PATJ Low Frequency Variants Are Associated With Worse Ischemic Stroke Functional Outcome

A Genome-Wide Meta-Analysis

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Rationale: **Ischemic stroke is among the leading causes of adult disability. Part of the variability in functional outcome after stroke has been attributed to genetic factors but no locus has been consistently associated with stroke outcome.**

Objective: **Our aim was to identify genetic loci influencing the recovery process using accurate phenotyping to produce the largest GWAS (genome-wide association study) in ischemic stroke recovery to date.**

Methods and Results: **A 12-cohort, 2-phase (discovery-replication and joint) meta-analysis of GWAS included anterior-territory and previously independent ischemic stroke cases. Functional outcome was recorded using 3-month modified Rankin Scale. Analyses were adjusted for confounders such as discharge National Institutes of Health Stroke Scale. A gene-based burden test was performed. The discovery phase (n=1225) was followed by open (n=2482) and stringent joint-analyses (n=1791). Those cohorts with modified Rankin Scale recorded at time points other than 3-month or incomplete data on previous functional status were excluded in the stringent analyses. Novel variants in *PATJ* (Pals1-associated tight junction) gene were associated with worse functional outcome at 3-month after stroke. The top variant was rs76221407 (G allele, β=0.40, $P=1.70\times10^{-9}$).**

Conclusions: **Our results identify a set of common variants in *PATJ* gene associated with 3-month functional outcome at genome-wide significance level. Future studies should examine the role of *PATJ* in stroke recovery and consider stringent phenotyping to enrich the information captured to unveil additional stroke outcome loci. (*Circ Res*. 2019;124:114-120. DOI: 10.1161/CIRCRESAHA.118.313533.)**

Key Words: allele ■ genetic loci ■ genetic variant ■ genome-wide association study ■ ischemic stroke

In September 2018, the average time from submission to first decision for all original research papers submitted to *Circulation Research* was 14.06 days.

The full list of affiliations is available in the Appendix.

This study was supported by Genetic Contribution to Functional Outcome and Disability After Stroke (GODS) Project, NINDS Stroke Genetics Network (SiGN), International Stroke Genetics Consortium (ISGC), and GeneStroke Consortium

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The online-only Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/CIRCRESAHA.118.313533.

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Ischemic stroke (IS) is the leading cause of adult disability[1] and the second cause of death worldwide.[2] Approximately 15 million people per year have a stroke, 5 million results in long-term disability.[3] More than a thousand potential targets for neuronal recovery have been identified,[4] although few have been tested in clinical trials. As no trials had positive results it is of high priority to find new drug targets for clinical practice.

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Functional outcome after IS varies between individuals irrespective of clinical factors as initial stroke severity, stroke subtype, and vascular risk factors.[5] Multiple metabolic pathways are important in the response to cerebral ischemic damage and their activity may be modulated by variation in the genes of the involved components. New synaptic connections have been observed in areas surrounding a cerebral infarct within days after a stroke and this response is correlated with functional recovery.[6]

It is reasonable to presume that genetic variation may influence stroke recovery.[7] Candidate-gene studies reported the association between several genes and disability after stroke, but no locus has been found through a hypothesis-free genome-wide approach and most of the reported candidates failed to replicate in other cohorts.[8]

Genome-wide association studies (GWASs) have identified multiple single nucleotide polymorphisms (SNPs) contributing with a small effect to the risk of complex diseases, and different genes have arisen as potential therapeutic targets to reduce the risk of stroke.[9,10] Studying the genetic component of stroke outcome is of great scientific interest but requires very accurate phenotyping and proper attention to potentially confounding factors that may hide the true genetic contribution.

We aimed to find the genetics influencing the stroke recovery process in a dataset of first-ever IS patients, using highly accurate phenotyping and producing the largest GWAS in stroke mid-term functional outcome to date to identify new potential drug targets.

Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Detailed Methods are provided in the online-only Data Supplement.

Study Design

We conducted a 2-phase analysis; discovery-replication and joint association of IS cases. The discovery consisted of a meta-analysis of 4 GWAS. The replication was performed as an in silico analysis of the top SNPs ($P<1\times10^{-5}$) identified in the discovery. Two final joint meta-analyses according to open or stringent criteria were performed. The open joint meta-analysis (n=2482) included 4 discovery and 8 replication cohorts and the stringent joint meta-analysis (n=1791) 4 discovery and 3 replication cohorts. This joint approach has proved to be more efficient than discovery-replication alone in increasing the statistical power.[11] A gene-based burden test was conducted.

Study Sample

European ancestry patients with a diagnosis of IS according to World Health Organization criteria were selected from the Spanish Stroke Genetics Consortium (GeneStroke) and the International Stroke Genetics Consortium.

Genome-Wide Association Study

After GWAS quality controls and imputation, association analyses with 3-month modified Rankin Scale (mRS) were performed using an additive model and a multivariate linear regression. The common variants between the individual GWAS were joined into weighted z-score meta-analyses for 3-month mRS. All the SNPs with $P<5\times10^{-8}$ were considered statistically significant for a genome-wide approach.

Results

Genome-Wide Association Study

GWAS—Discovery Phase

We meta-analyzed 4 individual GWAS with 1225 individuals and 4480015 SNPs in the discovery phase. In the bivariate analysis (Table II in the online-only Data Supplement), 3-month mRS showed association with age at stroke onset, sex, smoking status, stroke subtype by Trial of Org 10172 in acute stroke treatment,[12] and discharge National Institutes of Health Stroke Scale. The results include 79 variants in 18 independent ($r^2<0{\cdot}001$) genomic regions associated with impaired 3-month mRS at $P<1\times10^{-5}$ (Figure 1; Table III in the online-only Data Supplement), selected for replication. Global genomic inflation was $\lambda=1032$. Best evidence was for 3 SNPs in *PATJ* (Pals1-associated tight junction) on chromosome 1 exceeding $P<5\times10^{-8}$: rs76221407 ($P=1.087\times10^{-8}$, $\beta=0.42$); rs150862264 ($P=1.539\times10^{-8}$, $\beta=0.41$); and rs182008837 ($P=1.825\times10^{-8}$, $\beta=0.40$). The top variant was imputed with $r^2=89.9\%$ and imputation certainty=99.2%.

GWAS—Replication Phase

The SNPs selected for replication were analyzed in 8 cohorts (Table IV in the online-only Data Supplement). The stringent replication meta-analysis with 3 cohorts that strictly fulfill our selection criteria showed 5 SNPs in *PATJ* nominally associated with 3-month mRS ($P<0.05$), including the 3 top SNPs from the discovery (Table V in the online-only Data Supplement). The effect size in the replication is slightly lower than in the discovery, indicating widespread consistency of results among the cohorts.

GWAS—Joint

A stringent joint meta-analysis was performed with the 4 discovery and the 3 stringent replication cohorts (1791 individuals). The analysis revealed strong genetic association between 18 low-frequency SNPs in *PATJ* and worse 3-month mRS (Table). The most striking SNP was rs76221407 ($P=1.72\times10^{-9}$, $\beta=0.40$), as shown in the forest plot (Figure IV in the online-only Data Supplement), driven by a variant with low frequency (3%) in our European ancestry cohorts (which is consistent with 1 kg data). Figure 2 shows the percentage of patients per group of 3-month mRS depending on the presence of the risk allele.

An open joint meta-analysis was performed with all 12 cohorts (2482 individuals) independently of meeting criteria for stringent analysis. Results revealed less significant *P* values than the stringent analysis (rs76221407, $P=1.3\times10^{-8}$, $\beta=0.37$; Figure 3; Table VI and Figure V in the online-only Data Supplement). The percentage of the phenotypic variation in 3-month mRS accounted by the lead SNP is 0.27% in the whole sample (12 cohorts).

The same analyses through an ordinal regression showed loss of statistical power (stringent, $P=3.60\times10^{-5}$; open,

Novelty and Significance

What Is Known?

- Disability because of stroke has a significant impact on public health as it affects the quality of life of both—the patients and the caregivers.
- Irrespective of clinical factors, functional recovery after ischemic stroke (IS) varies widely among individuals.
- Variability in functional outcome has been attributed, in part, to genetic factors, but to date, no locus has been consistently associated with stroke outcome.

What New Information Does This Article Contribute?

- Genetic variants in *PATJ* gene are associated with IS functional outcome at 3 months.
- Association of *PATJ* gene variants with IS functional outcome was discovered through a meta-analysis of genome-wide association studies and was validated in a multiple-cohort replication study.

Cerebrovascular disease is the leading cause of adult disability. Mid-term functional recovery after stroke varies significantly among individuals, independent of infarct size, stroke subtype, vascular risk factors, or clinical status after acute treatment. Identifying genetic factors that contribute to this variability requires a hypothesis-free, comprehensive genotyping approach, such as genome-wide association studies, as well as accurate phenotyping to identify confounding factors that may obscure the genetic effects. Using a restrictive inclusion criteria to obtain a less heterogeneous patient population, in a multi-cohort genome-wide association studies we found that *PATJ* gene variants were associated with functional outcomes in patients with IS. The accumulation of risk alleles in the *PATJ* gene was associated with a worse functional outcome at 3-month after IS. This evidence for a genetic contribution to mid-term stroke prognosis provides a new platform for understanding the mechanisms that determine functional recovery after stroke, and may also help in better prediction of functional outcomes after IS.

Nonstandard Abbreviations and Acronyms

GWAS	genome-wide association study
IS	ischemic stroke
mRS	modified Rankin Scale
SNPs	single nucleotide polymorphisms

P=4.33×10^{-5}). Evidence of the robustness of the genetic association presented in this work is clearly shown in Figure 3, where the top SNP has a consistent effect direction in 11 out of 12 cohorts.

Gene-Based Study

The gene-based association test revealed the *PATJ* gene as significantly associated with 3-month mRS. The 4.48 million genetic variants from the discovery GWAS were clustered in 23 972 genes, of which the only significant gene was *PATJ* (P=1.99×10^{-6}) (Table VII in the online-only Data Supplement). Significance threshold was set at P<0.05/23 972=2.086×10^{-6}.

Discussion

We report the first genetic findings in IS outcome using a GWAS. Our results show a novel association between low-frequency genetic variants in *PATJ* gene and worse IS functional outcome measured with 3-month mRS. Genetic studies on IS outcome had previously focused on candidate loci (see Lindgren and Maguire).[7] However, findings had shown contradictory results and failed in consistent replications.[8] We performed a meta-analysis of 12 independent cohorts within the International Stroke Genetics Consortium, applying open and stringent criteria. Several low-frequency variants in *PATJ* were significantly associated with worse functional outcome at 3 months. The lead variant, rs76221407, presents a consistent effect direction in 11 of 12 cohorts, providing convincing evidence of its robust genetic association with stroke outcome. The SNP is located in an intronic region of *PATJ* and shows linkage disequilibrium with 17 other variants found through the stringent joint meta-analysis (r^2>0.3, in 1000 Genomes Project for European ancestry individuals). The fact that the variants are intronic may suggest that its effect on protein synthesis is carried out through the regulation of gene expression, similar to other common variants identified by GWAS that are linked to diseases by their modulation of the activity of DNA regulatory elements.[13] No previously established risk locus for stroke[10] has been related to stroke outcome in this work. Considering our restrictive inclusion criteria, which widely differ from the case/control studies, it is reasonable that we do not find overlaps in the top SNPs. None of our significant variants have been associated with another phenotype yet, although other *PATJ* polymorphisms have been related to sleep disturbance[14] and obesity-related traits.[15]

The results show a positive effect (β=0.4) for the G allele of rs76221407, indicating that an increase of 0.4 points in the mRS score is attributed to each copy of G allele (GG>GA>AA). This means that G allele is related to poor functional outcome at 3-month. The results from the gene-based test also revealed significance for this locus (P=1.99×10^{-6}) pointing out *PATJ* as a hot region of accumulated contributing variants. Although our *PATJ* SNPs are low frequency (≈3%), the power of the association is strong enough to persist in the replication, evidencing the consistency of the results presented and the suitability of this gene as a therapeutic target.

PATJ, also known as INADL (inactivation-no-afterpotential D-like), localized at tight junctions and at the apical membrane of epithelial cells, encodes a protein with 7 PDZ domains, interaction modules that regulate multiple biological processes like ion channel signaling and transport.

To study the genetic component of a complex trait as IS functional outcome, it is key to be precise in characterizing the phenotype. The exclusion of posterior and lacunar strokes was considered necessary because these locations show a poor correlation between infarct size and clinical symptoms, and thus functional outcome.[16] A small lesion can be asymptomatic or show very severe symptoms with great disability depending on a variation of just few millimeters in its location. In these cases, recovery processes and tissue regeneration mechanisms could be masked by this random location effect.

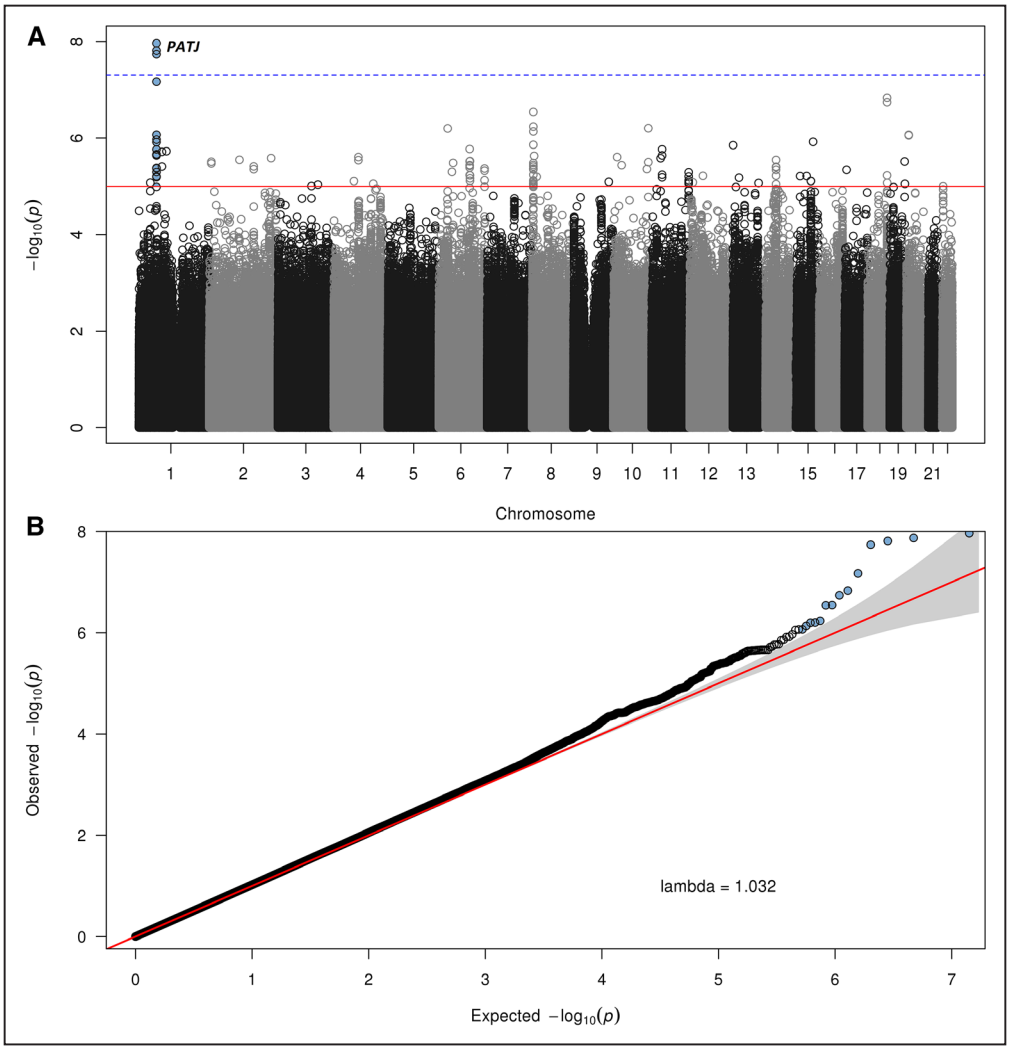

Figure 1. Manhattan plot and Q-Q plot of the discovery results. Association testing was performed using a linear regression model adjusted for the first 2 PCs, sex, age, smoking, stroke subtype, and discharge National Institutes of Health Stroke Scale (NIHSS). The red line shows $P=1\times10^{-5}$ and the blue line the genome-wide association study (GWAS) significance threshold ($P=5\times10^{-8}$; **A**). The *x*-axis is the expected $-\log_{10} P$ under the null hypothesis and lambda is the observed median χ^2 test statistic divided by the median expected χ^2 test statistic under the null hypothesis (**B**).

Minor strokes (initial National Institutes of Health Stroke Scale ≤4) and individuals with a dependent status previous to the stroke event (mRS score of >2) were also excluded. This was done to permit the analyses of an equivalent recovery process, without taking into consideration the degree of disability before stroke or those patients with only minimal damage to be recovered. In both cases, it would be difficult to evaluate the significance of recovery at 3-month. The achievement of a highly homogeneous sample was one of the main priorities during the study design. This led to the performance of 2 types of joint meta-analyses. The more permissive inclusion criteria of the open analysis led to a larger sample size (1.5× greater) but less significant results compared with the stringent analysis. The reduction of phenotypic heterogeneity by properly defining the study cohort increases statistical power.[17] While the phenotypic homogeneity of the sample is the main strength of our study, it also limited the sample size, as very few cohorts worldwide have the complete data needed for the stringent analysis[8] and this may prevent the discovery of other loci. However, our work demonstrates the value of prioritizing homogeneity and phenotyping accuracy over a larger sample size. The mRS, the most widely used scale in stroke patients to assess functional outcome, has only 7 categories but it offers the advantages of being easy to apply and having good inter-observer reproducibility.[18] Analyzing the mRS score as a continuous instead of an ordinal value,[19] according to Rhemtulla et al,[20] is the preferable choice for ordinal data with >4 categories in contrast to robust categorical methodology, and the statistical power is improved.

This project generated extensive genotyping and phenotyping of individual-level data that can help to disentangle the genetic architecture of the stroke recovery process. The use of whole exome or genome sequencing would provide information about rare variants, which may account for a greater proportion of the stroke outcome's genetic component than common variants. Additional functional studies are warranted to establish whether *PATJ* can reveal biological pathways that could be novel therapeutic targets to improve post-IS rehabilitation strategies.

Table. Intronic Variants in *PATJ* Associated With 3-Mo mRS at $P<5\times10^{-8}$ in the Stringent Joint Analysis

SNP	Position	Gene	SNP Type	MA	MAF	β	β (80% power)	SE	*P* Value
rs76221407	1:62131826	*PATJ*	intronic	G	0.03	0.40	0.44	0.07	1.72×10^{-9}
rs150862264	1:62132045	*PATJ*	intronic	C	0.03	0.40	0.44	0.07	1.76×10^{-9}
rs182008837	1:62107102	*PATJ*	intronic	C	0.03	0.39	0.43	0.07	2.93×10^{-9}
rs117335978	1:62140649	*PATJ*	intronic	T	0.02	0.46	0.54	0.08	1.96×10^{-8}
rs137999692	1:62092932	*PATJ*	intronic	A	0.03	0.36	0.43	0.07	2.98×10^{-8}
rs75717958	1:62141064	*PATJ*	intronic	A	0.02	0.47	0.55	0.08	3.71×10^{-8}
rs7546744	1:62141462	*PATJ*	intronic	A	0.02	0.47	0.55	0.08	3.71×10^{-8}
rs7513982	1:62141944	*PATJ*	intronic	C	0.02	0.47	0.55	0.08	3.72×10^{-8}
rs17123133	1:62142178	*PATJ*	intronic	A	0.02	0.47	0.55	0.08	3.72×10^{-8}
rs7514107	1:62142096	*PATJ*	intronic	C	0.02	0.47	0.55	0.08	3.72×10^{-8}
rs141479296	1:62140561	*PATJ*	intronic	G	0.02	0.47	0.55	0.08	3.77×10^{-8}
rs11805802	1:62142912	*PATJ*	intronic	C	0.02	0.47	0.55	0.08	3.78×10^{-8}
rs10157504	1:62140248	*PATJ*	intronic	G	0.02	0.47	0.55	0.08	3.80×10^{-8}
rs74469018	1:62144275	*PATJ*	intronic	T	0.02	0.47	0.56	0.08	3.86×10^{-8}
rs77007585	1:62144350	*PATJ*	intronic	A	0.02	0.47	0.56	0.08	3.88×10^{-8}
rs11806656	1:62144566	*PATJ*	intronic	C	0.02	0.47	0.56	0.08	3.88×10^{-8}
rs7542598	1:62145477	*PATJ*	intronic	T	0.02	0.47	0.56	0.08	3.95×10^{-8}
rs118168181	1:62141702	*PATJ*	intronic	A	0.02	0.47	0.56	0.09	3.98×10^{-8}

β, β-value for the association; β (80% power), β that could be detected as statistically significant under 80% power; Position, chromosome: genomic coordinates according to human genome reference version 19 (build GRCh38/hg38). MA indicates minor allele; MAF, minor allele frequency; and SNP, single nucleotide polymorphism.

Sources of Funding

Genetic contribution to functional outcome and disability after stroke (GODS) project, Fundació Marató-TV3 Grant 2011 (76/C/2011), Recercaixa'13; Generación Project, Instituto de Salud Carlos III; GENISIS (Genetics of Early Neurological InStability After Ischemic Stroke) project, National Institutes of Health (NIH); National Institute of Neurological Disorders and Stroke Stroke Genetics Network (SiGN) Project, NIH. See information about funding for each cohort in the online-only Data Supplement (p 20).

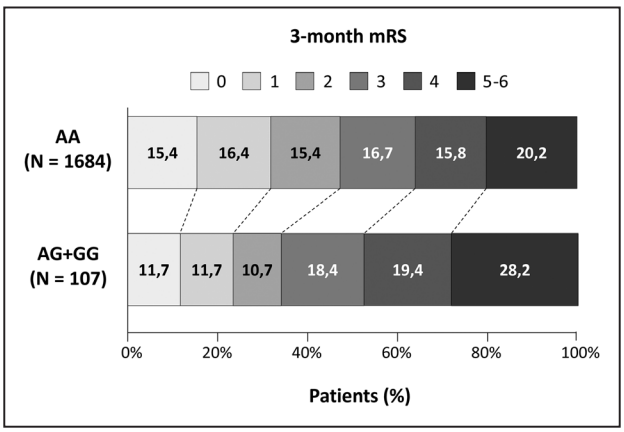

Figure 2. Distribution of 3-mo mRS score in the stringent joint-analysis. *Joining in the same category the mRS values 5–6 and in the same group the G allele carriers (heterozygous and homozygous) is uniquely suited for facilitating the visualization of this figure, although the analyses are done without joining these categories.

Disclosures

None.

Appendix

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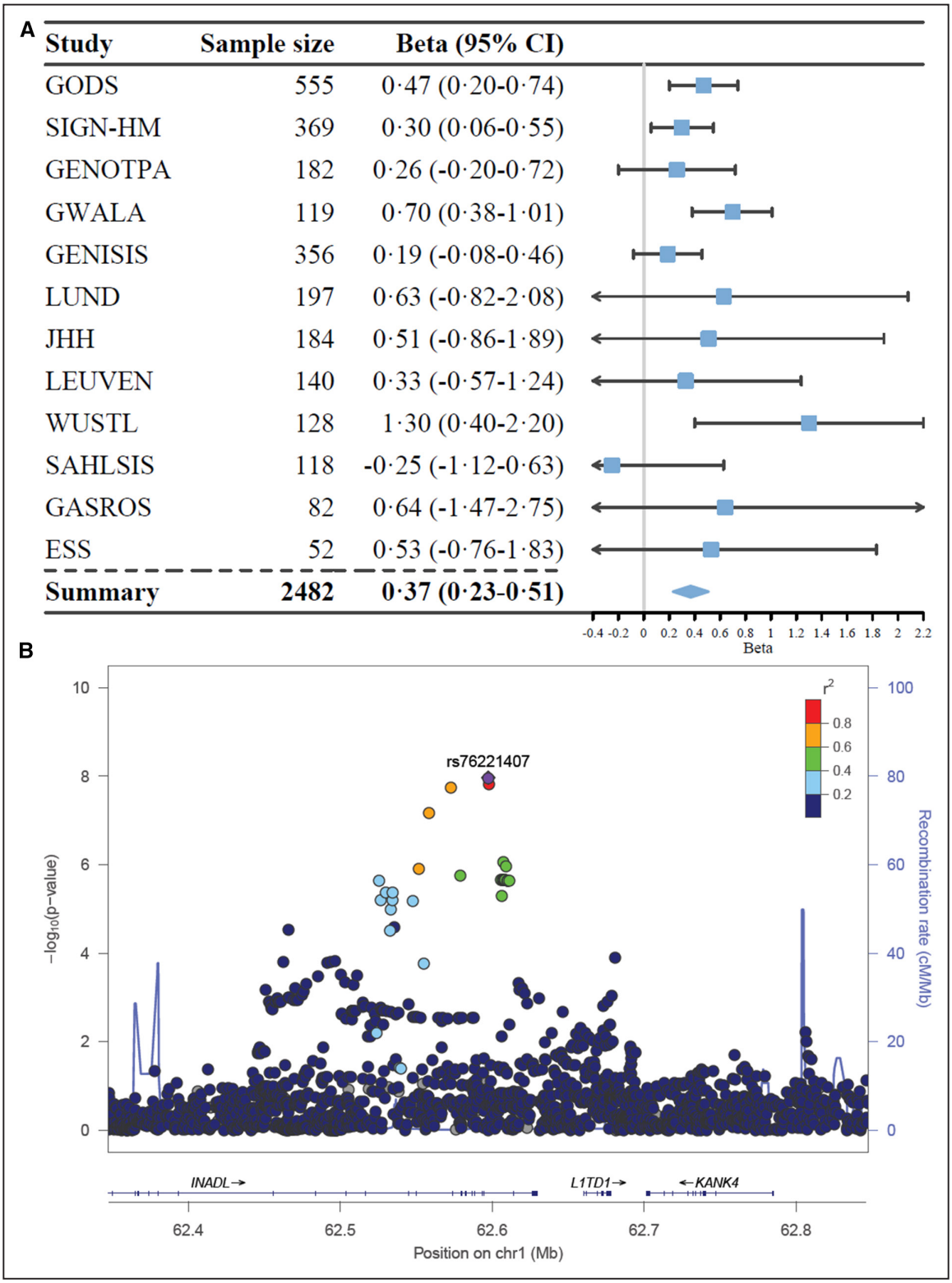

Figure 3. Forest plot and regional association plot for rs76221407 in the open joint-analysis. Plot of the effect size of the association with 3-mo mRS across the 12 cohorts (open joint-analysis; **A**). Association of rs76221407 and other single nucleotide polymorphisms (SNPs) in the region was plotted with $-\log_{10} P$ values (left *y*-axis), the estimated local recombination rate in blue (right *y*-axis). The signals are distinguished by color and shape. Linkage disequilibrium (r^2) of nearby SNPs is shown by color gradient (**B**). chr1 indicates chromosome 1; CI, confidence interval; cM, centimorgan; INADL, inactivation-no-afterpotential D-like (*PATJ* gene); KANK4, KN motif and ankyrin repeat domains 4; L1TD1, LINE1 type transposase domain-containing 1; and Mb, megabase.

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