

Genetic Predisposition to Ischemic Stroke A Polygenic Risk Score

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Background and Purpose—The prediction of genetic predispositions to ischemic stroke (IS) may allow the identification of individuals at elevated risk and thereby prevent IS in clinical practice. Previously developed weighted multilocus genetic risk scores showed limited predictive ability for IS. Here, we investigated the predictive ability of a newer method, polygenic risk score (polyGRS), based on the idea that a few strong signals, as well as several weaker signals, can be collectively informative to determine IS risk.

Methods—We genotyped 13214 Japanese individuals with IS and 26470 controls (derivation samples) and generated both multilocus genetic risk scores and polyGRS, using the same derivation data set. The predictive abilities of each scoring system were then assessed using 2 independent sets of Japanese samples (KyushuU and JPJM data sets).

Results—In both validation data sets, polyGRS was shown to be significantly associated with IS, but weighted multilocus genetic risk scores was not. Comparing the highest with the lowest polyGRS quintile, the odds ratios for IS were 1.75 (95% confidence interval, 1.33–2.31) and 1.99 (95% confidence interval, 1.19–3.33) in the KyushuU and JPJM samples, respectively. Using the KyushuU samples, the addition of polyGRS to a nongenetic risk model resulted in a significant improvement of the predictive ability (net reclassification improvement=0.151; $P<0.001$).

Conclusions—The polyGRS was shown to be superior to weighted multilocus genetic risk scores as an IS prediction model. Thus, together with the nongenetic risk factors, polyGRS will provide valuable information for individual risk assessment and management of modifiable risk factors. (*Stroke*. 2017;48:253-258. DOI: 10.1161/STROKEAHA.116.014506.)

Key Words: genome-wide association study ■ genotype ■ risk assessment ■ stroke

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Ischemic stroke (IS) is a leading cause of death and long-term disability in the world.¹ Although a large proportion of IS events could be prevented by appropriate management of modifiable risk factors, such as high blood pressure and tobacco use,² the burden attributable to these modifiable risk factors remains problematic.³ To lower this burden, it is important to apply both population and high-risk approaches.⁴ Genetic information can be a useful tool for the identification of high-risk individuals.

The effects of individual genetic markers are relatively small for common polygenic disorders, and therefore, a well-studied approach, weighted multilocus genetic risk score (wGRS), typically integrates tens of weak genetic markers into a single risk score, based on summary statistics from genome-wide association (GWA) studies.⁵ For hypertension and coronary artery disease, wGRSs have been derived from GWA data of the target traits.⁵ However, previous IS GWA studies have identified only a few replicable susceptibility loci,^{6–9} possibly because of the etiologic heterogeneity of IS. Therefore, wGRSs for IS in previous studies have been derived from GWA data on hypertension,¹⁰ atrial fibrillation,¹¹ and coronary artery disease,^{12–14} and their predictive abilities have been limited.^{10–14}

We hypothesized that the lower predictive abilities of wGRSs are related to the polygenic nature of IS. A previous analysis on international IS GWA data inferred that genetic predispositions to IS are shared among the different subtypes,¹⁵ which may be related to susceptibilities to arteriosclerosis, hypertension, hyperlipidemia, and their combinations. Thus, we sought to develop a statistical model to predict the genetic predispositions shared among IS subtypes, rather than identify genetic markers specific to IS subtypes. To accomplish this, we created a polygenic risk score (polyGRS) based on the assumption that, in addition to a few genome-wide signals obtained from IS GWA data, numerous weaker signals can be collectively informative for predicting IS incidence. In previous studies, polyGRSs showed remarkable predictive abilities for schizophrenia, bipolar disorder, hypertension, and coronary artery disease.^{16,17} However, the predictive ability of polyGRS for IS remains to be determined. The complexity of IS subtypes requires the validation of the genetic model; therefore, we derived both a wGRS and polyGRS from the same large-scale derivation samples and compared their predictive abilities in 2 sets of independent samples.

Methods

Cohorts and Case Definition

In this study, we used 3 sets of Japanese samples. The first set (derivation data set) was used to derive a wGRS and polyGRS. The second set (KyushuU data set) was used to assess the predictive abilities of the 2 GRSs with detailed clinical information. The third set (JPJM data set) was used as an additional data set for the validation of the predictive abilities. All 3 sets of samples were independent from each other.

For the derivation data set, patients with IS were recruited by the BioBank Japan Project from 2003 to 2008.¹⁸ All participants provided written informed consent, as approved by the ethical committees of the BioBank Japan Project and the University of Tokyo. Clinical information on the subjects was collected from medical charts, neuroimaging results (including computed tomography and magnetic resonance imaging), and self-reported questionnaires. Controls in the derivation data set were enrolled from participants in Japanese

prospective cohort studies, including the Tohoku Medical Megabank Project,¹⁹ the Japan Public Health Center–based prospective study,²⁰ and the Japan Multi-Institutional Collaborative Cohort Study.²¹ Details of the study design and recruitment methods of the 3 cohort studies were described previously.^{19–21}

For the KyushuU data set, details of the recruitment methods and diagnostic criteria were described previously.^{6,22} Briefly, affected individuals with IS were recruited from 7 hospitals affiliated with Kyushu University in 2004. For all cases, diagnoses of IS and its subtypes were made by stroke neurologists from the affiliated hospitals by referencing clinical presentation and ancillary laboratory examinations—namely, cerebral angiography, brain imaging, echocardiography, and carotid duplex imaging. Participants in the Hisayama study were enrolled as control subjects. The Hisayama study is a population-based cohort study established in 1961.^{23,24} Of 3328 Hisayama residents aged ≥ 40 years who consented to participate in the Hisayama study between 2002 and 2003, we selected age-matched (within 5 years) and sex-matched control subjects by 1:1 matching using random numbers, after excluding subjects with a history of stroke or coronary heart disease. For the subjects in the KyushuU data set, hypertension was defined as systolic blood pressure ≥ 140 mm Hg and diastolic blood pressure ≥ 90 mm Hg on at least 3 different occasions or as current treatment with antihypertensive drugs.²⁵ Diabetes mellitus was determined by a 75-g oral glucose tolerance test, casual blood glucose levels (>11.1 mmol/L), or a medical history of diabetes mellitus. Hyperlipidemia was defined as a total cholesterol level ≥ 5.69 mmol/L or current treatment with a cholesterol-lowering drug. Atrial fibrillation was diagnosed based on electrocardiographic findings.

For the JPJM data set, nested case–control subjects were enrolled from the participants of the Japan Public Health Center and Japan Multi-Institutional Collaborative Cohort studies in the Saga and Takashima regions.^{20,21,26} IS cases were confirmed by imaging studies, and age- and sex-matched controls were extracted from a pool of individuals with no history of stroke.²⁷

Genotyping

All subjects from the 3 data sets were genotyped using a HumanOmniExpressExome BeadChip array (Illumina, Inc, San Diego, CA).

Quality Control Filters for Derivation Samples

Samples with low call rate (<0.98), single-nucleotide polymorphisms (SNPs) with low call rate (<0.99), and close relationships characterized by the identity-by-state method were excluded, as well as subjects whose estimated ancestries outside of the Hondo cluster of the Japanese population²⁸ by PCA.^{29,30} Variants with a Hardy–Weinberg equilibrium exact test P value of $<1 \times 10^{-6}$ and a minor allele frequency <0.01 were also excluded. Ultimately, 39 684 individuals (Table 1) with 537 999 autosomal SNPs were included in our analyses.

Genetic Risk Score Derivation

Our statistical analysis workflow is shown in Figure 1. The wGRS included 5 SNPs selected at the end of the replication and exploratory analyses from the derivation samples.

Additional quality control filters were applied to the derivation data set to generate the polyGRS: (1) samples with a call rate of <0.99 , (2) SNPs with a Hardy–Weinberg equilibrium exact test P value of <0.05 , (3) SNPs with a P value in the test for nonignorable difference between cases and controls of <0.05 were excluded, according to a previous study.³¹ Ultimately, no individuals were excluded by these filters, and 357 367 autosomal SNPs were retained.

According to a previous study,³¹ polyGRS by genotyped data shows higher predictive ability than the model using all imputed data. Therefore, we also used the genotyped variants (357 367 variants) to generate the polyGRS. As such, we used a dual-formula technique to minimize overfitting to the derivation data set. The polyGRS was generated via 2 models: (1) restricted maximum likelihood and (2) best

Table 1. Age and Sex Distributions of the Derivation, KyushuU, and JPJM Samples

	Derivation		KyushuU		JPJM	
	Cases	Controls	Cases	Controls	Cases	Controls
Study design	Case–Population		Case–Control		Nested Case–Control	
Subjects	13 214	26 470	1097	1097	336	336
Age, y*, mean±SD	69±10	56±10	70±10	70±10	59±7	59±7
Women, %	35.7	60.6	39.1	39.1	37.5	37.5

*Age at enrollment.

linear unbiased prediction. A mixed linear model was assumed in both cases,³¹ which accounts for genotype data via the genetic relationship matrix, leaving only the variances of genetic and nongenetic effects as free parameters. More specifically, the restricted maximum likelihood model estimates the 2 free parameters based on derivation samples (N=39 684), whereas the best linear unbiased prediction model converts the variance parameter estimates into the weight parameters for the 357 367 SNPs in conjunction with the genotype data of derivation samples. In total, only 2 parameters were estimated through the restricted maximum likelihood and best linear unbiased prediction steps. Further details are provided in the [online-only Data Supplement](#).

Predictive Ability Assessment

Because previous studies on wGRS typically evaluated the odds ratio (OR) of the highest score quintile versus the lowest score quintile,^{11,12} we estimated the OR for each score quintile using Fisher exact test. In addition, the OR per 1 SD, the corresponding 95% confidence interval (CI), and the overall P value were estimated by the conditional logistic regression analysis. To compare the predictive abilities of the 2 GRSs, a continuous version of net reclassification improvement (NRI), an integrated discrimination improvement, and the C-index were calculated. The NRI, integrated discrimination improvement, and C-index were also calculated to assess the improvements in predictive ability obtained by adding the 2 GRSs to a nongenetic risk model.

Results

Derivation of Genetic Risk Scores

Of the 6 well-studied variants, the associations with IS were nominally significant regarding 4 variants (rs6843082

[*PITX2*]; rs2383207 [*CDKN2B-CDKN2A*]; rs2107595 [*HDAC9*]; and rs879324 [*ZFX3*]) in our derivation samples (Tables I and II in the [online-only Data Supplement](#)).

For exploratory identification of IS-susceptibility loci, based on our derivation samples, the associations between 6204 347 imputed genetic variants and IS were subjected to a genome-wide analysis (Figure I and Table III in the [online-only Data Supplement](#)). The results showed genome-wide significance ($P < 5 \times 10^{-8}$) for one variant, rs1275923, which is located in an intron of *KCNK3* (Figure II in the [online-only Data Supplement](#)) and is reportedly associated with blood pressure.³²

Based on these results, we chose 5 variants as model variables for our wGRS (Table IV in the [online-only Data Supplement](#)). Additionally, from the same derivation data set, we created a polyGRS, based on the assumption that, in addition to several genome-wide signals, numerous weaker signals can be collectively informative for the prediction of IS incidence. All genotyped variants that passed our quality control filters (357 367 variants) were used to derive the polyGRS.

Predictive Ability of Genetic Risk Scores

The predictive abilities of the wGRS and polyGRS were assessed using the KyushuU and JPJM samples. The power to detect significance with an OR of 1.2 per 1 SD was 99% and 66% for the KyushuU and JPJM data sets, respectively (Table 2). This was comparable to the OR of 1.17 per SD at $P_T=1$ by the score-profiling model (Table V in the [online-only Data Supplement](#)), indicating that all genotyped variants in the polyGRS contributed useful signal to the score.

Although the polyGRS significantly associated with IS in both validation data sets, this was not observed with wGRS (Table 2). Moreover, the OR for the highest polyGRS quintile compared with the lowest polyGRS quintile was 1.75 (95% CI, 1.33–2.31) and 1.99 (95% CI, 1.19–3.33) in the KyushuU and JPJM samples, respectively (Figure 2), with a significant improvement observed in the KyushuU samples (NRI=0.179; Figure 3; Table VI in the [online-only Data Supplement](#)).

Predictive Ability of Genetic Risk Scores for Each Etiologic Subtype

In the KyushuU samples, the predictive abilities of the 2 GRSs were investigated for each etiologic subtype: large-vessel disease, small-vessel disease, and cardioembolic stroke (Figure 3). The wGRS failed to associate with any subtype, whereas the polyGRS was significantly associated with all 3. Furthermore, the predictive ability of the subtype-mixture

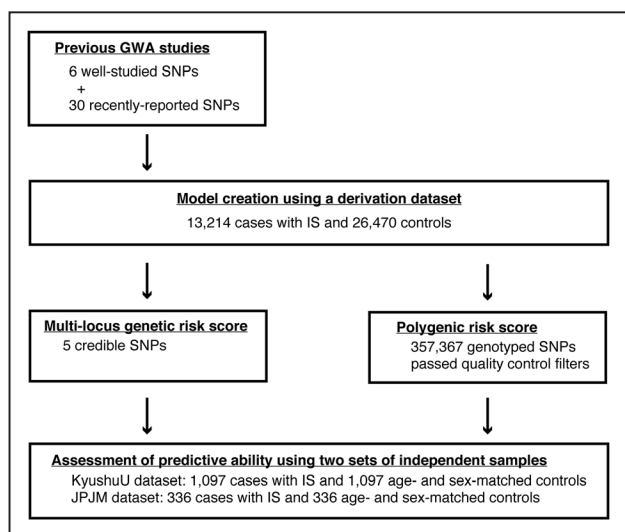


Figure 1. Statistical analysis flow diagram. GWA indicates genome-wide association; IS, ischemic stroke; and SNP, single-nucleotide polymorphism.

Table 2. Predictive Ability of the Multilocus and the Polygenic Risk Scores in the KyushuU and JPJM Samples

Validation Samples	Model	Q1 OR (95% CI)	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)	Q5 OR (95% CI)	OR per SD* (95% CI)	Overall* P Value	C-index (95% CI)
KyushuU (N=2194)	wGRS	Reference	1.09 (0.83–1.43)	1.08 (0.82–1.42)	1.03 (0.78–1.35)	1.17 (0.89–1.54)	1.04 (0.96–1.14)	0.313	0.510 (0.486–0.534)
	polyGRS	Reference	1.08 (0.82–1.42)	1.10 (0.84–1.45)	1.41 (1.08–1.86)††	1.75 (1.33–2.31)††	1.20 (1.10–1.31)††	<0.001‡	0.555 (0.531–0.579)††
JPJM (N=672)	wGRS	Reference	1.37 (0.82–2.28)	2.02 (1.21–3.39)††	1.39 (0.83–2.32)	1.45 (0.87–2.42)	1.11 (0.96–1.29)	0.172	0.530 (0.487–0.574)
	polyGRS	Reference	1.96 (1.18–3.29)††	1.69 (1.01–2.83)†	1.33 (0.80–2.23)	1.99 (1.19–3.33)††	1.20 (1.01–1.41)†	0.033‡	0.536 (0.492–0.580)

CI indicates confidence interval; OR, odds ratio; polyGRS, polygenic risk score; Q1–Q5, quantiles 1–5; and wGRS, weighted multilocus genetic risk score.

*Considering the genetic risk scores as continuous variables.

†Significant after multiple corrections.

‡Results are nominally significant ($P < 0.05$).

polyGRS was higher than that of the subtype-specific model (Table VII in the [online-only Data Supplement](#)).

Integration of Genetic Risk Scores Into a Nongenetic Risk Model

Based on the KyushuU samples, the wGRS or polyGRS was added to a nongenetic risk model that included hypertension, diabetes mellitus, hyperlipidemia, and atrial fibrillation as model variables. Notably, the polyGRS showed an improved predictive ability, whereas the wGRS did not (Figure 4; Table VIII in the [online-only Data Supplement](#)). For all IS cases, the polyGRS NRI was estimated to be 0.151 (95% CI, 0.068–0.235), the integrated discrimination improvement was 0.004 (95% CI, 0.001–0.006), and the Δ C-index was 0.700 (95% CI, 0.679–0.722). When stratified by subtype, the NRI was significant for large-vessel disease and small-vessel disease but not cardioembolic stroke.

Discussion

Based on large-scale genotyping of Japanese samples (N>40 000 in total), we showed that the polyGRS has a superior ability to predict IS compared with the wGRS. Given that the estimates of ORs of the highest score quintile compared with the lowest score quintile were at most ≈ 1.3 in previous studies,^{8–12} the higher OR for polyGRS (>1.75) indicated that the polygenic approach was well suited to derive genetic risk scores for IS. The difference in incident risk between the highest and lowest score quintiles (>75% difference)—which was consistently estimated from both retrospective (case–control) and prospective (nested case–control) designs—supports the utility of polyGRS methodology for healthcare and preventive purposes. Moreover, the polyGRS

significantly associated with all etiologic subtypes, suggesting that it effectively predicts the genetic predispositions shared among IS subtypes. Furthermore, the significant NRI estimates for all IS cases indicated that the integration polyGRS into nongenetic risk models would be valuable, whereas the nonsignificant NRI for cardioembolic stroke suggested that the adjustment by atrial fibrillation and other nongenetic factors attenuated the predictive ability of the polyGRS.

One aspect of the clinical utility of the polyGRS is the individualization of clinical criteria. Japanese clinical guidelines for the management of hypertension published in 2014 recommended the grouping of patients with hypertension into risk strata based on blood pressure levels and other cardiovascular risk factors, including age, smoking, dyslipidemia, obesity, diabetes mellitus, and family history of young-onset cardiovascular disease.³³ For each risk stratum, a distinct therapeutic strategy was recommended. Here, we showed that polyGRS may be a valuable predictor of IS. Accordingly, hypertensive patients whose risks have been underestimated without the additional genetic information would be reclassified into higher risk strata after including polyGRS as a part of the clinical criteria.

To advance further, prospective cohort studies on the predictive ability of the polyGRS would be essential. It would also be interesting to investigate whether the polyGRS derived from Japanese samples can predict IS in other East Asians and other ethnicities. Additionally, ethical, legal, social, and policy issues, including the responsibility for the management of the genetic information, should be discussed in future studies.

The difference between the predictive abilities of polyGRS and wGRS elucidates the polygenic nature of IS. An important methodological difference between wGRS and polyGRS

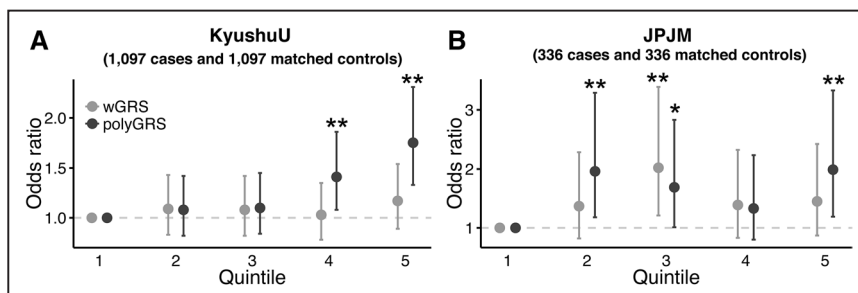


Figure 2. Predictive ability of the weighted multilocus genetic risk scores (wGRS) and polygenic risk scores (polyGRS) in the (A) KyushuU and (B) JPJM samples. Odds ratio for each score quintile compared with the lowest score quintile. *Nominal significant odds ratios ($P < 0.05$). **Significant odds ratios after multiple corrections.

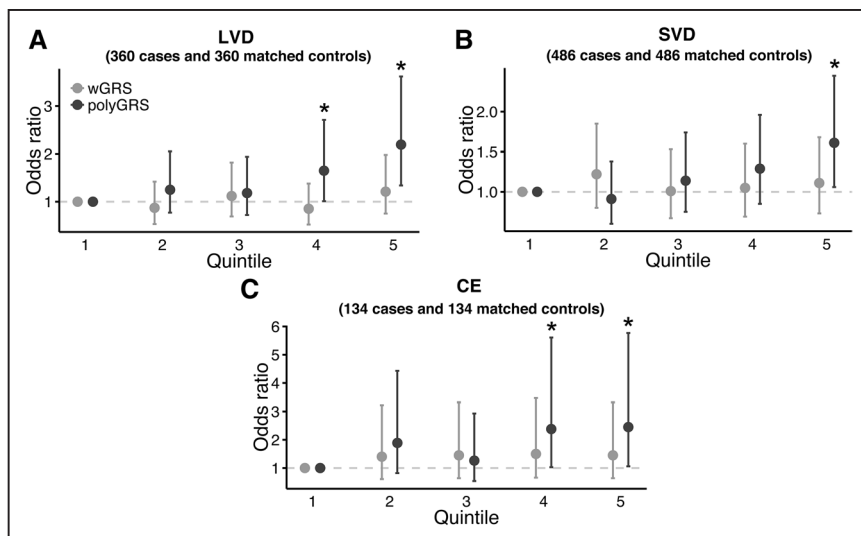


Figure 3. Predictive ability of the weighted multilocus genetic risk scores and polygenic risk scores for each etiologic subtype in the KyushuU samples. Odds ratio for each score quintile compared with the lowest score quintile. **A**, Large-vessel disease (LVD). **B**, Small-vessel disease (SVD). **C**, Cardioembolic stroke (CE). *Significant odds ratios.

is that the wGRS only included 5 credible SNPs as model variables, whereas polyGRS used all genotyped SNPs. The superior predictive ability of polyGRS demonstrates the validity of our assumption that, in addition to a few genome-wide signals, numerous weaker signals are collectively informative for predicting IS. Furthermore, in the score-profiling model, the predictive ability improved as P_T threshold increased. This result implies that usage of more SNPs is essential for improvement of the predictive ability. Our results suggest that a large number of IS-susceptibility loci with small effect size have yet to be discovered and that larger derivation and replication of GWA data sets would be advantageous for discovering novel susceptibility loci in future studies. Furthermore, given that we are currently unable to identify all IS-susceptibility variants with small effect size, polyGRS approach may represent a fascinating method to use the valuable information contained in weak GWA signals to predict IS.

Conclusions

We demonstrated that the polyGRS approach is superior to that of wGRS as a method of choice for the assessment

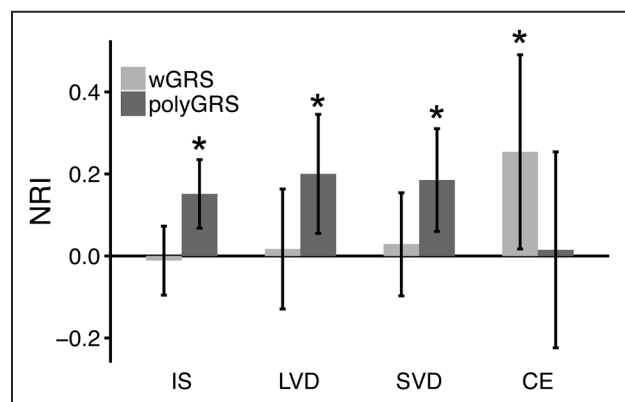


Figure 4. Predictive ability improvement offered by the addition of weighted multilocus genetic scores (wGRS) or polygenic risk score (polyGRS) to a nongenetic risk model in the KyushuU samples. CE indicates cardioembolic stroke; IS, ischemic stroke; LVD, large-vessel disease; NRI, net reclassification improvement; and SVD, small-vessel disease. *Significant differences.

of IS genetic risks. This is clinically important because the polyGRS approach is promising for the individualization of clinical criteria in the era of precision medicine.

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Disclosures

None.

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