Validation of a clinical-genetics score to predict hemorrhagic transformations after rt-PA.

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ABSTRACT:

Aim

We aimed to validate the Genot-PA score, a clinical-genetic logistic regression score that stratifies the thrombolytic therapy safety in a new cohort of stroke patients.

Methods

1482 rt-PA treated stroke patients were enrolled in Spain and Finland from 2003 to 2016. Cohorts were analyzed based on ethnicity and therapy: Spanish patients treated with intravenous rt-PA within 4.5 hours of onset (cohort A and B), or rt-PA in combination with mechanical thrombectomy within 6 hours of onset (cohort C); and Finnish participants treated with intravenous rt-PA within 4.5 hours of onset (cohort D). Genot-PA score was calculated, hemorrhagic transformation (HT) and parenchymal hematoma (PH) risk were determined for each score stratum.

Results

Genot-PA score was tested in 1324 (Cohort A, n=726; B, n=334; C, n=54; and D, n=210) patients who had enough information to complete the score. 213 (16.1%) participants developed HT and 85 (6.4%) PH. In cohorts A, B and D the HT occurrence was predicted by the score (p-value: $2.02x10^{-6}$; p-value=0.023; p-value=0.033); PH prediction was associated in cohorts A, B and C (p-value= 0.012; p-value=0.034; p-value=$5.32x10^{-4}$). Increased frequency of PH events from the lowest to the highest risk group was found (A: 4-15.7%; B:1.5-18.2%; C: 0-100%). The best odds-ratio for PH prediction in the highest risk group was obtained in Cohort A (OR: 5.16; 95%CI: 1.46-18.08. p-value=0.009)

Conclusions

The Genot-PA score predicts HT in stroke patients treated with intravenous rt-PA. Moreover, by an exploratory study, the score was associated with PH risk in mechanical thrombectomy treated patients.

INTRODUCTION

Intravenous tissue-plasminogen activator (rt-PA) is the first-line treatment for acute ischemic stroke (AIS). Despite its benefits, only15.6% of patients are treated with rt-PA[1] due to the narrow therapeutic time-window[2]and side effects such as hemorrhagic transformation (HT)[3-5] which influence patients' outcome[6]. Mechanical thrombectomy has demonstrated its efficacy in patients with large vessel occlusion; however, according to current guidelines eligible patients for intravenous rt-PA should receive this drug even if other treatments are considered[7].

Individualizing thrombolytic therapy is a challenge; clinical predictors[8], plasma biomarkers[9-11], imaging parameters[12] and risk models[13, 14] of post-thrombolytic HT were described. Two single nucleotide polymorphisms (SNPs), rs669 in Alpha-2-macroglobulin (*A2M)* and rs1801020 in Coagulation Factor XII (*FXII)*, were associated with rt-PA safety[15]. Additionally, a logistic regression-based score (Genot-PA score) generated with medical history of atrial fibrillation (AF), admission diastolic blood pressure, baseline National Institutes of Health Stroke Score (NIHSS)[16], onset-to-treatment time and the presence of rs669 and rs1801020 risk alleles, predicted HT in the Spanish population[15]. The score stratified the HT risk into four groups (G0-G3), where G0 represented the lowest risk and G3 the highest risk group[15]. The Genot-PA score was the first study that combined genetics and clinical variables to detect patients at high risk of a hemorrhagic event.

We aimed to validate the Genot-PA score in a new cohort of rt-PA treated AIS patients to determine its predictive capability. Furthermore, as an exploratory aim, we tested the predictive value of the score in a cohort of patients who received thrombolysis and mechanical thrombectomy.

METHODS:

Study Population

Our target group was Caucasian patients suffering from an acute ischemic stroke admitted to an emergency room treated with intravenous (IV) rt-PA alone or in combination with mechanical thrombectomy. IV rt-PA was administered in a standard dose of 0.9 mg/kg dose (10% bolus, 90% continuous 1-hour infusion), initiated within 4.5 hours after symptom onset. Endovascular treatment decision was considered according to the REVASCAT trial eligibility criteria[17].

AIS patients treated with rt-PA were collected. We excluded patients with remote parenchymal hematoma and without HT occurrence data. Cohort A (n=726) and B (n=334) consisted of ischemic stroke patients treated with intravenous thrombolysis in Spanish hospitals (Table1) between 2003 and 2016; overlapping with previous participants, where the score was tested previously, was avoided. Onset-to-treatment time was not available in cohort B, onset-to-door (OTD) time for calculating the score was used instead. Cohort C (n=36) was formed by AIS patients with eligibility for endovascular therapy admitted to Vall d'Hebron University Hospital from 2012 to 2015. Cohort D (n=210) was part of the Helsinki 2000 Ischemic Stroke Genetics Study, all ischemic stroke cases with positive neuroimaging for the presence of a new-infarction relevant to the symptoms were recruited at the Helsinki University Central Hospital, which is the only neurological emergency unit for a population of 1.5 million inhabitants[18]. Patients treated exclusively with IV rt-PA were selected in this cohort.

Clinical Protocol

A detailed history of vascular risk factors and current medication was obtained from each patient. On admission, clinical examination and stroke severity were assessed with

the National Institutes of Health Stroke Score (NIHSS)[16]. Participants who underwent endovascular therapy were registered. Presence of HT was excluded by CT scan prior rt-PA administration. HT was evaluated in a follow-up CT scan performed 24 hours after symptom onset or whenever a neurological worsening was detected, defined as an increase of ≥4 points on NIHSS score. Neuroradiologists or neurologists evaluated the CT scans at each center.

Endovascular treatment decision was considered in selected AIS patients with persistent arterial occlusion determined by CTA or MRA after 30 minutes of rt-PA therapy. Eligible patients for mechanical thrombectomy consisted of: AIS patients with carotid artery occlusion at T or M1 vessels; an Alberta Stroke Program Early Computed Tomography Score (ASPECTS) higher than 6 on CT scan; pre-stroke functional disability score using the modified Rankin scale of 1 or less; and baseline NIHSS score of at least 6 points.

HT was radiologically classified according to the ECASS criteria[19]. Hemorrhagic infarct (HI) was defined as small or confluent petechiae along the periphery of the infarct without space-occupying effect (HI1 and HI2). Parenchymal hematoma (PH) was established as a bleeding in less than 30 percent of the infarcted area with mild space-occupying effect (PH1) or hematoma over 30 percent of this area and a significant mass effect (PH2). Symptomatic Intracerebral Hemorrhage (sICH) was defined according to the ECASS-II criteria[20] as an increase of ≥4 points in the NIHSS score due to hemorrhage on CT brain scan within the first 24-36 hours after thrombolytic therapy. The radiological, clinical and genetic evaluations were blinded to each other.

Standard protocol approvals, registrations, and patient consents

The study was reviewed and approved by the appropriate Ethics Committee of each center (Hospital Vall d'Hebron Clinical Research Ethics Committee Reference [IFC-ALT-2011-01]), and all patients or relatives gave informed written consent.

Genetic Analysis

1482 stroke patients were collected. Genotyping of 1210 participants were conducted using commercial panels of SNPs as Human Core Exome, Human Omni Quad 5 M from Illumina (San Diego, CA, SA), and Axiom Biobank from Affymetrix (Santa Clara, CA, USA) platforms. Additionally, direct genotyping for *A2M* rs669 and *F12* rs1801020 SNPs were performed in 272 strokes from cohort A with Sequenom™ iPLEX® technology at the Spanish National Genotyping Centre (Santiago de Compostela, Spain).

Prior to the association analysis, all the samples (cohort A – D) genotyped with commercial panels underwent a rigorous quality control with PLINK v 1.7. Samples cleaning process included sex mismatch, duplicate or kinship among the participants (Pihat >0.18), missing filter (95%) and batch effects. Samples were projected onto the HapMap3 data using principal component analysis to confirm European-ancestry[21]. High-quality genotype call rates (>95%), minor allele frequency (MAF 0.01), duplicate errors, and Hardy Weinberg equilibrium (p $1x10^{-4}$) were used. Due to a call rate below 95% of *F12* rs1801020 in the cohort genotyped in Axiom Biobank array, imputation was performed in 110 samples. After estimating the phased haplotypes, imputation was performed with Impute2 v2.3.0[22] and 1000 genome data (March 2012 release). Post-imputation quality controls included info >0.5 and MAF >0.01 [23]. Besides, 99.99% concordance was found between a random selection of 58 paired samples sequenced by genome-wide platforms and PCR - Sanger technique.

We assigned the 272 sequenom approach samples from cohort A in 96 well plates. Hapmap samples (NA10830, NA10831, and NA12147) were included as a quality control for validating genotype accuracy, Hardy Weinberg equilibrium (p 0.001) and a mean call rate of 95% was employed. Minor allele frequencies were compared with the reference population groups from 1000 Genome Project: IBS, Iberian in Spain; and FIN, Finnish in Finland.

Statistical Analysis

Statistical Analyses were performed using SPSS statistical package, version 17.0 (IBM, Chicago, US). Univariate analysis for cases-controls was evaluated by $\chi 2$ for categorical variables. T-test or Mann-Whitney U was used for continuous variables. The Genot-PA score was created based on logistic regression beta coefficients[15] and tested with the previously described formula[15]: Score=0.053 x baseline NIHSS + 0.534 x AF + 0.039xOTT (10 min) + 0.0149 x DBP (10 mm Hg) + 0.711 x rs18010120 *F12* (C allele) + 0.784 x rs669 *A2M* (A allele). Risk categories from the lowest risk group G0 to the highest group G3 (G0: lower than 3.95 points; G1:3.95-5.10 points; G2:5.10-6.10 points; G3: higher than 6.10 points) were stablished by the mathematic algorithm Chi-square Automatic Interaction Detector (CHAID) algorithm as detailed before[15]. We can better explain how to calculate the score with a clinical case; for example: A patient is evaluated in the emergency room and registered baseline NIHSS = 13, presence of AF, OTT = 170 minutes, DBP = 85 mmHg and the absence of any of the risk alleles for rs1801020 and rs669. To estimate the HT risk, the Genot-PA score is calculated using: 0.053 x 13 (NIHSS) + 0.534 x 1 (AF) + 0.039 x 17 (OTT) + 0.0149 x 8.5 (DBP) + 0.711 x 0 (*F12* C allele) + 0.784 x 0 (*A2M* A allele) = 3.15, identifying this patient in the lowest risk group G0. However, under the same clinical presentation but the

presence of 2 risk alleles in both genotyped genes, the score generated is 6.14, leading to the highest group G3.

The area under the receiver operating characteristic curve (AUC-ROC) was assessed. Sensitivity and specificity were calculated through MedCalc software. (https://www.medcalc.org/calc/diagnostic_test.php).

Data availability

Anonymized data will be shared by request from any qualified investigator, only for purposes of replicating procedures and results.

RESULTS

Among 1482 rt-PA treated strokes, 8 AIS without HT report and 22 remote parenchymal hematomas were excluded. 68 participants did not pass the quality controls previously described. Moreover, *F12* and *A2M* variants were not successfully genotyped or imputed in 9 participants; 51 participants did not fulfill all the clinical predictors needed for the score and after clean-up 1324 participants were included in the study.

Briefly, in the whole study 55.5% (735) participants were men with a median age of 74 years. The prevalence of all HT was 16.1% (213): HI1 4.3 % (57), HI2 5.4 % (71), PH1 3.5% (46), PH2 2.9% (39). Meanwhile the prevalence for Symptomatic Intracerebral Hemorrhage (sICH) was 1.7% (23) [Table 2]. Minor allele frequencies (MAF) did not differ significantly from their respective reference population (1000 Genome Project).

Cohort A (n=726) showed a prevalence of global HT of 15% (109) with an occurrence of PH of 7.1% (51) and moderate to severe strokes (median baseline NIHSS=12). Cohort B (n=334) presented a global HT and PH prevalence of 9.3% (31) and 4.5% (15) respectively, with moderate to severe AIS (median baseline NIHSS=11); an OTD median time of 90 minutes was used instead of OTT for calculating the score. Cohort C (n=54), which included patients who received rt-PA in combination with mechanical thrombectomy, reported 37% (20) hemorrhagic events, 4.5% (3) of PH and severe strokes (median baseline NIHSS=18). Meanwhile, cohort D (n=210) showed 25.2% (53) HT, 7.6% (16) PH and mild strokes (baseline NIHSS=7). [Table 2]

HTstudy

Analysis of the Genot-PA score components found high baseline NIHSS associated with the occurrence of HT in the three cohorts (cohort A, B, and D). AF was statistically significant in cohort A and D (p-value<0.05). Other clinical predictors of the score such as DBP, OTT or OTD, did not show significant association in our study [Table 3]. The variant rs669 was not significantly associated with HT occurrence in the dominant-recessive nor in the allelic model in any cohorts, whereas rs1801020 was significantly associated only in cohort D in the dominant-recessive model with HT (CC=31.1%; CT+TT=19.2% p-value=0.047. OR; 1.90; 95% CI: 1.00-3.60) [Table 4 and Table 5].

Increasing rates of HT in the four risk groups of each cohort were found. Global HT risk was predicted by Genot-PA score in the cohorts treated with intravenous rt-PA exclusively (A, B, D) (cohort A: 9.1% (G0) to 42.2% (G3), p-value=2.02 $x10^{-6}$; cohort B: 3% (G0) to 18.2% (G3), p-value=0.023; cohort C: 25% (G0) to 100% (G3), p-value=0.51; cohort D: 25.7% (G0) to 33.3% (G3), p-value=0.033). However, hemorrhagic events in the Finnish cohort (D) were more prevalent in the lowest risk

groups (G0-G1) compared to the other cohorts. [Figure 1] [Table 3]. Analysis of sICH was not performed, due to the small number of cases in each cohort.

Furthermore, the mean value of the score in the hemorrhagic event group was higher compared with the non-HT group in all the cohorts (cohort A: 5.14±0.92 HT vs. 4.80±0.80 non-HT, p-value= 0.001; cohort B: 5.09±0.69 HT vs. 4.61±0.85 non-HT, p-value= 0.002; cohort C: 1.40±0.75 HT vs. 1.17±0.72 non-HT, p-value=0.28; cohort D: 4.67±0.89 HT vs. 4.28±0.88 non-HT, p-value= 0.006).

Overall, the performance of the score over the HT risk in all cohorts were comparable to the previously reported that was AUC ROC=0.72, 95% CI: 0.66-0.77. (Cohort A: AUCROC = 0.68, 95% CI: 0.62-0.74, Cohort B: AUCROC = 0.74, 95% CI: 0.65-0.83, Cohort C: AUCROC = 0.66, 95% CI: 0.46-0.82, cohort D: AUCROC = 0.74, 95% CI: 0.67-0.82). Risk group G3 (cut-off 6.10) showed the highest risk of hemorrhagic events compared to G0 groups, with cohort A having the best OR (OR: 7.31 (95% CI: 2.95-18.07, p-value=3.22 $x10^{-6}$) with HT prediction specificity of 77.59% (95% CI: 68.91-84.81), whereas the sensitivity was 67.86% (95% CI:47.65-84.12) [Table 6].

PH study

PH phenotype was associated with stroke severity (Baseline NIHSS) in cohort A, and atrial fibrillation (AF) in cohort B (p-value<0.05), other predictors of the score were not significantly associated [Table 3].

Genetic variant studies revealed that rs669 was significantly associated with PH occurrence in the dominant-recessive and allelic model only in cohort D, nevertheless the SNP exhibited a protection effect (AA=1.2%; AG+GG=11.6% p-value=0.006. OR: 0.09; 95% CI: 0.01-0.73) (A=5.2%; G=11.2% p-value 0.02. OR: 0.43; 95% CI:0.21-

189 0.90). Other associations for rs669 and rs1801020 were not found [Table 4 and Table
190 5].

191 The PH occurrence was predicted by the score only in the Spanish cohorts (A, B, C)
192 where increasing number of events for each stratum were found. On the other hand, the
193 Finnish cohort (D) exhibited an opposite pattern of PH identification (cohort A 4% (G0)
194 to 17.8% (G3), p-value= 0.012; cohort B 1.5% (G0) to 18.2% (G3), p-value=0.034;
195 cohort C 0% (G0) to 100% (G3), p-value=$5.32x10^{-4}$; cohort D (G0) 10% to 0% (G3), p-
196 value=0.64). [Figure 1] [Table 3].

197 The mean value of the score in the case group was significantly higher compared with
198 non-PH group in 2 cohorts (A, B). Moreover, nominal significance association is
199 reported in cohort C, (cohort A: 5.22±0.91 PH patients vs. 4.83±0.81 non-PH patients,
200 p-value= 0.001; cohort B: 5.21±0.81 PH patients vs. 4.63±0.84 non-PH patients, p-
201 value=0.010 ; cohort C: 2±1 PH patients vs. 1.2±0.70 non-PH patients, p-value=0.07;
202 cohort D: 4.29±0.66 PH patients vs. 4.39±0.92 non-PH patients, p-value=0.67).

203 The performance of the score over the PH risk was Cohort A: AUCROC = 0.67, 95%
204 CI: 0.59-0.75, Cohort B: AUCROC = 0.72, 95% CI: 0.60-0.84, Cohort C: AUCROC =
205 0.79, 95% CI: 0-1, cohort D: AUCROC = 0.75, 95% CI: 0.63-0.83). The risk group G3
206 (cut-off 6.10) showed the higher risk of PH events compared to G0 group, except for the
207 Finnish cohort. The best OR was detected in cohort A (OR: 5.16 (95% CI: 1.46-18.08,
208 p-value=0.009) with PH prediction specificity of 71.97% (95% CI: 63.49-79.43),
209 whereas the sensitivity was 66.67% (95% CI:34.89-90.08) [Table 6].

210 **DISCUSSION**

211 In the present study, we were able to validate the Genot-PA score in new cohorts treated
212 with intravenous rt-PA alone although the use of onset-to-door as one of the

components of the risk scale. Moreover, the parenchymal hematoma occurrence was predicted by the score in IV thrombolysis use alone or in addition to mechanical thrombectomy cohorts by an exploratory study.

Spanish cohorts treated exclusively with intravenous thrombolysis (A, B) exhibited a performance of the score over global HT risk like the previously reported [15]. Even though cohort B exhibited lower rates of HT than cohort A, the highest risk group still englobed the biggest percentage of hemorrhagic events. Most importantly, parenchymal hematoma occurrence in the highest risk group (G3) was similar in cohort A and B.

Higher rates of hemorrhagic events in the risk groups G0, G1 compared to Spanish cohorts who underwent the same thrombolytic therapy were found in cohort D. Furthermore, the Finnish group (D) was formed by younger and less severe rt-PA treated strokes. A previous study identified different baseline characteristics involved in HT risk in the Finnish population, such as blood sugar levels or radiological parameters[14]; we hypothesized that these parameters could generate a big influence over the hemorrhagic risk in the Finnish cohort. Surprisingly, the genetic variant of *A2M* (rs669) acted as a protector for PH in this cohort, contrary to that described in the original score[15]. Previous studies reported a bottlenecked effect in this population [24],[25] where the genetic architecture differs from the rest of Europeans and could modulate the effect of the SNPs studied in the Genot-PA score.

A small cohort of 54 patients treated with IV thrombolysis in combination with thrombectomy (cohort C) was included in this study. The worrisome percentage of global HT in C cohort was explained by the accumulation of HI subtype events. Previous studies linked HI as a marker of successful recanalization[26] which is achievable after the use of endovascular devices in selected patients. On the other hand,

the prevalence of PH remains stable and comparable with the clinical trial performed in the same population[17]. Moreover, the prediction of PH showed a complete separation between cases and controls; nevertheless, the score should be analyzed in a larger cohort.

After reviewing risk factors for the occurrence of HT after IV thrombolysis, stroke severity measured by NIH Stroke Scale appeared to be a strong predictor of hemorrhagic events and its subtype PH, with the greatest risk seen in scores higher than 20[27]. The presence of atrial fibrillation in the cohorts who receive IV rt-PA alone was higher than the original score group, AF leads to greater hypoperfusion, generating damage to the integrity of blood vessels and increasing the risk of intracerebral bleeding [28]. However, we failed to find an association between diastolic blood pressure, time from onset to treatment, *A2M* variant (rs669) and HT which were predictors in the original score cohort and other previous studies[8]. *F12* polymorphisms (rs1801020) was associated in the dominant-recessive model with HT occurrence in cohort D. Recently the contribution of *F12* to blood-brain barrier leakage, intracerebral hemorrhage, edema, and infarct volume was confirmed in a murine thrombotic stroke model treated with rt-PA[29]. Additionally, the inhibition of *F12* in mice reduced the size of hemorrhagic transformations induced by thrombolysis[29].

One of the limitations of our study is the small sample size of the combination therapy cohort who underwent mechanical thrombectomy preceded by IV rt-PA. However, highly selected patients with strict inclusion criteria were selected, that permitted a more homogenous sample of patients in which the therapy outcome is well studied[17]. On the other hand, this kind of participants was excluded in previous risk scores[13], [14],[30],[31].

Another limitation of the study is the use of onset-to-door time in cohort B. OTD, that was not included in the original score could introduce heterogeneity and possible reduce points in the score. The Telestroke network on acute stroke care in Catalonia, the same geographical area from most of the participants, reported the mean OTD of 71 minutes and OTT of 120 min during AIS treatment[32]. A difference of 49 minutes could represent a reduction of 0.191 points in the Genot-PA score (12-7.1*0.039).

Furthermore, as the Genot-PA score is calculated based on the presence of two SNPs, we should consider the genetic variability between populations as polymorphisms could be associated to traits in specific populations[33]. The current study counts with the participation of only one cohort of non-Spanish AIS, which is a limitation and we cannot fully generalize our findings to other groups more than the ones which participate in the study.

Despite advances in endovascular therapies, the procedure requires highly trained staff, technical equipment, and specialized centers[34]. Intravenous rt-PA is still the recommended treatment of AIS[7]. The implementation of predictive scores may aid to identify patients with high or low risk of hemorrhagic complications to help physicians in decision making, avoiding treatment delays, adjust the rt-PA dose [35], initiate mechanical clot removal alone[36] or implement additional support or therapy.

Considering the interaction of the genetic background and clinical data[37], genetics could improve the effectiveness of the scores in order to enhance the risk prediction. This could be particularly important to treat patients out of the window time with low HT risk, the creation of treatments which prevent hemorrhagic events among other circumstances. Nowadays, advances in the molecular diagnosis using point-of-care genotyping devices, such as QuantuMDX Q-POC[38], would provide a fast-accessible

tool with genotyping data in less than 20 minutes that could be performed in the ambulance before arriving at hospital. This short period of time suggests that the use of genetics for the management of therapies during the acute phase of ischemic stroke is possible.

The present study validated the Genot-PA score in new cohorts of stroke patients treated with intravenous thrombolysis. Furthermore, by an exploratory study, the score predicted the most severe HT subtype occurrence, parenchymal hematoma in stroke patients treated with rt-PA and mechanical thrombectomy. In addition, the score should be analyzed in an extended cohort of endovascular treated patients selected by the current guidelines from The American Heart Association and the American Stroke Association as well as in other populations.

APPENDIX 1

Name	Location	Role	Contribution
Caty Carrera MSc	Neurovascular Research Laboratory, Vall d'Hebron Research Institute (VHIR), Universitat Autònoma de Barcelona	Author	analysis or interpretation of data, statistical analysis, drafting the manuscript, accepts responsibility for conduct of research.
Natalia Cullell MSc	Stroke Pharmacogenomics and Genetics, Fundació Docència i Recerca Mútua Terrassa	Author	revision of the manuscript, contribution of patients.
Nuria Torres-Águila MSc	Stroke Pharmacogenomics and Genetics, Fundació Docència i Recerca Mútua Terrassa	Author	revision of the manuscript, contribution of patients.
Elena Muiño MD, MSc	Stroke Pharmacogenomics and Genetics, Fundació	Author	revision of the manuscript,

	Docència i Recerca Mútua Terrassa		contribution of patients.
Alejandro Bustamante MD, PhD	Neurovascular Research Laboratory, Vall d'Hebron Research Institute (VHIR), Universitat Autònoma de Barcelona	Author	revision of the manuscript, interpretation of data, contribution of patients.
Antonio Dávalos MD, PhD	Department of Neuroscience, Hospital Germans Trias i Pujol	Author	revision of the manuscript, interpretation of data, contribution of patients.
Elena López-Cancio MD	Stroke Unit, Hospital Universitario Central de Asturias (HUCA)	Author	revision of the manuscript, interpretation of data, contribution of patients.
Marc Ribó MD, PhD	Stroke Unit, Department of Neurology, Hospital Universitari Valld'Hebron	Author	revision of the manuscript, contribution of patients.
Carlos A. Molina MD, PhD	Stroke Unit, Department of Neurology, Hospital Universitari Valld'Hebron	Author	revision of the manuscript, contribution of patients.
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Carolina Soriano-Tárraga PhD	IMIM-Hospital del Mar	Author	revision of the manuscript, contribution of patients.
Marina Mola-Caminal PhD	IMIM-Hospital del Mar	Author	revision of the manuscript, contribution of

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Jordi Jiménez-Conde MD, PhD	IMIM-Hospital del Mar	Author	revision of the manuscript, interpretation of data, contribution of patients.
Jaume Roquer MD, PhD	IMIM-Hospital del Mar	Author	revision of the manuscript, interpretation of data, contribution of patients.
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Daniel Strbian MD, PhD	Helsinki University Hospital	Author	revision of the manuscript, interpretation of data, contribution of patients.
Joan Montaner MD, PhD	Virgen del Rocío & Macarena Hospitals, IBIS	Author	study concept or design, drafting / revision of the manuscript, interpretation of data, obtaining funding
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299 **APPENDIX 2**

Name	Location	Role	Contribution
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Jonathan González	Stroke Pharmacogenomics and Genetics, Fundació Docència i Recerca Mútua Terrassa	Site Investigator	contribution of patients
Lucía Muñoz	Department of Neuroscience, Hospital Germans Trias i Pujol	Site Investigator	contribution of patients
Elisa Cortijo-	Hospital Clínico	Site	contribution of

García	Universitario, University of Valladolid	Investigator	patients
Rebeca Marín Bueno	Hospital de la Santa Creu i Sant Pau	Site Investigator	contribution of patients
Aki Havulinna	Institute for Molecular Medicine Finland	Site Investigator	contribution of patients
Veikko Salomaa	Institute for Molecular Medicine Finland	Site Investigator	contribution of patients

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TABLES AND FIGURES

Table 1: Spanish University Hospitals participated in the study

Table 2: Demographic data and baseline clinical findings of the cohorts

§onset-to-door. OTT was not was not available, onset-to-door was used instead.

For categorical variables, frequencies as percentage were described.

For continuous variables, median values, interquartile range (IQR) were calculated.

HT: Hemorrhagic Transformation; HI: Hemorrhagic infarct; PH: Parenchymal hematoma; sICH: Symptomatic Intracerebral Hemorrhage; OTT: Time from onset to treatment; DBP: Diastolic blood pressure.

Table 3: Univariate analysis of the clinical components of the Genot-PA score in the cohorts and percentage of Hemorrhagic Transformation per risk group

*p-value <0.05. §onset-to-door. OTT was not was not available, onset-to-door was used instead.

P-value for HT risk by the Genot-PA score in cohort A: 2.02 x10-6; cohort B: 0.023; cohort C: 0.51; cohort D: 0.033.

P-value for PH risk by the Genot-PA score in cohort A: 0.012; cohort B: 0.034; cohort C: 5.32x10-4; cohort D: 0.64.

For categorical variables, $\chi 2$ and frequencies of HT and PH (as percentage) in presence of absence of the clinical predictor were described.

For continuous variables, median values, interquartile range (IQR) and Mann-Whitney p-values for HT and non-HT groups were calculated.

AF: Atrial Fibrillation; HT: Hemorrhagic Transformation; PH: Parenchymal hematoma; OTT: Time from onset to treatment; DBP: Diastolic blood pressure.

Table 4. Development of hemorrhagic transformation and parenchymal hematoma according to the dominant-recessive model

*p-value <0.05

SNP ID, identification number in the dbSNP database; HT: Hemorrhagic transformation; PH: Parenchymal hematoma; MA: Minor allele; RA: Risk allele; OR: Odds Ratio; 95% CI: 95% confidence interval.

Table 5. Development of hemorrhagic transformation and parenchymal hematoma according to the allele model.

*p-value <0.05

OR: Odds Ratio; 95% CI, 95% confidence interval; HT: Hemorrhagic transformation; PH: Parenchymal hematoma.

Table 6. **Sensitivity and specificity for hemorrhagic transformation and parenchymal hematoma in the Genot-PA score risk groups**

§ Sensibility was not calculated due to the lack of cases.

*p-value <0.05

OR: Odds Ratio; 95% CI, 95% confidence interval; HT: Hemorrhagic transformation; PH: Parenchymal hematoma.

Figure 1: Occurrence of hemorrhagic transformation after rt-PA treatment per increasing group of the Genot-PA score.

A) Cohort A; B) Cohort B; C) Cohort C; D) Cohort D. Score groups G0 ≤3.95 points, G1 3.95-5.10 points, G2 5.10-6.10 points, G3 ≥6.10 points. HI: Hemorrhagic infarct; PH: Parenchymal hematoma

Table 1: Spanish University Hospitals participated in the study

1	Hospital Universitario Vall d'Hebron
2	Hospital Germans Trias i Pujol
3	Hospital del Mar
4	Hospital Son Espasas
5	Hospital Clinic
6	Hospital Universitario de Valladolid
7	Hospital Universitario de Albacete
8	Hospital de la Santa Creu i Sant Pau
9	Hospital Universitari Mútua de Terrassa
10	Hospital Virgen del Rocío
11	Hospital Virgen de la Macarena
12	Hospital de Basurto

Table 2. Demographic data and baseline clinical findings of the cohorts

	Cohort A (n=726)	Cohort B (n=334)	Cohort C (n=54)	Cohort D (n=210)	All cohorts (n=1324)
Sex, male (%)	383 (52.8)	195 (58.4)	28 (51.9)	129 (61.4)	735 (55.5)
In-hospital mortality (%)	64 (9.3)	15 (4.5)	7 (13)	0 (0)	86 (6.7)
Atrial fibrillation (%)	225 (31)	109 (32.9)	10 (18.5)	57 (27.1)	401 (30.3)
TOAST (%)					
Cardioembolism	311 (44)	161 (48.2)	23 (42.6)	100 (47.6)	595 (45.6)
Large-artery atherosclerosis	109 (15.4)	52 (15.6)	15 (27.8)	29 (13.8)	205 (15.7)
Small-vessel occlusion	30 (4.2)	25 (7.5)	-	24 (11.4)	79 (6.1)
Other	18 (2.5)	2 (0.6)	3 (5.6)	10 (4.8)	33 (2.5)
Undetermined etiology	239 (33.8)	94 (28.1)	13 (24.1)	47 (22.4)	392 (30.1)
Applied thrombolysis therapy (%)					
Intravenous only	726 (100)	334 (100)	-	210 (100)	1270 (95.9)

Intravenous + Mechanical treatment	-	-	54 (100)	-	54 (4.1)
Endpoint (%)					
HT	109 (15)	31 (9.3)	20 (37)	53 (25.2)	213 (16.1)
HI1	28 (3.9)	7 (2.1)	5 (9.3)	17 (8.1)	57 (4.3)
HI2	30 (4.1)	9 (2.7)	12 (22.2)	20 (9.5)	71 (5.4)
PH1	27 (3.7)	7 (2.1)	1 (1.9)	11 (5.2)	46 (3.5)
PH2	24 (3.3)	8 (2.4)	2 (3.7)	5 (2.4)	39 (2.9)
sICH	15 (2.1)	4 (1.2)	2 (3.7)	2 (1)	23 (1.7)
Age (Years. IQR)	76(66-82)	75 (66-82)	73 (56-81)	65 (57-72)	74 (64-81)
Baseline NIHSS(IQR)	12 (7-19)	11 (6-18)	18 (12-20)	7 (4-14)	11 (6-18)
OTT (min. IQR)	135(100-180)	90 (60-139)§	106 (80-175)	115 (81-165)	120 (85-174)
DBP (mm Hg. IQR)	80(70-88)	77 (68-87)	77 (69-84)	85 (78-93)	80 (70-89)

§onset-to-door. OTT was not was not available, onset-to-door was used instead.
For categorical variables, frequencies as percentage were described. For continuous variables, median values, interquartile range (IQR) were calculated.
HT: Hemorrhagic Transformation; HI: Hemorrhagic infarct; PH: Parenchymal hematoma; sICH: Symptomatic Intracerebral Hemorrhage; OTT: Time from onset to treatment; DBP: Diastolic blood pressure.

Table 3. Univariate analysis of the clinical components of the Genot-PA score in the cohorts and percentage of Hemorrhagic Transformation per risk group.

		Cohort A (n= 726)			Cohort B (n=334)			Cohort C (n=54)			Cohort D (n= 210)		
		Absence	Presence	p	Absence	Presence	P	Absence	Presence	p	Absence	Presence	p
Baseline	HT	11 (6-18)	18 (12-20)	<0.001*	10 (6-17)	19 (15-22)	<0.001*	16 (10-20)	18 (16-21)	0.38	6 (4-11)	12 (9-16)	<0.001*
NIHSS(IQR)	PH	12 (6-18)	18 (11-21)	<0.001*	10 (6-18)	17 (11-22)	0.05	18 (12-20)	16 (16-19)	0.75	7 (4-13)	12 (7-16)	0.09
DBP	HT	80 (70-88)	79 (68-88)	0.83	77 (68-87)	79 (74-85)	0.60	80 (70-85)	73 (67-82)	0.15	85 (78-93)	86 (77-92)	0.62
(mmHg.IQR)	PH	80 (70-88)	78 (68-91)	0.90	77 (68-87)	82 (72-88)	0.50	77 (69-84)	76 (72-88)	0.72	85 (77-93)	86 (80-92)	0.96
OTT	HT	135	135	0.60	85	98	0.43	98	135	0.26	115	115	0.61
(min. IQR)		(104-180)	(100-180)		(60-139) §	(68-133) §		(75-161)	(80-178)		(81-168)	(81-162)	
	PH	135	145	0.37	85	105	0.07	100	157	0.48	115	102	0.25
		(100-180)	(105-182)		(60-135) §	(80-160) §		(80-17|1)	(116-221)		(81-170)	(78-134)	
AF (%)	HT	178 (28.8)	47 (43.1)	0.003*	95 (31.4)	14 (45.2)	0.12	17 (38.6)	3 (30)	0.73	38 (24.2)	19 (35.8)	0.01*
	PH	207 (30.7)	18 (35.3)	0.49	100 (31.3)	9 (60)	0.02*	83 (6.8)	0 (0)	1	53 (27.3)	4 (25)	1
Risk groups													
G0	HT	(%)	9 (9.1)			2 (3)			2 (25)			18 (25.7)	

	PH (%)	4 (4)	1 (1.5)	0 (0)	7 (10)
G1	HT (%)	4 (13.9)	12 (7.5)	9 (36)	15 (16.9)
	PH (%)	19 (5.6)	5 (3.1)	1 (4)	7 (7.9)
G2	HT (%)	34 (14.1)	15 (16)	8 (40)	18 (40)
	PH (%)	20 (8.3)	7 (7.4)	1 (5)	2 (4.4)
G3	HT (%)	19 (42.2)	2 (18.2)	1 (100)	2 (33.3)
	PH (%)	8 (17.8)	2 (18.2)	1 (100)	0 (0)

*p-value <0.05. §onset-to-door. OTT was not was not available, onset-to-door was used instead.
P-value for HT risk by the Genot-PA score in cohort A: 2.02×10^{-6}; cohort B: 0.023; cohort C: 0.51; cohort D: 0.033.
P-value for PH risk by the Genot-PA score in cohort A: 0.012; cohort B: 0.034; cohort C: 5.32×10^{-4}; cohort D: 0.64.
For categorical variables, $\chi 2$ and frequencies of HT and PH (as percentage) in presence of absence of the clinical predictor were described.
For continuous variables, median values, interquartile range (IQR) and Mann-Whitney p-values for HT and non-HT groups were calculated.
AF: Atrial Fibrillation; HT: Hemorrhagic Transformation; PH: Parenchymal hematoma; OTT: Time from onset to treatment; DBP: Diastolic blood pressure.

Table 4. Development of hemorrhagic transformation and parenchymal hematoma according to the dominant-recessive model

						Genotype n (%)			
Gene	SNP ID	MA	RA			AA	AG+GG	OR (95% CI)	p
A2M	rs669	G	A	Cohort A	HT	60 (16)	49 (14)	1.17 (0.78-1.76)	0.46
					PH	30 (8)	21 (6)	1.36 (0.76-2.42)	0.30
				Cohort B	HT	19 (10.9)	12 (7.5)	1.50 (0.7-3.18)	0.30
					PH	10 (5.7)	5 (3.1)	1.86 (0.62-5.58)	0.26
				Cohort C	HT	8 (36.4)	12 (37.5)	0.95 (0.31-2.93)	0.93
					PH	2 (9.1)	1 (3.1)	3.10 (0.26-36.48)	0.56
				Cohort D	HT	19 (23.5)	34 (26.4)	0.86 (0.45-1.63)	0.64
					PH	1 (1.2)	15 (11.6)	0.09 (0.01-0.73)	0.006*
Gene	SNP ID	MA	RA			CC	CT+TT	OR (95% CI)	P
F12	rs1801020	T	C	Cohort A	HT	76 (15.9)	33 (13.4)	1.22 (0.79-1.90)	0.37
					PH	36 (7.5)	15 (6.1)	1.26 (0.67-2.34)	0.47
				Cohort B	HT	19 (8.7)	12 (10.3)	0.83 (0.39-1.77)	0.63

	PH	9 (4.1)	6 (5.2)	0.79 (0.28-2.28)	0.66
Cohort C	HT	13 (36.1)	7 (38.9)	0.88 (0.27-2.85)	0.84
	PH	2 (5.6)	1 (5.6)	1 (0.09-11.82)	1
Cohort D	HT	33 (31.1)	20 (19.2)	1.90 (1.00-3.60)	0.047*
	PH	12 (11.3)	4 (3.8)	3.19 (0.99-10.24)	0.07

*p-value <0.05

SNP ID, identification number in the dbSNP database; HT: Hemorrhagic transformation; PH: Parenchymal hematoma; MA: Minor allele; RA: Risk allele; OR: Odds Ratio; 95% CI: 95% confidence interval.

Table 5. Development of hemorrhagic transformation and parenchymal hematoma according to the allele model.

							Genotype n (%)			
Gene	SNP ID	MA	RA	MAF			A	G	OR (95% CI)	p
A2M	rs669	G	A	28.8	Cohort A	HT	158 (15.3)	60 (14.4)	1.07 (0.78-1.48)	0.66
						PH	77 (7.4)	25 (6)	1.27 (0.79-2.02)	0.32
				27.4	Cohort B	HT	49 (10.1)	13 (7.1)	1.47 (0.77-2.78)	0.23
						PH	24 (4.9)	6 (3.3)	1.54 (0.61-3.82)	0.35
				36.1	Cohort C	HT	27 (39.1)	13 (33.3)	1.29 (0,57-2,93)	0.55
						PH	5 (7.2)	1 (2.6)	2.69 (0.33-26.37)	0.42
				40.2	Cohort D	HT	63 (25.1)	43 (25.4)	0.98 (0.63-1.54)	0.93
						PH	13 (5.2)	19 (11.2)	0.43 (0.21-0.90)	0.02*
Gene	SNP ID	MA	RA				C	T	OR (95% CI)	P
F12	rs1801020	T	C	18.8	Cohort A	HT	183 (15.5)	35 (12.8)	1.25 (0.84-1.84)	0.26
						PH	86 (7.3)	16 (5.9)	1.26 (0.73-2.56)	0.40
				19.3	Cohort B	HT	49 (9.1)	13 (10.1)	0.89 (0.47-1.70)	0.73
						PH	24 (4.5)	6 (4.7)	0.96 (0.38-2.39)	0.92

18.5	Cohort C	HT	32 (36.4)	8 (40)	0.86 (0.32-2.32)	0.76
		PH	5 (5.7)	1 (5)	1.15 (0.12-10.37)	1
27.6	Cohort D	HT	83 (27.3)	23 (19.8)	1,52 (0.90-2-56)	0.12
		PH	27 (8.9)	5 (4.3)	2.16 (0.81-5.76)	0.11

*p-value <0.05

OR: Odds Ratio; 95% CI, 95% confidence interval; HT: Hemorrhagic transformation; PH: Parenchymal hematoma.

Table 6. Sensitivity and specificity for hemorrhagic transformation and parenchymal hematoma in the Genot-PA score risk groups

HT	Cut-off	Presence (%)	OR (95% CI)	p-value	Sensitivity (95% CI)	Specificity (95% CI)
Cohort A	1.95	47 (13.9)	1.61 (0.76-3.42)	0.21	83.93 (71.67-92.38)	23.62 (19.45-28.21)
	2.5	34 (14.1)	1.64 (0.76-3.57)	0.21	79.07 (63.96-89.96)	30.30 (25.13-35.88)
	6.10	19 (42.2)	7.31 (2.95-18.07)	<0.001*	67.86 (47.65-84.12)	77.59 (68.91-84.81)
Cohort B	1.95	12 (7.5)	2.61 (0.57-12.01)	0.24	85.71 (57.19-98.22)	30.33 (24.21-37.02)
	2.5	15 (16)	6.08 (1.34-27.55)	0.009*	88.24 (63.56-98.54)	44.76 (36.44-53.29)
	6.10	2 (18.2)	7.11 (0.89-56.95)	0.095	50 (6.76-93.24)	87.67 (77.88-94.20)
Cohort C	1.95	9 (36)	1.69 (0.28-10.17)	0.69	81.82 (48.22-97.72)	27.27 (10.73-50.22)
	2.5	8 (40)	2 (0.32-12.51)	0.67	80.00 (44.39-97.48)	33.33 (13.34-59.01)
	6.10	1 (100)	-	0.13	33.33 (0.84-90.57)	100 (54.07-100)
Cohort D	1.95	15 (16.9)	0.59 (0.27-1.26)	0.17	45.45 (28.11-63.65)	41.27 (32.58-50.38)
	2.5	18 (40)	1.93 (0.86-4.29)	0.11	50 (32.92-67.08)	65.82 (54.29-76.13)
	6.10	2 (33.3)	1.44 (0.24-8.56)	0.65	100 (1.23-31.70)	92.86 (82.71-98.02)
PH						
Cohort A	1.95	19 (5.6)	1.42 (0.47-4.26)	0.54	82.61 (61.22-95.05)	22.95 (18.98-27.30)

	2.5	20 (8.3)	2.15 (0.72-6.46)	0.16	83.33 (62.62-95.26)	30.06 (25.06-35.45)
	6.10	8 (17.8)	5.16 (1.46-18.08)	0.009*	66.67 (34.89-90.08)	71.97 (63.49-79.43)
Cohort B	1.95	5 (3.1)	2.11 (0.24-18.42)	0.67	83.33 (35.88-99.58)	29.68 (23.71-36.21)
	2.5	7 (7.4)	5.23 (0.63-43.56)	0.14	87.50 (47.35-99.68)	42.76 (34.78-51.03)
	6.10	2 (18.2)	14.44 (1.19-175.9)	0.05	87.84 (78.16-94.29)	66.67 (9.4-99.16)
Cohort C	1.95	0 (0)	-	1	100 (2.5-100)	25 (11.46-43.40)
	2.5	1 (5)	-	1	100 (2.5-100)	29.63 (13.75-50.18)
	6.10	1 (100)	-	0.11	100 (2.5-100)	100 (63.06-100)
Cohort D	1.95	7 (7.9)	0.76 (0.26-2.31)	0.64	50 (23.04-76.96)	43.45 (35.25-51.92)
	2.5	2 (4.4)	0.42 (0.08-2.11)	0.48	22.22 (2.87-60.01)	59.43 (49.46-68.87)
	6.10	0 (0)	-	1	-§	91.30 (82.03-96.74)

§ Sensitivity was not calculated due to the lack of cases.

*p-value <0.05

OR: Odds Ratio; 95% CI, 95% confidence interval; HT: Hemorrhagic transformation; PH: Parenchymal hematoma

Figure 1: Occurrence of hemorrhagic transformation after rt-PA treatment per increasing group of the Genot-PA score.

A) Cohort A; B) Cohort B; C) Cohort C; D) Cohort D. Score groups G0 ≤3.95 points, G1 3.95-5.10 points, G2 5.10-6.10 points, G3 ≥6.10 points. HI: Hemorrhagic infarct; PH: Parenchymal hematoma