis indicated a slightly increased incidence of lung cancer in schizophrenic patients, but after the data were adjusted for smoking prevalence, this was not seen (54).

In conclusion, the present study identifies BRM as potentially a key molecule in a wide range of pathophysiology associated with schizophrenia.

MATERIALS AND METHODS

Human subjects

Subjects of schizophrenia for screening, replication 1 and replication 2 were 100 (mean age ± SD: 57.5 ± 14.9 years, 58 males and 42 females), 576 (mean age ± SD: 51.6 ± 14.8 years, 322 males and 254 females) and 1344 (mean age ± SD: 46.7 ± 14.4 years, 733 males and 611 females) and control subjects in replications 1 and 2 were 576 (mean age ± SD: 46.8 ± 12.5 years, 268 males and 322 females) and 1344 (mean age ± SD: 47.8 ± 13.8 years, 783 male and 561 female). The replication samples were independent from the sample set used for screening. For every possible paring of individuals, the mean and variance in number of alleles shared identity-by state (IBS) across markers was estimated using the GRR tool (55). All subjects were of Japanese descent and were recruited from the main island of Japan. All schizophrenic subjects were given a best-estimate lifetime diagnosis according to DSM-IV criteria with obtained consensus from at least two experienced psychiatrists on the basis of all available sources of information, including unstructured interviews, clinical observations and medical records. Control subjects were mentally healthy and had no family history of mental illness within second-degree relatives as self-reported. The study was approved by the Ethics Committees of the University of Tsukuba, Niigata University, Fujita Health University, Nagoya University, Okayama University and Seiwa Hospital, and all participants provided written informed consent.

Postmortem brains

Brain specimens were from European-descent Australian individuals and Japanese individuals. The Australian sample comprised 10 schizophrenic patients and 10 age- and gender-matched controls (56). The diagnosis of schizophrenia had been made according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria (American Psychiatric Association 1994) by a psychiatrist and a senior psychologist. Control subjects had no known history of psychiatric illness. Tissue blocks were cut from gray matter in an area of the prefrontal cortex referred to as Brodmann's area 9 (BA9). Japanese samples of BA9 gray matter were from 6 schizophrenic patients and 11 age- and gender-matched controls (56). In addition, postmortem brains of 37 deceased Japanese patients with schizophrenia were also analyzed (56). The Japanese subjects met the DSM-III-R criteria for schizophrenia. The study was approved by the Ethics Committees of the Central Sydney Area Health Service, University of Sydney, Niigata University, University of Tsukuba, Tokyo Metropolitan Matsuzawa Hospital and the Tokyo Institute of Psychiatry.

Genotyping

Association screening was performed using the Illumina Sentrix Human-1 Genotyping 109 k BeadChip and HumanHap370 BeadChip according to the manufacturer's instructions (Illumina, San Diego CA, USA). All DNA samples were subjected to rigorous quality control to check for fragmentation and amplification. Approximately 750 ng of