ONLINE METHODS

Subjects. The subjects enrolled in the GWAS (n = 26,620) and replication sets 1 and 2 (n = 3,763 and n = 4,147, respectively) were obtained from the BioBank Japan Project[14] at the Institute of Medical Science, the University of Tokyo, which consisted of subjects with 1 of 32 diseases (**Supplementary Table 1**). Subjects with ages <18 or >85 years, subjects who had undergone dialysis treatment or subjects who were determined to be of non-Japanese origin by self-report or by PCA in the GWAS or our previous study[15] were not included. Clinical information on the subjects including age (61.3 ± 12.9 years (mean ± s.d.)), gender (47.2% female) and smoking history (49.8% 'ever-smoker') were collected by a standard questionnaire. BMI values (22.7 ± 3.59 (mean ± s.d.)) were calculated based on self-reported height and weight. BMI based on self-reported data is known to be highly correlated (r > 0.94) with that based on measurements[32], and any potential bias induced by self-reported data may have little impact on the analyses[32,33]. All participants provided written informed consent as approved by the ethical committees of the RIKEN Yokohama Institute and the Institute of Medical Science, University of Tokyo. The subjects enrolled in the replication set 3 (n = 27,715) consisted of subjects from the eight cohorts of east Asian populations, and these subjects were enrolled in the discovery stage of the concurrent meta-analysis for BMI[17]. The subjects in our GWAS were also enrolled in the replication stage of the meta-analysis[17].

Genotyping and quality control. We used the data from 32 GWAS performed for the BioBank Japan Project, in which subjects with each of the 32 diseases were genotyped (**Supplementary Table 1**)[14]. In the GWAS, genotyping was performed using the Illumina HumanHap610-Quad Genotyping BeadChip. After excluding the subjects with call rates <0.98, we excluded SNPs with call rates <0.99, SNPs with ambiguous calls and non-autosomal SNPs. We excluded subjects in close kinships based on estimations using identity by state. We considered the subject pairs with an average proportion of alleles shared by identity by state >1.7 to be in first- or second-degree kinship and excluded the member of the pair with the lower call rates. We also excluded subjects whose ancestries were estimated to be distinct from the other subjects using PCA performed using EIGENSTRAT version 2.0. We performed PCA for the genotype data from our study along with the genotype data of unrelated European (CEU), African (YRU) and east Asian (Japanese and Han Chinese (JPT + CHB)) individuals obtained from the Phase II HapMap database (release 24)[34]. Based on the PCA plot, we excluded the outliers in regard to ancestry from the JPT + CHB clusters (**Supplementary Fig. 1**). We then excluded the SNPs with MAF < 0.01 or the SNPs with an exact Hardy-Weinberg equilibrium $P < 1.0 \times 10^{-7}$ and obtained genotype data for 480,103 SNPs in 26,620 subjects.

Genotype imputation was performed using MACH 1.0 in a two-step procedure. The individuals from the JPT and CHB populations obtained from the Phase II HapMap database (release 24)[34] were used as references. In the first step, recombination and error-rate maps were estimated using 500 subjects randomly selected from the GWAS data. In the second step, genotype imputation of all subjects was conducted using the rate maps estimated in the first step. We excluded the imputed SNPs with MAF < 0.01 or Rsq < 0.7 and obtained genotype data for 2,178,018 SNPs.

In the replication study sets 1 and 2, we used genotyping data that we obtained using the Illumina HumanHap550v3 Genotyping BeadChip and the Illumina HumanOmniExpress Genotyping BeadChip, respectively. We applied the same quality control criteria and imputation procedure as was used for the GWAS data. Details of the genotyping, quality control and imputation procedure used in replication set 3 are described elsewhere[17].

Statistical analyses. *Genome-wide association study and the replication studies of BMI.* A rank-based inverse-normal transformation was applied to the BMI values of the subjects. In the GWAS, associations of the SNPs with transformed values of BMI were assessed by linear regression assuming additive effects of allele dosages (bound between 0.0 and 2.0) using mach2qtl software, and a genomic-control correction was applied[35]. In the regression model, gender, age, age-squared, smoking history, the affection statuses of the diseases and the demographic classifications of the medical institutes in Japan where the subjects were enrolled[36] were used as covariates. For the loci that satisfied $P < 5.0 \times 10^{-5}$ in the GWAS, replication studies were conducted that consisted of three replication sets (**Supplementary Table 1** and **Supplementary Fig. 1**). In replication sets 1 and 2, the associations of the SNPs were assessed in the same manner as they were in the GWAS. In replication set 3, we referred to the results from the discovery stage of the concurrently conducted genome-wide meta-analysis of BMI[17]. The combined results of the studies were obtained using an inverse-variance method from the summary statistics β and the standard error (SE). Details of our examination of the tag copy number variants and our expression analysis of the *KLF9* locus using publicly available database for HapMap Phase II east Asian individuals[34,37] are described in **Supplementary Figure 4**. Associations of the SNPs that satisfied $P < 5.0 \times 10^{-5}$ in the combined study of the GWAS and the replication studies were further evaluated using the results of the meta-analysis for BMI in European populations by the GIANT consortium[12]. For the evaluation of the associations in the previously reported BMI-associated loci[3–12,18], the loci that had FDR < 0.05 based on the number of loci reported with non-monomorphic SNPs were considered to be significant. The statistical power of the study was estimated using Quanto version 1.2.4.

The inter-individual variance in BMI explained by each of the identified loci ($P < 5.0 \times 10^{-8}$ in the combined study) was estimated using $2f(1-f)\beta^2$, where f is the frequency of the variant in the HapMap east Asian populations and β is its additive effect size on the BMI obtained from the replication studies. To estimate the variance explained by the combination of the identified loci, we calculated the genetic risk scores for the subjects in the GWAS by summing the dosages of the alleles associated with higher BMI carried by the subjects weighted by the effect sizes of the SNPs obtained from the replication studies. The explained variance was estimated from a linear regression model incorporating this score as the predictor and the covariate-adjusted inverse normal transformed BMI residuals as the outcome.

Gene-gene interaction analysis of BMI. The gene-gene interactions of the SNPs were evaluated using a multivariate linear regression model assuming additive × additive effects of two SNPs[20]. The allele dosages of the respective SNPs and the product of the allele dosages were included in the model, in addition to the covariates. The product of the allele dosages was considered an interaction term. For each of the landmark SNPs in the loci confirmed to be associated with BMI, the gene-gene interactions were evaluated with all of the genome-wide SNPs (7 × 2,178,018 SNP pairs; **Supplementary Fig. 5**), and genomic-control corrections were applied[35]. For SNP pairs that had $P < 5.0 \times 10^{-6}$ for the interaction term, replication studies using replication sets 1 and 2 were performed. The SNP pair that satisfied $P < 5.0 \times 10^{-8}$ in the combined study of the GWAS and the replication studies was considered to be significant.

Association study of metabolic and other related traits. Associations with obesity (BMI ≥ 27.5 (ref. 38); 3,058 cases and 31,472 controls), T2D (6,526 cases and 22,689 controls), systolic and diastolic blood pressure (n = 13,049), total cholesterol (n = 12,565), high-density–lipoprotein cholesterol (n = 4,924), low-density–lipoprotein cholesterol (n = 4,219) and triglyceride (n = 9,747) were evaluated using the subjects enrolled in the GWAS and in replication sets 1 and 2 (**Supplementary Table 6**). In addition to the two new loci associated with BMI (*CDKAL1* and *KLF9*), we assessed the *GIPR* locus, where associations with T2D and its related traits have been reported[25]. A case-control analysis and analyses of the quantitative traits were performed using logistic and linear regression models, respectively, that included the covariates. In the association analysis of T2D, subjects not affected with cardiovascular diseases were enrolled as controls, and BMI was additionally incorporated as a covariate.

R statistical software was used for the general analysis. Details of the study design are also included in **Supplementary Figure 1**.

32. Wada, K. *et al.* Validity of self-reported height and weight in a Japanese workplace population. *Int. J. Obes. (Lond)* **29**, 1093–1099 (2005).
33. Nakamura, K., Hoshino, Y., Kodama, K. & Yamamoto, M. Reliability of self-reported body height and weight of adult Japanese women. *J. Biosoc. Sci.* **31**, 555–558 (1999).
34. The International HapMap Consortium. The International HapMap Project. *Nature* **426**, 789–796 (2003).

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35. Devlin, B. & Roeder, K. Genomic control for association studies. *Biometrics* **55**, 997–1004 (1999).
36. Yamaguchi-Kabata, Y. *et al.* 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 (2008).
37. Stranger, B.E. *et al.* Population genomics of human gene expression. *Nat. Genet.* **39**, 1217–1224 (2007).
38. W.H.O. Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* **363**, 157–163 (2004).

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Meta-analysis identifies common variants associated with body mass index in east Asians

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Multiple genetic loci associated with obesity or body mass index (BMI) have been identified through genome-wide association studies conducted predominantly in populations of European ancestry. We performed a meta-analysis of associations between BMI and approximately 2.4 million SNPs in 27,715 east Asians, which was followed by *in silico* and *de novo* replication studies in 37,691 and 17,642 additional east Asians, respectively. We identified ten BMI-associated loci at genome-wide significance ($P < 5.0 \times 10^{-8}$), including seven previously identified loci (*FTO, SEC16B, MC4R, GIPR-QPCTL, ADCY3-DNAJC27, BDNF* and *MAP2K5*) and three novel loci in or near the *CDKAL1, PCSK1* and *GP2* genes. Three additional loci nearly reached the genome-wide significance threshold, including two previously identified loci in the *GNPDA2* and *TFAP2B* genes and a newly identified signal near *PAX6*, all of which were associated with BMI with $P < 5.0 \times 10^{-7}$. Findings from this study may shed light on new pathways involved in obesity and demonstrate the value of conducting genetic studies in non-European populations.

Genome-wide association studies (GWAS) have thus far identified 37 genetic loci associated with obesity or BMI[1–11]. Virtually all of these studies were conducted in populations of European ancestry and included limited data from Asian populations[9,11]. Asians, who account for over 60% of the world's population, have higher percentages of body fat and increased metabolic disease risk than individuals of European ancestry with the same BMI[12]. Therefore, studies conducted in Asian populations, in addition to allowing an evaluation of the extent to which genetic markers of obesity identified in North American and European populations can be generalized, also facilitate the dissection of the genetic architecture of obesity and the identification of genetic variants of particular importance in Asians.

The initial genome-wide association meta-analysis of BMI included approximately 2.4 million genotyped or imputed SNPs generated from eight GWAS including 27,715 east Asians (stage 1). This was followed by an *in silico* replication analysis conducted among 37,691 east Asians from an additional seven GWAS (stage 2) and a subsequent *de novo* replication study conducted among 17,642 east Asians from three studies (stage 3). Details of the study designs are provided in **Supplementary Figure 1**, **Supplementary Tables 1–3** and the **Supplementary Note**.

The stage 1 meta-analysis was performed using the METAL program, and study-specific genomic control adjustment was applied (see Online Methods). The stage 1 analysis revealed that three well-established loci (*FTO*, *SEC16B* and *MC4R*) were associated with BMI at or near the level of genome-wide significance ($P < 5 \times 10^{-8}$)[13] (**Fig. 1** and **Table 1**).

In stage 2, we analyzed 798 SNPs that were associated with BMI at $P < 1.0 \times 10^{-4}$ in stage 1 and 50 additional SNPs that were previously reported to be associated with BMI but which did not reach $P < 1.0 \times 10^{-4}$ in stage 1. Seven additional GWAS conducted in east Asian populations participated in the stage 2 study. We combined data from stage 2 with the stage 1 meta-analysis results in meta-analyses with adjustment for both study-specific genomic control inflation and estimated

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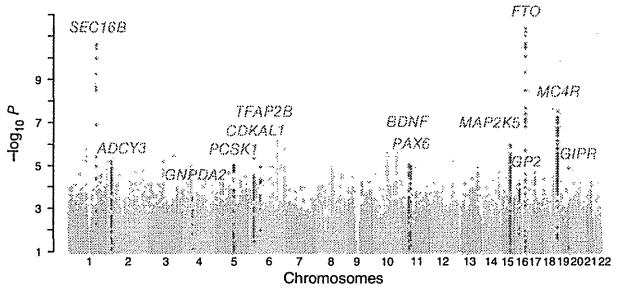

Figure 1 Manhattan plot showing the significance of associations between BMI and SNPs in the stage 1 data. The SNPs in previously reported genes that show significant associations with BMI are highlighted in red. The SNPs in newly identified loci that are significantly associated with BMI are highlighted in blue.

genomic control inflation for the stage 1 meta-analysis results (1.056; see Online Methods). These analyses revealed that the index SNPs in six previously reported loci (*FTO*, *SEC16B*, *MC4R*, *GIPR-QPCTL*, *ADCY3-DNAJC27* and *BDNF*) were associated with BMI with genome-wide significance ($P < 5.0 \times 10^{-8}$), whereas those in three other previously reported loci (*GNPDA2*, *TFAP2B* and *MAP2K5*) were near genome-wide significance ($P < 5.0 \times 10^{-7}$) (**Table 1** and **Supplementary Table 4**). In addition, the index SNPs in nine other previously reported loci were associated with BMI with nominal significance ($P < 0.05$) (**Supplementary Table 4**).

We compared two SNPs at each of the *GIPR-QPCTL*, *ADCY3-DNAJC27* and *MAP2K5* loci (**Supplementary Table 5**), one identified by our study and another by the GIANT consortium (published during the course of our study)[8]. The SNPs at *ADCY3-DNAJC27* and *MAP2K5* identified in our study are in linkage disequilibrium (LD) with the ones identified by the GIANT consortium. At *GIPR-QPCTL*, our identified SNP, rs11671664, is not in LD with the GIANT-identified SNP, rs2287019, in Asians ($r^2 = 0.026$) and is in weak LD with rs2287019 in Europeans ($r^2 = 0.264$). rs2287019 did not have a statistically significant association with BMI in east Asians (**Supplementary Table 4**). Conditional analyses with the two SNPs at each locus included in the same model for mutual adjustment revealed a statistically significant association with BMI only for the SNP identified by our study (Online Methods and **Supplementary Table 5**), suggesting that the SNP we identified represents an independent association signal in Asians at the same locus.

The reported effect sizes for BMI-related SNPs in studies of populations of European ancestry are usually greater than 3% of the s.d. of BMI[4]. Given the sample sizes of our study, we had adequate statistical power (>0.8) to detect a SNP with such an effect size and with a MAF of >0.2 in stage 1 or a MAF of >0.08 in the combined stage 1 and 2 data at a significance of $P < 0.05$. The index SNPs in the 19 previously identified loci that were not replicated in our study at $P < 0.05$ had either very small effect sizes or very low MAFs in east Asians (**Supplementary Table 4**).

One representative SNP from the four newly identified loci at or near the *CDKAL1*, *PCSK1*, *PAX6* and *GP2* genes and the three loci at the *GIPR-QPCTL*, *ADCY3-DNAJC27* and *MAP2K5* genes that were reported by the GIANT consortium[8] (**Supplementary Table 4**) were selected for further replication in stage 3 using *de novo* genotyping in three studies that included a total of 17,642 subjects (**Supplementary Tables 1** and **2**). In stage 3 analyses, the directions of the associations between BMI and the seven SNPs were consistent with the corresponding associations in stages 1 and 2. The final results derived from combined data from all three stages showed that six SNPs at or near *GIPR-QPCTL*, *ADCY3-DNAJC27*, *MAP2K5*, *CDKAL1*, *PCSK1* and *GP2* were associated with BMI with genome-wide significance ($P = 1.02 \times 10^{-8}$ to 5.93×10^{-14}) (**Table 1**), and rs652722 near the *PAX6* gene was found to be associated with BMI with $P = 7.65 \times 10^{-8}$ (**Supplementary Table 6**). The variance in BMI explained by these SNPs is presented in **Table 1**.

We also evaluated the association of BMI with these seven SNPs in the GIANT consortium data. Four of these SNPs (rs654581, rs261967, rs4776970 and rs1167166) at or near the *AGCY3-DNAJC27*, *PCSK1*, *MAP2K5* and *GIPR-QPCTL* loci, respectively, showed a significant association with BMI at $P < 0.007$ ($P = 0.05/7$, to account for seven tests) (**Supplementary Table 7**). Although the effect sizes of these seven loci were smaller than those of the well-established variants in the *FTO*, *MC4R* and *SEC16B* loci (2.55–4.22% of s.d. versus 5.51–7.92%; **Table 1**), their effect sizes were larger and the explained variances were larger in east Asians than in Europeans (**Supplementary Table 7**), with the exception of rs4776970 in the *MAP2K5* gene, which was independently identified by both our study and the GIANT

Table 1 Identified loci associated with BMI variation in east Asian populations

Gene	Chr.	SNP	Genotype[a]	EAF[b]	β (s.e.m.)[c]	*P* value by stage[d]				Explained variance[f]
						1	2	3	Final[e]	
Previously identified BMI loci										
FTO	16	rs17817449	G/T	0.17	7.92 (1.06)	6.13×10^{-12}	8.18×10^{-14}		4.60×10^{-27}	0.18%
SEC16B	1	rs574367	T/G	0.20	5.93 (0.92)	2.38×10^{-11}	1.28×10^{-10}		9.47×10^{-20}	0.11%
MC4R	18	rs6567160	C/T	0.21	5.51 (0.93)	6.92×10^{-8}	3.35×10^{-9}		2.76×10^{-15}	0.10%
GIPR-QPCTL	19	rs11671664	G/A	0.50	4.22 (0.76)	1.29×10^{-5}	2.57×10^{-8}	3.57×10^{-3}	5.93×10^{-14}	0.09%
ADCY3-DNAJC27	2	rs6545814	G/A	0.45	3.26 (0.76)	1.20×10^{-5}	1.62×10^{-5}	1.05×10^{-5}	1.35×10^{-13}	0.05%
BDNF	11	rs6265	C/T	0.44	4.97 (0.83)	1.18×10^{-5}	2.72×10^{-9}		3.56×10^{-13}	0.12%
MAP2K5	15	rs4776970	A/T	0.22	2.55 (0.90)	1.10×10^{-6}	4.63×10^{-3}	2.90×10^{-3}	2.33×10^{-9}	0.02%
GNPDA2	4	rs10938397	G/A	0.29	3.72 (0.85)	1.60×10^{-3}	1.30×10^{-5}		9.69×10^{-8}	0.06%
TFAP2B	6	rs4715210	T/C	0.21	3.05 (0.91)	1.12×10^{-5}	7.64×10^{-4}		1.61×10^{-7}	0.03%
Newly identified BMI loci										
CDKAL1	6	rs9356744	T/C	0.58	3.39 (0.76)	3.21×10^{-5}	7.67×10^{-6}	3.02×10^{-3}	2.00×10^{-11}	0.06%
PCSK1	5	rs261967	C/A	0.41	3.77 (0.77)	1.22×10^{-5}	9.36×10^{-7}	8.46×10^{-1}	5.13×10^{-9}	0.07%
GP2	16	rs12597579	C/T	0.80	4.09 (0.96)	7.13×10^{-5}	2.07×10^{-5}	1.45×10^{-1}	1.02×10^{-8}	0.05%
PAX6	11	rs652722	C/T	0.61	2.75 (0.77)	2.84×10^{-5}	3.70×10^{-4}	1.89×10^{-1}	7.65×10^{-8}	0.04%

Chr., chromosome.
[a]Effect allele/other allele. [b]Effect allele frequency in Asians, estimated from stages 1 and 2. [c]Per allele effect of SNPs (in percentage) on BMI, obtained from stage 2 data only. [d]Derived from meta-analysis. *P* values for the combined data were adjusted for both study-specific inflation factors and the estimated inflation factor for the stage 1 meta-analysis statistic. [e]Combination of all available data from the three stages. [f]The effect sizes obtained from stage 2 data were used to estimate the explained variance.

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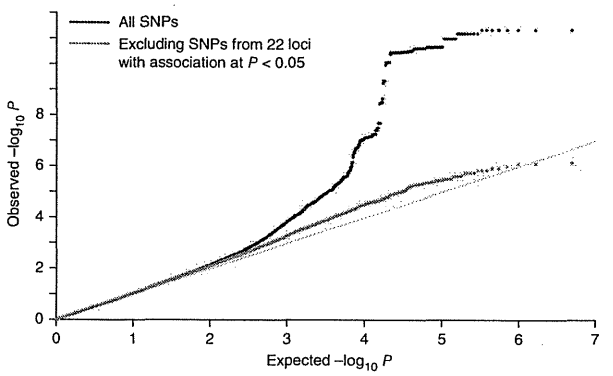

Figure 2 Quantile–quantile plot for the association of BMI with SNPs in all stage 1 data (black) and in stage 1 data that excluded the 22 SNPs with associations at $P < 0.05$ (red) (**Supplementary Table 4**).

consortium study. The explained variance of this SNP is 0.03% in Europeans (**Supplementary Table 7**) and 0.02% in Asians (**Table 1**).

As shown in **Table 1**, the SNP in *FTO* had the greatest effect on BMI and accounted for the largest proportion of the variance (0.18%) in our study population. Together, the 10 loci associated with BMI that reached genome-wide significance explained 0.87% of the interindividual variation in BMI, and all 22 loci that were associated with BMI at $P < 0.05$ explained 1.18% (Online Methods and **Supplementary Table 4**). These explained variance values are lower than those reported by the GIANT consortium (1.45% for the SNPs overall and 0.34% for *FTO*)[8]. After exclusion of SNPs within these 22 loci that were associated with BMI at $P < 0.05$, the number of SNPs with small observed *P* values for an association with BMI still exceeded the expected number (**Fig. 2**), suggesting that additional BMI-related loci remain to be uncovered in east Asians.

The newly identified associations of four SNPs at or near the *CDKAL1*, *PCSK1*, *PAX6* and *GP2* genes with BMI were consistent across studies, gender and ancestry and remained little changed after subjects with chronic diseases (cancer or diabetes) were excluded (**Supplementary Table 6**). Meta-analyses of obesity as a dichotomous outcome (BMI ≥ 27.5)[14] also showed similar associations, with odds ratios per allele ranging from 1.05 to 1.10 (**Supplementary Table 8**). Of the studies participating in our analyses, one stage 2 study, the Singapore Cohort study Of the Risk factors for Myopia (SCORM), only included children (9 year olds). In data from the SCROM study, all four loci had an association with BMI consistent with the meta-analysis, and the rs652722 SNP near *PAX6* was associated with nominal significance ($P = 0.0335$) (**Supplementary Table 6**). Excluding the SCORM study from the analysis had little effect on the results.

The consistency of the findings across studies and populations suggests that population structure alone did not account for the significant associations we identified. In addition, multiple SNPs in LD with each other showed similar associations in the combined stage 1 and 2 data at each locus (**Fig. 3** and **Supplementary Table 9**). This finding, together with the identification of similar associations in the *de novo* replication stage, suggests that our results are unlikely to have been caused by genotyping or imputation errors.

The locus represented by the rs9356744 SNP (6p22.3) contains the *CDKAL1* gene, which has been reported to affect type 2 diabetes risk in a number of studies[15–17]. A recent study identified an association between a SNP in *CDKAL1*, rs4712526, and BMI in 8-year-old children[18]. rs4712526 was not included in stage 2, but stage 1 data for this SNP were consistent with the previous report (**Supplementary Table 10**). This SNP is in strong LD with rs9356744 ($r^2 = 0.87$) in Asians. Excluding participants with type 2 diabetes resulted in a similar association ($P = 4.01 \times 10^{-8}$) (**Supplementary Table 6**), indicating that the association of rs9356744 with BMI was not driven by inclusion of individuals with diabetes in the study. Additionally, two SNPs in the *CDKAL1* gene (rs9356744 and rs9368222; **Supplementary Table 9**) are *cis* expression quantitative trait loci (eQTLs) for the nearby *E2F3* gene, a transcription factor and tumor suppressor[19]. In an accompanying paper, Okada *et al.*[20] identified another SNP (rs2206734) in the

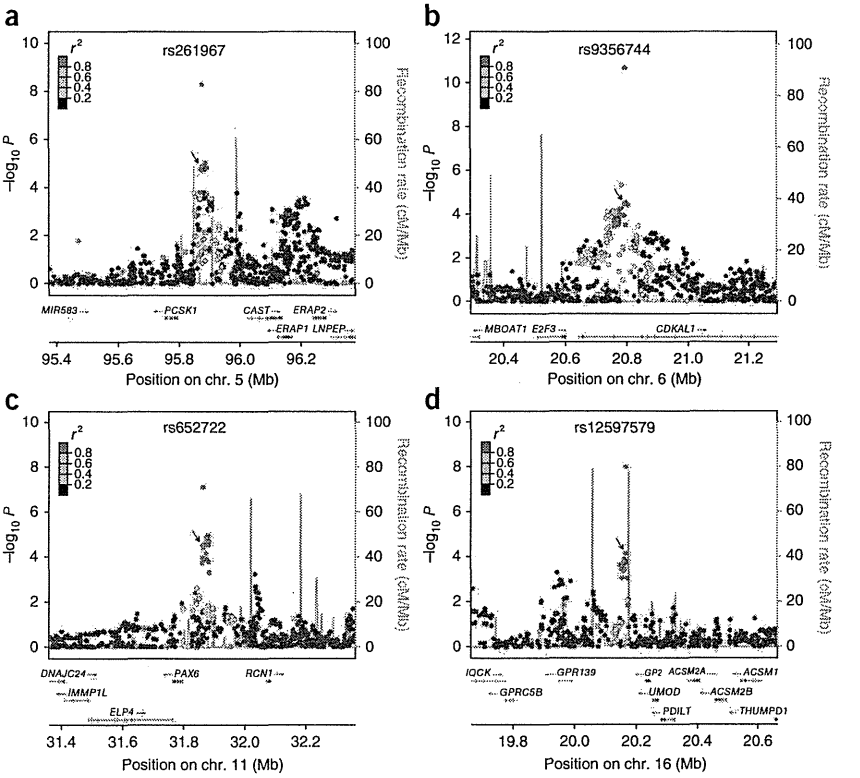

Figure 3 Regional plots of four newly identified loci in this study. (**a–d**) Results are shown for the rs261967 (**a**), rs9356744 (**b**), rs652722 (**c**) and rs12597579 (**d**) SNPs. SNPs are plotted by their position on the chromosome against their association ($-\log_{10} P$) with BMI using stage 1 (GWAS meta-analysis) data. The names are shown for the top SNPs, which were selected on the basis of combined data from all studies with full genomic control adjustment (**Table 1**). The *P* value in stage 1 for the same SNP is denoted by a purple circle and indicated with an arrow. Estimated recombination rates (from HapMap) are plotted in cyan to reflect the local LD structure. The SNPs surrounding the top SNP are colored to reflect their LD with the top SNP (using pairwise r^2 values from HapMap Han Chinese in Beijing (CHB) and Japanese in Tokyo (JPT) data). The positions of genes and exons, as well as the direction of transcription, are shown below the plots (using data from the UCSC Genome Browser). Plots were generated using LocusZoom.

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CDKAL1 gene that is associated with BMI. rs9356744 and rs2206734 are in strong LD in Asians ($r^2 = 0.932$) and in weaker LD in Europeans ($r^2 = 0.396$). In data from the GIANT consortium, rs9356744 was not associated with BMI ($P = 0.186$; **Supplementary Table 7**), but rs2206734 was ($P = 0.0049$). These findings suggest that the functional SNP encoding risk for obesity is in LD with both rs9356744 and rs2206734 in east Asians but only with rs2206734 in populations of European ancestry.

At the chromosome 5 locus (5q15), the top SNP, rs261967, along with 13 other SNPs that are in strong LD ($r^2 = 1.0$) with it, reached genome-wide significance in the combined stage 1 and 2 data (**Supplementary Table 9**). The nearest gene to this locus is *PCSK1* (81.3 kb upstream). A candidate-gene study reported two common nonsynonymous coding variants (rs6234 and rs6235) in the *PCSK1* gene that were associated with obesity[21]. However, these two SNPs showed no association with BMI in our study (**Supplementary Table 10**), nor were they in LD with the 14 SNPs we identified ($r^2 = 0$) according to HapMap Asian data. rs261967 showed an association with BMI ($P = 0.00158$; **Supplementary Table 7**) in the data provided by the GIANT consortium[8].

At the chromosome 16 locus (16p12.3), the identified SNP, rs12597579, is near the *GPR139* and *GP2* genes. Multiple SNPs in this region showed an association with BMI in stage 1 that nearly met the significance threshold that was required for them to be evaluated in stage 2 (**Fig. 3d**). One of these SNPs, rs12598578 ($P = 1.63 \times 10^{-4}$; **Supplementary Table 10**), which is in LD ($r^2 = 0.968$ in Asians) with the identified rs12597579 SNP, is highly conserved across species according to the TRANSFAC database[22], and the common G allele creates a Ying-Yang transcription factor binding site.

The top SNP at the chromosome 11 locus (11p13), rs652722, is approximately 66.0 kb from the nearest gene, *PAX6*. This SNP is in LD with several SNPs that, according to the SCAN database[23], are predicted to be eQTLs for a number of genes potentially important in the regulation of body weight. Among the potentially regulated genes is *MIF*, according to HapMap lymphoblastoid cell lines. According to data from these cell lines, two SNPs (rs621611 and rs679887) in LD with rs652722 are significantly associated with *MIF* expression in SCAN. High plasma levels of the MIF protein are related to higher BMI[24]. Another gene whose expression is associated with rs652722 is *PFKP*, which, along with *FTO*, has been associated with increased BMI, hip circumference and weight[2].

We identified multiple signals at the *ADCY3-DNAJC27* locus (**Supplementary Table 4**). The rs11676272 SNP ($P = 5.88 \times 10^{-10}$) encodes a predicted missense mutation in the *ADCY3* gene that causes a p.Ser107Pro alteration in the protein, and this change is predicted to be potentially deleterious according to PolyPhen. This SNP is also associated with expression of the *POMC* gene, which regulates energy balance[8]. In addition, rs11676272 and rs6545814 at this locus ($r^2 = 0.98$ for LD between the two SNPs in Asians) are both eQTLs for the *ADCY3* gene[25].

In conclusion, our study identified ten loci that are associated with BMI at the genome-wide significance level ($P < 5.0 \times 10^{-8}$), including seven loci previously identified in populations of European ancestry (*FTO, SEC16B, MC4R, GIPR-QPCTL, ADCY3-DNAJC27, BDNF* and *MAP2K5*) and three newly identified loci in or near the *CDKAL1, PCSK1* and *GP2* genes. Three additional loci nearly reached genome-wide significance, including two previously identified SNPs in the *GNPDA2* and *TFAP2B* genes and a newly identified marker near *PAX6*, all having $P < 5.0 \times 10^{-7}$. Of the three previously reported loci at *GIPR-QPCTL, ADCY3-DNAJC27* and *MAP2K5*, conditional analyses showed that only the SNPs identified by our study were associated with BMI in east Asian populations. The representative SNP (rs261967) near the newly identified association with *PCSK1* showed a significant association ($P = 0.00158$) with BMI in a European population. As expected, the explained variance of the previously reported loci was generally lower in east Asians than in Europeans, whereas the explained variance for the newly identified loci from this study was generally higher in east Asians than in Europeans. The identification of new loci may shed light on new pathways involved in obesity. Future fine mapping of mixed-ancestry populations could lead to the identification of causal links.

URLs. METAL, http://www.sph.umich.edu/csg/abecasis/Metal/; ConSite, http://www.phylofoot.org/consite/; PolyPhen, http://genetics.bwh.harvard.edu/pph/; USCS Genome Browser, http://www.genome.ucsc.edu/; SNPinfo Web Server, http://snpinfo.niehs.nih.gov; MACH, http://www.sph.umich.edu/csg/abecasis/MACH/index.html; Impute, http://mathgen.stats.ox.ac.uk/impute/impute.html.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS
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1. Frayling, T.M. *et al.* A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* **316**, 889–894 (2007).
2. Scuteri, A. *et al.* Genome-wide association scan shows genetic variants in the *FTO* gene are associated with obesity-related traits. *PLoS Genet.* **3**, e115 (2007).
3. Loos, R.J. *et al.* Common variants near *MC4R* are associated with fat mass, weight and risk of obesity. *Nat. Genet.* **40**, 768–775 (2008).
4. Thorleifsson, G. *et al.* Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat. Genet.* **41**, 18–24 (2009).
5. Willer, C.J. *et al.* Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat. Genet.* **41**, 25–34 (2009).
6. Meyre, D. *et al.* Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nat. Genet.* **41**, 157–159 (2009).
7. Scherag, A. *et al.* Two new loci for body-weight regulation identified in a joint analysis of genome-wide association studies for early-onset extreme obesity in French and German study groups. *PLoS Genet.* **6**, e1000916 (2010).
8. Speliotes, E.K. *et al.* Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat. Genet.* **42**, 937–948 (2010).
9. Chambers, J.C. *et al.* Common genetic variation near *MC4R* is associated with waist circumference and insulin resistance. *Nat. Genet.* **40**, 716–718 (2008).
10. McCarthy, M.I. Genomics, type 2 diabetes, and obesity. *N. Engl. J. Med.* **363**, 2339–2350 (2010).
11. Cho, Y.S. *et al.* A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat. Genet.* **41**, 527–534 (2009).
12. Deurenberg, P., Deurenberg-Yap, M. & Guricci, S. Asians are different from Caucasians and from each other in their body mass index/body fat per cent relationship. *Obes. Rev.* **3**, 141–146 (2002).
13. de Bakker, P.I. *et al.* Practical aspects of imputation-driven meta-analysis of genome-wide association studies. *Hum. Mol. Genet.* **17**, R122–R128 (2008).
14. W.H.O. Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* **363**, 157–163 (2004).
15. Voight, B.F. *et al.* Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat. Genet.* **42**, 579–589 (2010).
16. Zeggini, E. *et al.* Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* **316**, 1336–1341 (2007).
17. Steinthorsdottir, V. *et al.* A variant in *CDKAL1* influences insulin response and risk of type 2 diabetes. *Nat. Genet.* **39**, 770–775 (2007).
18. Winkler, C. *et al.* BMI at age 8 years is influenced by the type 2 diabetes susceptibility genes *HHEX-IDE* and *CDKAL1*. *Diabetes* **59**, 2063–2067 (2010).
19. Veyrieras, J.B. *et al.* High-resolution mapping of expression-QTLs yields insight into human gene regulation. *PLoS Genet.* **4**, e1000214 (2008).
20. Okada, Y. *et al.* Common variants at *CDKAL1* and *KLF9* loci are associated with body mass index in east Asian populations. *Nat. Genet.* published online, doi:10.1038ng.1086 (12 February 2012).
21. Benzinou, M. *et al.* Common nonsynonymous variants in *PCSK1* confer risk of obesity. *Nat. Genet.* **40**, 943–945 (2008).
22. Wingender, E. *et al.* TRANSFAC: an integrated system for gene expression regulation. *Nucleic Acids Res.* **28**, 316–319 (2000).
23. Gamazon, E.R. *et al.* SCAN: SNP and copy number annotation. *Bioinformatics* **26**, 259–262 (2010).
24. Dandona, P. *et al.* Increased plasma concentration of macrophage migration inhibitory factor (MIF) and MIF mRNA in mononuclear cells in the obese and the suppressive action of metformin. *J. Clin. Endocrinol. Metab.* **89**, 5043–5047 (2004).
25. Yang, T.P. *et al.* Genevar: a database and Java application for the analysis and visualization of SNP-gene associations in eQTL studies. *Bioinformatics* **26**, 2474–2476 (2010).

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ONLINE METHODS

Study design. This study had three stages. Stage 1 was a meta-analysis of study-specific results on the association between SNPs and BMI from eight GWAS that participated in the consortium and included a total of 27,715 individuals of east Asian ancestry. Each participating study was approved by the local institutional review board, and informed consent was obtained from participants. Promising SNPs selected from the stage 1 meta-analysis were further examined by *in silico* (stage 2) and *de novo* (stage 3) replication analyses. Basic information for all participating studies is summarized in **Supplementary Figure 1**, **Supplementary Tables 1** and **2** and the **Supplementary Note**.

Stage 1 samples and genotyping. The sample sizes of the eight GWAS in stage 1 varied between 821 and 8,838 participants, with a total of 27,715 individuals. For genotyping, two studies used Affymetrix arrays, and six studies used an Illumina platform (detailed information is provided in the **Supplementary Note**). To allow for the combination of the data derived from different genotyping platforms and to improve coverage of the genome, genotype imputation was performed by each participating study using either MACH or IMPUTE.

Stage 1 statistical analysis. A uniform statistical analysis protocol was followed by each participating study. To calculate BMI, each study collected weight and height measurements. To improve the normality of the BMI distribution and alleviate the impact of outliers, the rank-based inverse normal transformation (INT) was applied separately to BMI values for each gender in each study. INT involves ranking all BMI values, transforming these ranks into quantiles and converting the resulting quantiles into normal deviates. The association between SNPs and the inverse normal transformed BMI values was analyzed with a linear regression model. The association between SNPs and obesity as a dichotomous outcome, which defined obesity as BMI ≥ 27.5 (ref. 14), was analyzed with a logistic regression model assuming an underlying additive genetic mode and adjusting for age (continuous), age squared, gender (if applicable) and ancestry (if applicable). Stratified analyses by gender and disease status (for cancer or type 2 diabetes) were also performed by each study.

Next, we carried out meta-analyses using two methods in parallel to cross-check the results: one approach combined effects weighted by the inverse variance and the second combined *P* values weighted by the square root of the sample size for each study. Both meta-analysis procedures were implemented in the METAL software package (see URLs). The final *P* values obtained from these two methods were highly congruent (Pearson correlation $r = 0.98$). *P* values derived from the effect-size–based method are reported here, as this method is preferred, in general, to the *P* value–based method and also provides combined regression coefficients and their standard errors[26]. The meta-analyses were carried out with all data combined and were also stratified by gender and disease status. The presence of heterogeneity across studies and between genders was tested with Cochran's *Q* statistics[27].

To correct each study for residual population stratification or cryptic relatedness, the meta-analyses were performed with genomic control correction[28] by adjusting for the study-specific inflation factor (λ), which ranged from 1.000 to 1.075 in stage 1 (**Supplementary Table 3**). After study-specific genomic control adjustment, the estimated inflation factor for the stage 1 meta-analysis statistic was 1.056, which was further adjusted when combining stage 1 results with stage 2 replication data.

On the basis of the stage 1 meta-analysis on the association between SNPs and BMI in all participants, we selected for stage 2 replication a total of 848 SNPs, which included 798 SNPs with $P < 1.0 \times 10^{-4}$ and 50 SNPs located in previously reported obesity-related loci that had $P > 1.0 \times 10^{-4}$. The cutoff of $P < 1.0 \times 10^{-4}$ was chosen so that the overall *P* value would reach genome-wide significance ($P < 5.0 \times 10^{-8}$) given the sample sizes of stages 1 and 2.

Stage 2 *in silico* replication. The 848 SNPs selected for replication were investigated in an independent set of 37,691 individuals of east Asian ancestry from seven additional GWAS. The sample sizes of the seven additional studies varied between 901 and 27,284 subjects. The RIKEN study was the main source of the replication data and included 27,284 individuals. One study (SCORM) included only children (9 year olds). All studies used the Illumina platform except for the GenSalt study, which used an Affymetrix array. Genotype imputation was also performed by each study using either MACH or IMPUTE, as for studies included in stage 1.

Each study individually conducted a similar analysis of the SNPs selected from stage 1, using the same protocol as in stage 1. The stage 2 data were combined using meta-analysis methods with study-specific genomic control adjustment in a manner similar to that performed in stage 1. Finally, we used meta-analysis to combine all data from stages 1 and 2, with further adjustment for the estimated inflation factor for the stage 1 meta-analysis statistic.

Stage 3 *de novo* replication. Seven SNPs that were associated with BMI according to the analysis of combined stage 1 and 2 data, including four SNPs at four newly identified loci and three loci that overlapped with loci that were reported by the GIANT consortium during the course of our study, were further validated in our stage 3 *de novo* replication studies. These analyses were conducted with data from three study sites (**Supplementary Table 1**), and the genotyping for the seven SNPs was carried out for a total of 17,642 east Asians. The results from stages 1, 2 and 3 were combined and analyzed using meta-analysis methods.

Quality control procedures. The following quality control procedures were recommended for each participating study. SNPs were excluded, either in the primary analysis conducted by each participating study or at the meta-analysis stage (**Supplementary Table 3**), if they (i) had a call rate of <90%, (ii) deviated from Hardy-Weinberg equilibrium with $P < 1.0 \times 10^{-6}$, (iii) had a MAF of <1%, (iv) had low imputation quality (for imputed SNPs; r-hat < 0.3 for MACH or proper-info < 0.5 for IMPUTE) or (v) were potentially contaminated. Samples from individuals were removed if they had a call rate of <90%, if they showed first-degree cryptic relationships in an identity-by-descent (IBD) analysis or if they were potentially contaminated. The specific quality control procedures adopted by each study are summarized in **Supplementary Table 3**.

Conditional analysis. To investigate the independent association of SNPs in the same locus, conditional analyses were conducted by including both SNPs at the same locus in the same regression model for mutual adjustment. The normal transformation of BMI values and the adjustment of covariates were applied in the same manner as in the stage 1 analysis. These conditional analyses were conducted among 57,931 (88.6%) subjects from 11 of the 15 studies in stages 1 and 2.

Estimation of the explained variance. The variation in BMI explained by an individual SNP was estimated by $2\beta^2 f(1-f)$ (ref. 29), where f is the frequency of the variant and β is its additive effect estimated from the stage 2 studies. We subsequently estimated the overall fraction of variance that can be explained by all significantly associated SNPs found in the current meta-analyses by calculating the genetic score with

$$\text{Score}_i = \sum_{j=1}^{m} \beta_j A_{ij}$$

where m is the number of SNPs, β_j is the effect of an allele at locus j, estimated from the stage 2 data, and A_{ij} is the number of reference alleles of individual i at locus j. The measure of variance explained (adjusted R^2) was estimated from a linear regression model incorporating the score as the predictor and the covariate-adjusted inverse normal transformed BMI residuals as the outcome. We reported the average explained variance weighted by the sample size of each study. These analyses to estimate the explained variance were conducted among 57,931 (88.6%) subjects from 11 of the 15 studies in stages 1 and 2.

Analyses of coding SNPs, eQTLs and copy-number variant (CNVs). Variants with potential functional impact were evaluated using the SNP Function Prediction tool, which is part of the SNPinfo Web Server[30]. This tool identifies SNPs with potential functional consequences, including those resulting in coding changes. The severity of a coding change was evaluated with PolyPhen, although SNPs reported to result in coding changes of consequence were independently verified using the PolyPhen website[31] (see URLs).

eQTLs were evaluated using the SCAN database[23] and the GeneVar program[25], with RNA sequencing and genotyping experiments conducted as described previously[32].

To test for CNVs in the proximity of the specific variants of interest, we used the UCSC Genome Browser[33]. Although direct access to individual-level SNP

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genotype information was unavailable to directly test for the presence of CNVs, we used the phased genotypes from HapMap 2 release 22 data[34], which show estimated LD values for the CHB and JPT HapMap populations (hapmapLdPhChbJpt table), in combination with the UCSC Genome Browser track from the Database of Genomic Variants (dgv table)[35] to identify the presence of known CNVs that intersected regions in strong LD with GWAS variants.

26. Zeggini, E. & Ioannidis, J.P. Meta-analysis in genome-wide association studies. *Pharmacogenomics* **10**, 191–201 (2009).
27. Cochran, W.G. The combination of estimates from different experiments. *Biometrics* **10**, 101–129 (1954).
28. Devlin, B. & Roeder, K. Genomic control for association studies. *Biometrics* **55**, 997–1004 (1999).
29. Park, J.H. *et al.* Estimation of effect size distribution from genome-wide association studies and implications for future discoveries. *Nat. Genet.* **42**, 570–575 (2010).
30. Xu, Z. & Taylor, J.A. SNPinfo: integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. *Nucleic Acids Res.* **37**, W600–W605 (2009).
31. Ramensky, V., Bork, P. & Sunyaev, S. Human non-synonymous SNPs: server and survey. *Nucleic Acids Res.* **30**, 3894–3900 (2002).
32. Montgomery, S.B. *et al.* Transcriptome genetics using second generation sequencing in a Caucasian population. *Nature* **464**, 773–777 (2010).
33. Fujita, P.A. *et al.* The UCSC Genome Browser database: update 2011. *Nucleic Acids Res.* **39**, D876–D882 (2011).
34. Frazer, K.A. *et al.* A second generation human haplotype map of over 3.1 million SNPs. *Nature* **449**, 851–861 (2007).
35. Feuk, L., Carson, A.R. & Scherer, S.W. Structural variation in the human genome. *Nat. Rev. Genet.* **7**, 85–97 (2006).

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J波症候群およびBrugada症候群の活動電位，心電図，電気生理学的検査の特徴

森田　宏

Ⅰ．J波症候群・早期再分極症候群

1．J波症候群・早期再分極症候群と特発性心室細動の関連

J波や早期再分極によるST上昇は，健常人，特に若年者に多くみられ，長らく無害な心電図所見と考えられてきた．病的な状態でみられるJ波としては低体温症によるOsborn波が知られているが，復温により消失する．1992年，Brugadaらは右側胸部誘導のST上昇と心室細動（VF）を呈する症例を報告した[1]．現在Brugada型心電図とされるこの心電図変化は，すでに1953年にOsherらが報告していたが，右室の早期再分極で無害性の変化と考えられていた[2]．Brugada症候群については次項で述べるため，この項では主に左側壁・下壁のJ波・早期再分極症候群と特発性VFの関係について概説する．

VFとJ波・早期再分極の関連性は，Aizawaらが1992年に初めて報告した．彼らはVF発生直前にJ波が増大するとともに期外収縮が頻発し，また同一の期外収縮からVFが誘発されることを示した[3]．こうした心電図所見は，Brugada症候群でも下壁・側壁誘導でみられることから[4]，Brugada症候群の亜型と考えられていた．2008年にHaïssaguerreらが，Brugada型波形を示さない特発性VF例206例のうち31%に早期再分極が認められたと報告した[5]．早期再分極を伴う例では，VF再発が短期間でみられ，リスクの高い所見とされた．この報告以降，早期再分極・J波と関連した特発性VFは，Brugada症候群の類縁疾患ないしは別のカテゴリーで分類するものと認識されるようになった．その後も，特発性VF例や健常人でのJ波・早期再分極症候群の頻度や予後との関連が多数報告された．2010年にAntzelevitchらが，これらをJ波症候群としてひとつの疾患概念としてまとめ，Brugada症候群もその1型であると提唱した[6]．

2．早期再分極の定義

J波・早期再分極の定義は研究者により多少異なるが，Haïssaguerreが報告した論文[5]に基づいているものが多い．すなわち，J点でのST上昇が0.1 mV以上のもので（ノッチ，スラーを含む），下壁・側壁誘導でふたつ以上の連続した誘導でみられるものとされる．J点でのST上昇がそのまま上行し，T波に移行する場合は早期再分極，J点付近でノッチを形成し，陽性の波形を呈するものをJ波，QRS終末部からなだらかにST部分に移行する波形をスラーと細分される（図1）．

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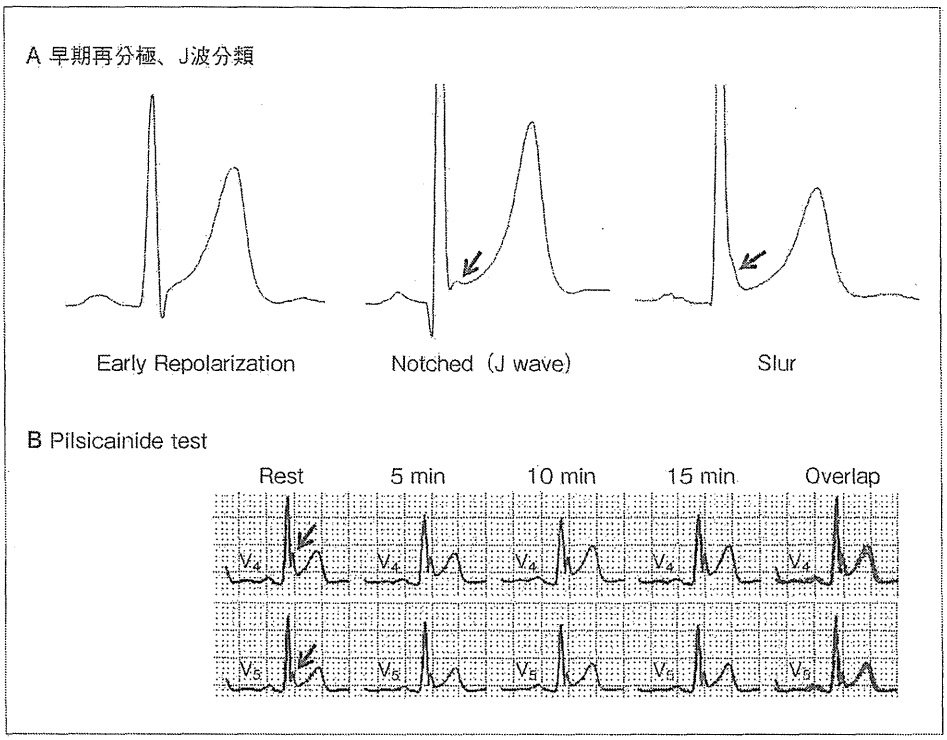

図1　早期再分極とJ波

A：早期再分極波形．矢印はJ波とスラーを示す．

B：ピルシカイニド負荷試験によるJ波の反応．ピルシカイニド投与により，J波の大きさはほとんど変化を示さなくなった．

3．早期再分極の頻度

従来より，早期再分極は健常人で頻度が高いと報告されており[7]，特に若年者（特に思春期以降），男性，黒人，運動家に多いことが知られていた．このため対象がどういった背景を有するかにより，各研究結果にかなりばらつきがみられる．主要な研究では健常人の早期再分極の頻度は3～32%，J波は2～13%，スラーは2～23%と報告されている．表1に示した主要な報告をまとめると，早期再分極は全体で11.9%（6,520/52,723名），男性で15.6%（4,326/27,670名），女性で6%（1,273/21,153名）となり，J波は5.4%，スラーは4.6%であった[5),7)]．一方，特発性VF例における早期再分極の頻度は23～43%とされ，主要な報告をまとめると，頻度は28.7%（246/858名），男性で29.8%（99/332），女性で23.9%（75/314）であった．またJ波の頻度は15～32%（平均22%），スラーの頻度は12～31%（平均23%）で，いずれも健常人よりも数倍高率であり[5]，特発性VF例では単なる良性の偶発的な所見ではないと考えられる．

このようにJ波・早期再分極はVFとの関連が報告されているが，もともと健常人では高頻度にみられる所見である．健常人での早期再分極と予後との関連を表2に示した．多くの論文ではJ点でのST上昇を0.1 mV以上で陽性とし，早期再分極所見のみでは全死亡や心事故死はむしろ少ないとしている[8]．健常人では早期再分極は若年者や運動家に多いことから，一般的な成人病や動脈硬化の予防による死亡・心事故死のリスク減少をみている可能性がある．一方，早期再分極の局在性（下壁・側壁誘導），早期再分極の形態，性別・人種などの項目を加えると，早期再分極を有する症例で心事故死・不整脈死・

表1　早期再分極の頻度

A

General population		Total		Male		Female		
		ER+	ALL	ER+	ALL	ER+	ALL	
Surawicz et al.	JACC 2002；40：1870	338	1,073	282	529	56	544	V_1-V_4 (5)
Klatsky et al.	Am J Med 2003；115：171	670	2,081	583	1,165	87	916	
Haissaguerre et al.	NEJM 2008：358：2016	21	412					
Rosso et al.	JACC 2008；52：1231	16	124					
Tikkanen et al.	NEJM 2009；361：2529 Circulation 2011；123：2666	630	10,864	422	5,693	208	5,171	
Num et al.	EHJ 2009；31：330	46	1,395	44	798	2	597	
Sinner et al.	PLoS Med 2010；7：1000314	811	6,213	439	3,035	372	3,178	MONICA/KORA
Noseworthy et al.	JACC 2011；57：2284	423	9,444	321	7,131	102	2,313	FHS, H2K
Olson et al.	EHJ 2011；24：3098	1,866	15,141	1,420	6,707	446	8,434	ARIC study
Haruta et al.	Circulation 2011；123：2931	1,429	5,976	815	2,612	614	3,364	
Total		6,250	52,723	4,326	27,670	1,273	21,153	
Percent（%）		11.9		15.6		6.0		

B

IVF		Total		Male		Female		
		ER+	ALL	ER+	ALL	ER+	ALL	
Haissaguerre et al.	NEJM 2008：358：2016	64	206	46	122	18	84	
Rosso et al.	JACC 2008；52：1231	19	45					
Merchant et al.	Am J Cardiol 2009；104：1402	9	39	7	22	2	12	
Cappato et al.	Circ A & EP 2010；3：305	10	23	8	16	2	5	J wave/slur
Abe et al.	Heart Rhythm 2010；7：675	7	22	6	17	1	5	J wave only
Lellouche et al.	JCE 2011；22：131	40	104					
Nunn et al.	JACC 2011；58：286	84	363	32	155	52	208	
Derval et al.	JACC 2011；58：722	13	56					
Total		246	858	99	332	75	314	
Percent（%）		28.7		29.8		23.9		

表2　健常人での早期再分極と予後

ER pattern	細項目	予後	HR	Reference
J≥0.1 mV		全死亡	0.8-0.85	Am J Med 2003, Circulation 2011 (Haruta)
		心事故死	0.75	Circulation 2011（Haruta）
		不慮の死	1.83	Circulation 2011（Haruta）
	inferior lead	心事故死	1.28-3.15	NEJM 2009, PLoS Med 2010
	infero-lateral lead（both）	不慮の死	2.50	Circulation 2011（Haruta）
	horizontal/descending ST	不整脈死	1.43	Circulation 2011（Tikkanen）
	slur/notch	不慮の死	2.09	Circulation 2011（Haruta）
	男性＋35-54 y. o.	心事故死	2.65	PLoS Med 2010（Sinner）
	男性＋35-54 y. o.＋Inferior	心事故死	4.27	PLoS Med 2010（Sinner）
	白人	突然死	2.03	EHJ 2011（Olson）
	女性	突然死	2.54	EHJ 2011（Olson）
J≥0.2 mV	inferior lead	心事故死	2.98	NEJM 2009（Tikkanen）
	horizontal/descending ST＋inferior lead	不整脈死	3.14	Circulation 2011（Tikkanen）

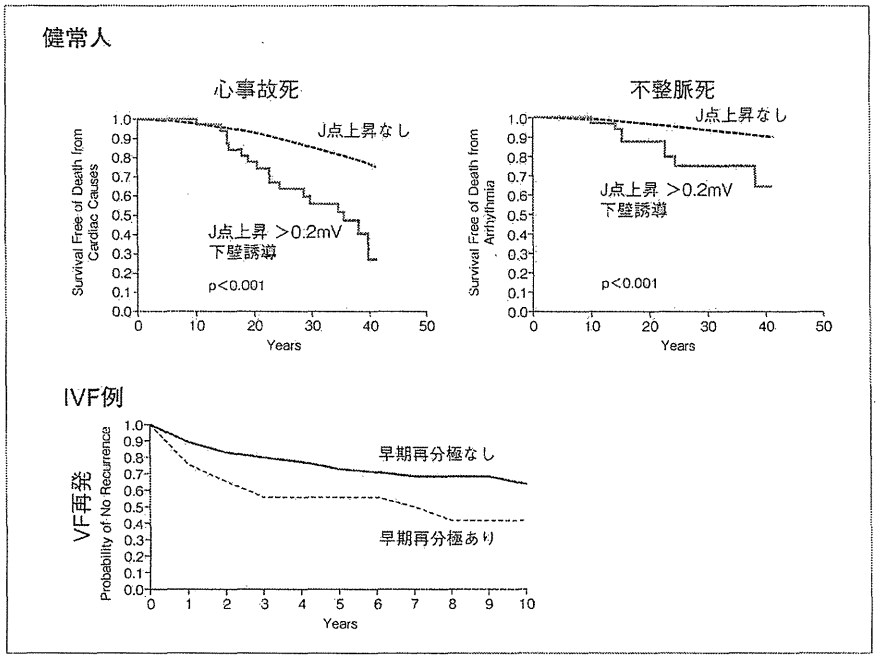

図2　早期再分極の有無による予後

上段は一般人口での心事故および不整脈死の発生，下段は特発性心室細動の再発を示す．

〔上段は文献9)，下段は文献5)より引用改変〕

突然死などが有意に増加する．さらにJ点でのST上昇を0.2 mV以上と定めると，早期再分極の局在性やST部分の形態により心事故死・不整脈死が約3倍に増加することが報告されている（図2）[9]．発症率からみると，早期再分極で不整脈による年間死亡率はJ点でのST上昇が0.1 mV以上では0.07%，ST上昇が0.1 mV以上で誘導の局在性や性別・人種などを項目に加えると0.1～0.3%，ST上昇が0.2 mV以上で何らかの追加項目が存在する場合は0.7～0.8%程度である．特発性VF例で早期再分極を伴う場合，VFの再発が高頻度にみられ再発のハザード比は2.1倍となり，ST上昇が高度なほど，リスクが高いことが報告されている（図2）[5]．

4．心電図の特徴

一般の若年者などでみられる早期再分極は，通常V_3～V_5誘導を中心として高さも0.1 mV前後であることが多い[10]．特発性VFと関連する早期再分極の波形はしばしば変動し，ST上昇やJ波増大が著明となるが，発作時から時間が経ち，安定していくとJ波が消失することも経験される（図3）[5,11]．徐脈や一過性のRR間隔の延長によりJ波は増大するが[11]．Na^+チャネル遮断薬の負荷試験ではJ波・ST上昇はほとんど影響を受けないか，やや縮小する（図1）[12]．運動負荷やカテコラミン負荷ではJ-ST上昇は軽快する[6]．STレベルやJ波高が高値（特に0.2 mV以上）であるほどリスクが高い[9]．

また，VF・心事故と関連する早期再分極・J波はしばしば下壁誘導にみられることが多く，さらにST上昇が下壁誘導のみならず，前壁・側壁まで広範囲にみられる例になると高リスクで，かつ頻回のVF発作（ストーム）とも関連する．Antzelevitchらは早期再分極・J波と関連する特発性VFを，ST上昇・J波の心電図での局在によってERS（Early repolarization syndrome）1型から3型まで分類し，Brugada症候群を4つめのタイプとした（表3）．さらに後天的なJ波症候群として虚血，低体温を含めている．J波の心電図誘導での局在はVFを引き起こす期外収縮の発生部位と関連しており，不整脈原

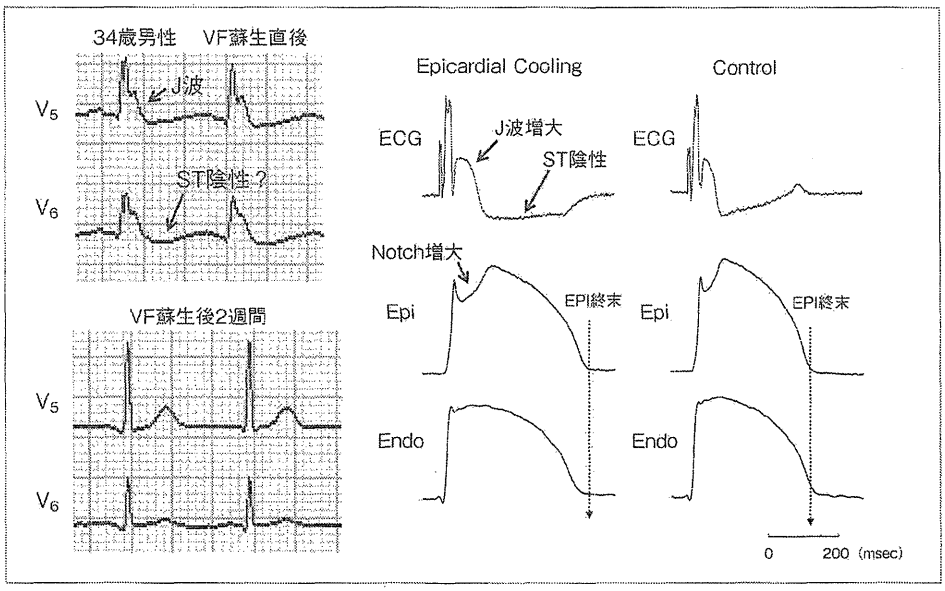

図3　J波の変動

左はJ波と関連した心室細動（VF）例の実例で，VF直後は著明なJ波を認めたが，2週後にはJ波が消失した（心電図は福山市民病院，渡邊敦之先生の御厚意により掲載）．右は動物モデルによるJ波の再現で，心外膜側（Epi）の活動電位第1相ノッチが増大するとJ波が増大する．心内膜面（Endo）では活動電位の変化は乏しい．

表3　J波症候群の分類

Type		遺伝性				後天性	
		ERS type 1	ERS type 2	ERS type 3	Brugada syndrome	虚血	低体温
電気的異常の局在		左室前側壁	左室下壁	左室，右室	右室	左室，右室	左室，右室
J wave	局在	I，V_4-V_6	II，III，aVF	広範囲	V_1-V_3	いずれか	いずれか
	徐脈	増大	増大	増大	増大	N/A	N/A
	Na channel 遮断薬	変化小/不変	変化小/不変	変化小/不変	増大	N/A	N/A
性差		男性	男性	男性	男性	男性	男性，女性
VFとの関連		まれ，健常男性ないし運動家	あり	あり，Strom	あり	あり	あり
薬剤	Quinidine	J点正常化，VT/VF抑制	J点正常化，VT/VF抑制	J点正常化，VT/VF抑制	J点正常化，VT/VF抑制	データ少	VT/VF抑制
	Isoproterenol	J点正常化，VT/VF抑制	J点正常化，VT/VF抑制	J点正常化，VT/VF抑制	J点正常化，VT/VF抑制	N/A	N/A
遺伝子変異		*CACNA1C*, *CACNB2B*	*KCNJ8*, *CACNA1C*, *CACNB2B*	*CACNA1C*	*SCN5A*, *CACNA1C*, *CACNB2B*, *GPD1-L*, *SCN1B*, *KCNE3*, *SCN3B*, *KCNJ8*	*SCN5A*	N/A

〔文献6）より引用改変〕

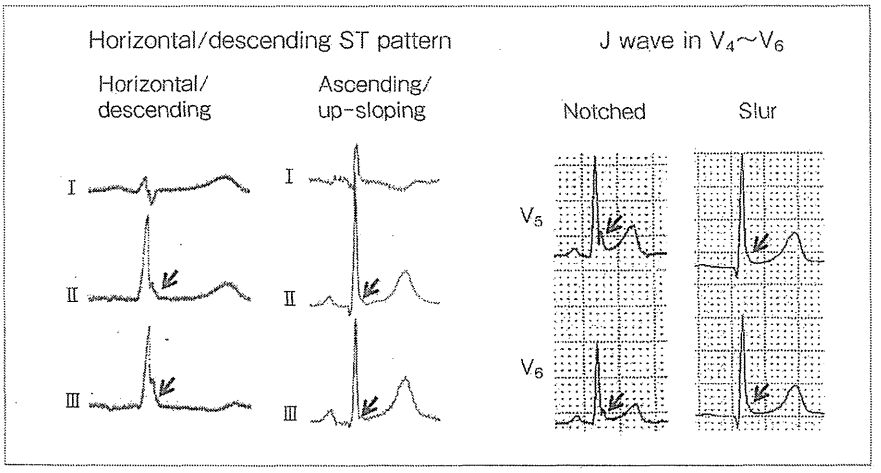

図4　Malignant type の早期再分極

J 点以降の ST 波形による分類で ST 部分が水平型・下降型のものは予後不良とされる（左）．J 点の形態でスラー型よりも J 波を示すもののほうが予後不良とされる（右）．

〔左は文献 9）より引用改変〕

性の電気的基質の局在を示すと考えられている[3),6)]．

J 点の形態についてはスラーよりもノッチ（J 波）を示すものが，VF と関連するとされる[13)]．J 点から ST 部分の形態については，健常人を対象とした研究では J 波やスラーに引き続き，水平型ないし下降型の ST 部分を示す場合に不整脈死と関連すると報告され（図4）[9)]，これは特発性 VF 例でも同様の所見が確認されている[14)]．QT 間隔は，早期再分極例では不変ないしやや短縮を示す．

5．加算平均心電図とプログラム刺激での心室細動誘発性

Abe らは特発性 VF 例の J 波を有するもの（7 例）は加算平均心電図で高率に遅延電位を認め，特に夜間に異常値が著明となることを示した[15)]．一方，遅延電位の陽性率はそれほど高くないとする報告もある[16)]．自検例では早期再分極・J 波関連の特発性 VF 例での遅延電位は 6 例中 1 例のみで陽性であった．これは記録法の違いも影響している可能性があり，今後症例を増やしての検討を要する．

Haïssaguerre らの報告では，特発性 VF のうち早期再分極を有する例にプログラム刺激を行うと 34％に VF が誘発されたが，早期再分極の有無では有意差はみられなかった[5)]．これは Brugada 症候群での VF 誘発率が 6～7 割と高率なのに比し，早期再分極症候群での誘発率はそれほど高くないことによると考えられる．また，HV 時間は明らかな延長を認めないものが多い．

6．早期再分極・J 波の機序

J 波は通常 QRS 終末部に存在し，QRS 波形の終了前から終了後までの間に位置する場合が多い．このため，J 波の機序として，QRS 波形の一部であるとする脱分極異常説と，再分極相早期の変化とする再分極異常説がある．Antzelevitch らは J 波の機序を心外膜側心筋の活動電位の第 1 相ノッチが反映するという，再分極異常説を提唱している[6),17)]．これは Brugada 症候群で想定されている機序と同様に，再分極相の変化および部位特異性から J-ST 上昇を説明し，活動電位のばらつきから機能的なリエントリーが生じるとする phase 2 Reentry 仮説である．図3 に示すように活動電位第 1 相ノッチは心外膜側心筋で大きく，心内膜側心筋では小さいため，再分極相早期では心内膜面から心外膜面へ電位差が生じ，心電図で J 波を呈する．第 1 相ノッチの増大に伴い活動電位長が延長するため，浅い陰性 T 波が

生じ，J波症候群の患者の心電図に類似する．さらに第1相ノッチが増大すると，心外膜側心筋の一部で活動電位が極端に短縮するloss of domeの状態となり，活動電位のばらつきからphase 2 Reentryが生じるとされる．こうした第1相ノッチの増大は，再分極相早期にかかわる心筋イオン電流の外向き電流の増大や内向き電流の減少が関連している(表3)．一方，J波はQRS終末部に重なるため，局所の脱分極異常・伝導障害が成因とする考え方もある．Brugada症候群でも同様の議論があり，臨床的には決着がついていない．

7．J波症候群・早期再分極症候群の治療，リスク評価

VFをきたし，かつJ波症候群・早期再分極症候群と考えられる症例は，植込み型除細動器（ICD）が適応となる[5]．急性期にVFを繰り返す場合はBrugada症候群と同様にイソプロテレノールの持続静注がVF抑制に有効である．一方，β遮断薬，リドカイン，ベラパミル，アミオダロンなどはVF抑制には限定的な効果しかもたらさない．慢性期に再発を繰り返す場合も，これらの抗不整脈薬の発作予防における効果は乏しいが，キニジンは高率にVF発作を予防する[18]．このような薬効評価についての報告は少なく，今後症例の蓄積が必要となる．また少数例ではあるがVFを引き起こす心室期外収縮のカテーテルアブレーションも試みられている[5]．

一方，症状のないJ波症候群・早期再分極症候群では積極的な検査，予防的なICD植込みはほとんどないと考えられる．特発性VFは一般若年成人の10万人に3人程度に発症するが，J波を伴う例の頻度は1万人に1人程度と考えられている[14]．大多数の早期再分極・J波は予後との関連は強くないため，不用意に突然死のリスクを説明すべきではない．確実なリスク評価の方法が定まっていない現時点では，無症候例では著明なJ波・早期再分極を有し，かつ濃厚な突然死の家族歴が存在する場合，電気生理学的検査や予防目的のICD植込みも考慮されることになるであろう．

Ⅱ．Brugada症候群

1．Brugada症候群概説

Burgada症候群は，1992年にBrugada兄弟らが右脚ブロック，恒常的な右側胸部誘導のST上昇を有し，かつ心臓突然死と関連する症例として報告し[1]，これ以降，多数の論文が発表されるようになった．Brugada症候群の機序・病態については，1999年にYanとAntzelevitchが右室心外膜側心筋の活動電位の変化からST上昇，心室不整脈が発生するとした再分極異常説を提唱したが[19]，臨床的に伝導障害を示す所見も多く，脱分極異常からST上昇が生じるとする説もある[20]．実際には脱分極異常と再分極異常は相互に関連し，切り離して考えることはできず，この両者が不整脈発生に関与していると考えられるようになってきた[21,22]．Brugadaらは最初の論文ですでに脱分極異常と再分極異常の両者が関与している可能性を述べている[1]．この項では再分極異常，脱分極異常の面からBrugada症候群の電気生理学的モデル，臨床所見や予後との関連性について概説する．

2．再分極異常の機序

Brugada症候群は，右側胸部誘導のST上昇を特徴とし，心室不整脈発生直前にはST上昇の増強もみられることから，不整脈発生とST上昇は密接に関連するとわかっている．ST上昇はRR間隔，薬剤[23]，運動[24]，発熱，食事[25,26]，自律神経緊張の変化などにより短期的に変動し，また日差変動で典型的なcoved型波形から正常な心電図波形まで変化することもある[27]．このようにダイナミックな変化を示すことから，ST上昇そのものは脱分極異常よりも，自律神経系などの影響で機能的に変化しやすい再分極異常の可能性が高いと考えられる．

イヌ右室心筋組織を使用した薬剤誘発性Brugada症候群モデルでは，Na^+遮断薬[28]，K^+チャネル開口薬[19]，Ca^{2+}チャネル遮断薬[29]などを用い，活動電位第1相付近の内向き電流の減少，外向き電流の増加により，活動電位第1相のノッチを増大させ，活

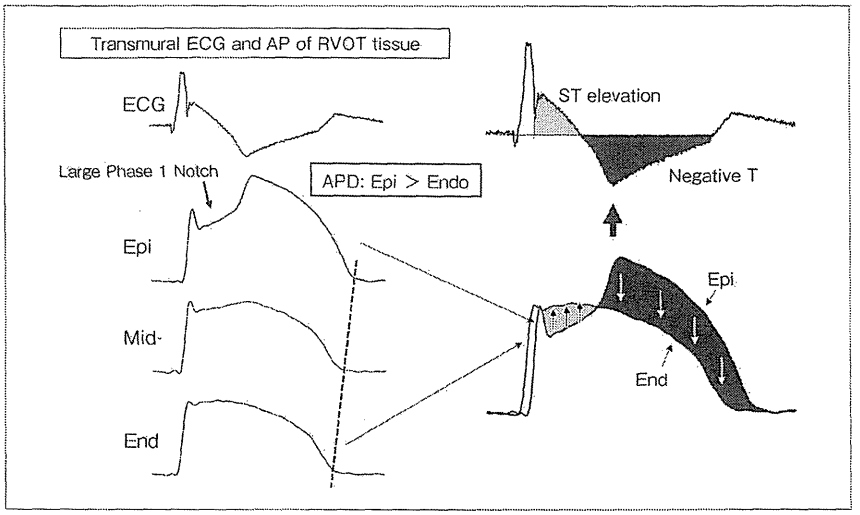

図5　Brugada 症候群モデルでの貫壁性活動電位のばらつきと電位差
心外膜―心内膜間での電位差の増大が ST 上昇，陰性 T 波を形成する．
〔文献 22) より引用改変〕

動電位波形を spike and dome 型に変える[22]．第 1 相ノッチが増大すると，内向き Ca^{2+} 電流の活性化が遅れ，第 2 相後半で大きなドームを形成し，活動電位長は延長する．この変化は主に心外膜側心筋でみられ，心内膜側心筋では変化が小さいため貫壁性に活動電位波形のばらつきが生じる（図5）[22),30)]．活動電位第 1 相ではノッチのため心外膜側心筋の電位が深く，心内膜から心外膜側へ電位勾配が生じる．第 2 相から第 3 相にかけては，心外膜側心筋が大きいドームを有し，活動電位長が延長するため，心外膜側から心内膜側への電位勾配が生じ，正常の状態と逆転する[22),30)]．この結果，心電図で活動電位第 1 相に対応して J-ST 上昇を，第 2 相から第 3 相に対応して陰性 T 波を形成し，Consensus Report[31] で報告された type 1 の Brugada 型心電図波形を生じる．臨床的には心外膜側心筋の活動電位の異常は，カテーテルによる単相性活動電位の記録や冠動脈内に挿入したガイドワイヤーによる電位記録で証明されている（図6）[32)~34)]．

心外膜側心筋の活動電位第 1 相ノッチがさらに増大し，早期再分極が進行すると，Ca^{2+} 電流の活性化が抑制され，第 1 相から第 2 相にかけての内向き電流が減少することで，急激な第 2 相ドームの消失をきたし，loss of dome 型の活動電位波形を生じる．この変化は心外膜側心筋内には起こらず，局所的に変化するため，活動電位長の長い spike and dome 型波形と活動電位長のきわめて短い loss of dome 型波形が混在し，心外膜側心筋内で著明な電位のばらつきを生じる．この心外膜側心筋内の電位差は，活動電位第 2 相で loss of dome 型活動電位の部位に新たな興奮を発生させ，機能的リエントリーを引き起こす（phase 2 reentry）[19),21),22),30)]．Loss of dome 型の活動電位波形がある程度広い範囲で出現すると，心電図では ST 上昇が増強し，陰性 T 波の浅化・消失がみられる[35]．この心電図波形は臨床的には VF の前後やピルシカイニド投与後に確認されることがあり，この特徴的な波形を，われわれは Consensus Report の type 1~3 波形に加え，type 0 波形として報告した（coved 型 ST 上昇≧2 mm かつ，陰性 T 波≦1 mm，図7）[36]．動物モデルでは活動電位の変化に伴い心電図波形が変化するが，心外膜側活動電位がダイナミックに変わるときに心外膜側心筋内の活動電位のばらつきが生じやすくなり，phase 2 reentry が発生する（図7，8）．電位のばらつきは，右

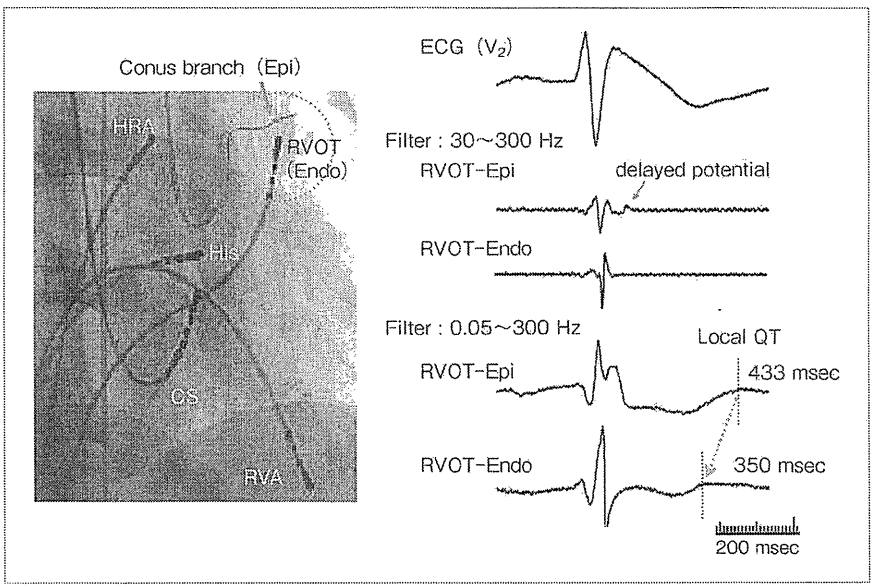

図6　冠動脈より記録した心外膜側電位

右室流出路心外膜側心筋では，相対する心内膜側で記録されない遅延電位と局所の再分極時間（QT 時間）の延長がみられる．　〔文献 33) より引用改変〕

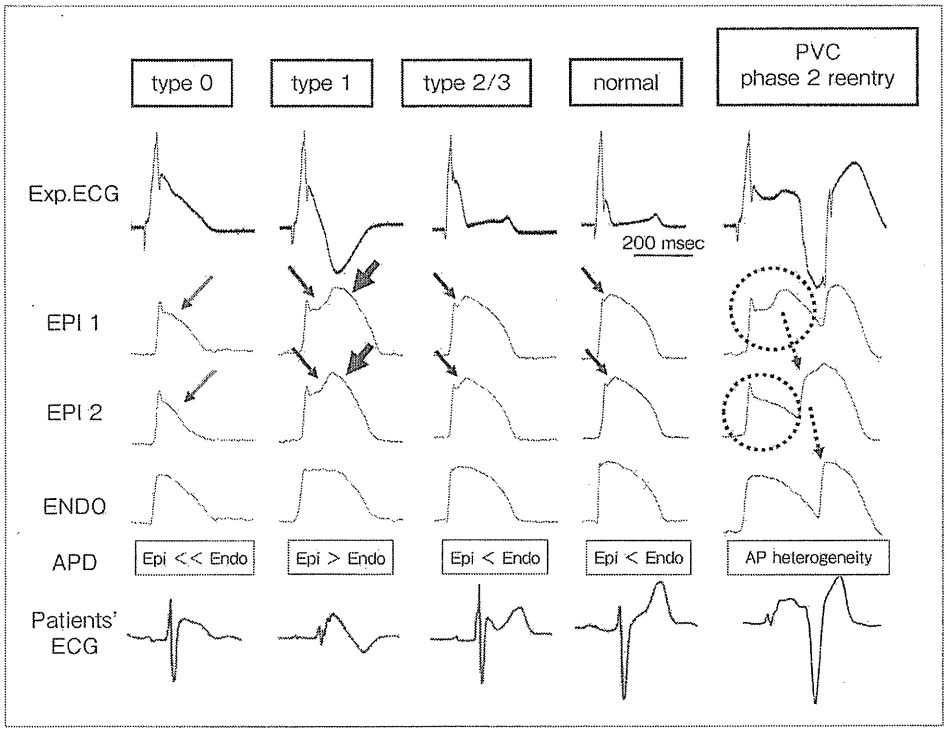

図7　Brugada 型心電図の分類と活動電位

Type 1 は典型的な coved 型 ST 上昇と陰性 T 波を，心外膜側心筋は spike and dome 型の活動電位を示す．Type 2，3 では活動電位第 1 相のノッチは小さく，ST 上昇も目立たない．心外膜側心筋が loss of dome 型になると，陰性 T 波は浅化・消失する．活動電位のばらつきが生じると phase 2 reentry が発生する．　〔文献 36) より引用改変〕

室心尖部付近の組織よりも右室流出路心筋組織で高頻度に発生する[37]．心室頻拍（VT）・VFが発生した組織では活動電位の変化の程度・ばらつきが強く，高頻度に心電図タイプの変動がみられる．一方，VT/VFが発生しなかった組織では電位の変化の程度，ばらつきも少なく，心電図のタイプ変動も2割程度にしかみられなかった．このように実験モデルでは，心電図タイプの変動は心外膜側心筋の活動電位の変化を表し，リエントリー性不整脈の発生に関与するひとつの指標であった[36]．

3．再分極変動の臨床所見

動物モデルから，心電図タイプの変動が不整脈リスクを表す可能性が示唆されたが，臨床的にも心電図変動と予後との関連性がいくつか報告されている．Makimotoらは93例のBrugada症候群患者のうちトレッドミル負荷試験後にST上昇が増強する例に着目し，負荷後のST上昇例では経過観察中にVFイベントが多いことを示した[24]．その他，満腹試験によるST上昇の増強とイベントの発生[25]，心電図タイプの自然変動とVF・ICD作動との関連性[27]なども報告されている．

われわれは，前項の実験モデルの結果から，心電図タイプ・STレベルの自然変動に着目し，予後との関連性について検討した[36]．VF例41例，無症候例33例のうち複数回安静時心電図記録がなされているが，各種負荷試験後，食後短時間のものなど，変動をきたす可能性のあるものは除外し，心電図の変動を検討した．各心電図タイプの出現およびタイプ間の変動，STレベルの変動（0.2 mV以上）を評価項目とした．心電図タイプはtype 1～3に加え，type 0および正常心電図とした（図7）．VF例では心電図タイプの変動は97%にみられたが，無症候例では44%にとどまった．またSTレベルの変動はVF例では91%が陽性であったのに対し，無症候例では15%のみが陽性であった．Type 0波形の出現率は高くないもののVF前後では18%の例で陽性となったが，最終発作から1年以上経過した安定期にはみられなかった．各種心電図指標とVFとの関連性を多変量解析すると，①type 0波形の出現，②心電図の正常化，③STレベル変動が独立した危険因子となった．典型的なtype 1からtype 0への波形変動やST形態の正常化は，心電図タイプの変動のなかでもmalignant typeの変動と考えてよいと思われる．このように心電図の変動はハイリスク群と関連し，活動電位のばらつきを表すものと考えられる．

4．脱分極異常と予後

Brugada症候群の最初の報告でも，しばしば1度房室ブロック，右脚ブロックを伴い，心内心電図でHV延長がみられるとされ[1]，脱分極異常が存在することが知られていた．その後も加算平均心電図で遅延電位が陽性となることや[38]，心筋障害（線維化，脂肪浸潤）や心筋炎の所見がみられることなど[39]の報告が相次ぎ，Brugada症候群における伝導障害の合併は決してまれではなく，予後と関連性があることがわかってきた．TakagiらはJ-IVF研究において，高リスク例ではQRS幅が延長すること，V_2およびV_5のQRS幅の延長（≧90 msec）が有症候例では経過中のVF発生と関連することを報告した[40]．同様の所見はBrugadaらによっても報告され，彼らはQRS≧120 msecを高リスクとしている[41]．加算平均心電図による遅延電位はIkedaらが予後との関連性を報告し[38]，Nagaseらは右室流出路心外膜側心筋に遅延電位が存在することを示した（図6）[33]．体表面電位図による検討では遅延電位とST上昇領域は右室流出路で重なり[42]，この部位で脱分極異常・再分極異常が発生し，心電図波形や不整脈発生に影響していることが推測される[21),22]．

Meregalliらは伝導障害の所見を重視し，ST上昇も，右室流出路の伝導遅延による右室内の電位勾配の変化で説明できるとした[20),43]．しかし，再分極異常のある心外膜側心筋部位の興奮が遅延すると，局所再分極の進行も遅れ，J-ST上昇と陰性T波の顕在化がみられる（図9）ことからも[32),44]，脱分極異常が再分極異常をより顕在化しうると考えられる．また，Aibaらは動物モデルを用い，伝導障害が存在す

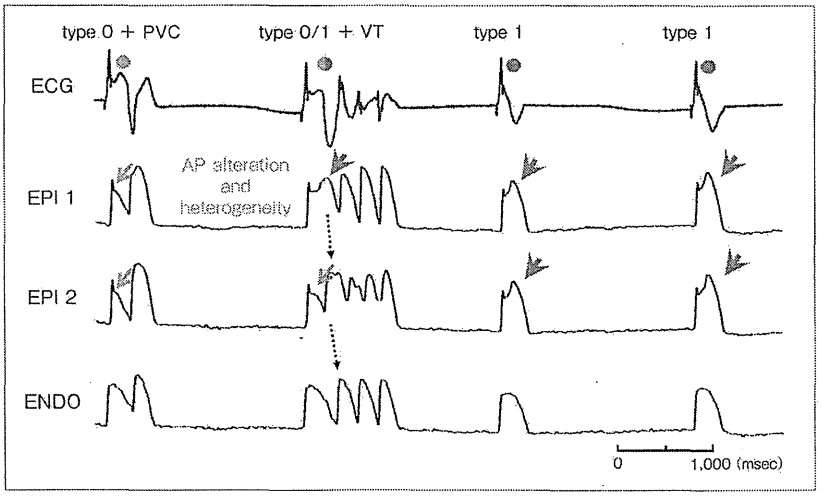

図8
心外膜側活動電位と心電図の変化
心外膜側活動電位が loss of dome から spike and dome に変化するときに，活動電位のばらつきが起こり，心室頻拍が発生した．心電図も type 0 から type 1 へ変化している．
〔文献 36) より引用改変〕

ると，phase 2 reentry で発生した VT の興奮前面が分裂し細動に移行することも示している[21]．再分極異常が心室不整脈発生のトリガーで，伝導障害が VF への移行・持続を引き起こす基質になると考えられる[22]．

Brugada 症候群で脱分極異常をきたす原因についてはいくつかの報告がみられる．Brugada 症候群の遺伝子変異は Na^+ 電流の減少と関連している場合が多く[45]，この電流の減少は活動電位第 0 相における電位の立ち上がりを抑え，伝導遅延をきたす原因となる．また心筋生検で脂肪浸潤や線維化，局所的な心筋炎がみられるとの報告もあるが[20]，一方で明らかな組織学的な異常はないとする報告もみられる．組織学的に心筋障害がみられなくとも，VF 例では酸化ストレスを示す所見が報告されており，膜電流の変化が起こっている可能性もある[46]．Coronel らは移植で摘出した Brugada 症候群患者の心臓に対し電気生理学的検査および組織学的検査を行い，心筋線維化・脂肪浸潤が右室流出路や自由壁に存在し，伝導障害をきたすことを報告した[47]．Nademanee らは Brugada 症候群患者の右室流出路心外膜面で低電位や分裂様電位が記録されたことから，同部位のアブレーションを行ったところ VF 発生の減少や心電図の正常化を得た[48]．このことから右室流出路心外膜面の心筋障害が脱分極異常・再分極異常をきたす基質となっていると考えられた．

5．新しい脱分極異常の指標：fragmented QRS

12 誘導心電図では QRS 幅や PQ 間隔で脱分極異常を診断することができるが，伝導障害がある程度強くないと反映されず，鋭敏な指標とはなりがたい．Das らは心筋梗塞や心筋症などの心筋障害を表す新しい心電図指標として fragemented QRS（fQRS）を提唱した[49]．これは QRS 波形の分裂，多棘などで定義され，虚血性心疾患や心筋症の診断・予後と関連する．Brugada 症候群でも 12 誘導心電図を詳細に観察すると，QRS 区間内に多棘性 R 波を認めることがあり，心筋障害の表れと考えられる．

われわれは，Brugada 症候群患者 115 例で fQRS 指標を用い伝導障害の評価を試みた[44]．Brugada 症候群患者で fQRS は 43% にみられた．とりわけ VF 例で高率（85%）に，失神のある患者では 50%，無症候例では 35% にみられた．fQRS 陽性の VF・失神患者では，経過観察中 58% に VF 発生を認めたが，fQRS 陰性例では VF 発生は 6% のみであった（図10）[44]．fQRS は加算平均心電図での遅延電位とは相関がみられなかった．実験的には右室心外膜側心筋の伝導遅延を想定することで fQRS 類似波形が再現され（図9），Brugada 症候群では伝導障害も不整脈発生に大きな影響を及ぼすとわかった．最近，報告されたイタリアの多施設共同研究（PRELUDE