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Global DNA Methylation of Ischemic Stroke Subtypes

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Parc de Salut MAR Barcelona
genneuvas
GeneStroke
prbb Parc Recerca Biomèdica Barcelona
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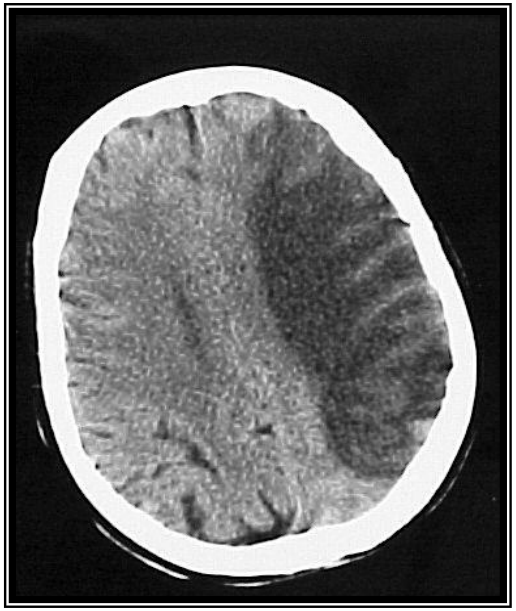
RIC red de investigación cardiovascular

Stroke classification

Ischemic stroke

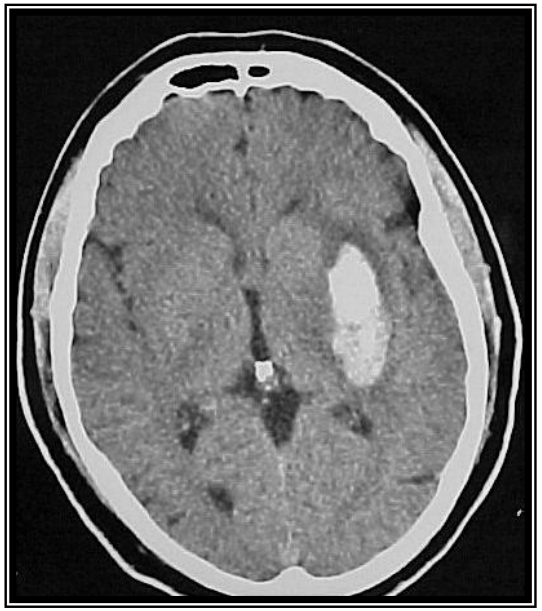
IS 85%



lack of blood flow

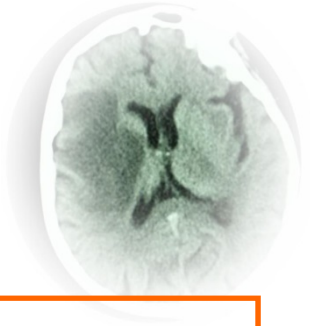
Intracerebral hemorrhage

ICH 15%



rupture of blood vessel

Ischemic stroke



Etiology
(***TOAST classification***):

- Atherosclerosis
- Cardioembolism
- Lacunar
- Other determined etiology
- Undetermined

Risk factors

- Age
- Gender
- Family history
- Hypertension
- Diabetes
- Smoking habit
- Hyperlipidemia
- Coronary artery disease
- Alcohol abuse
- Others

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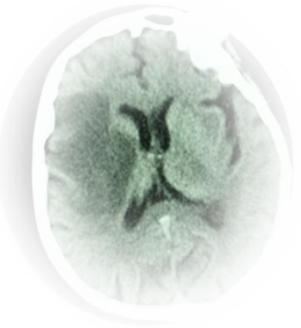
Genetics of Ischemic Stroke



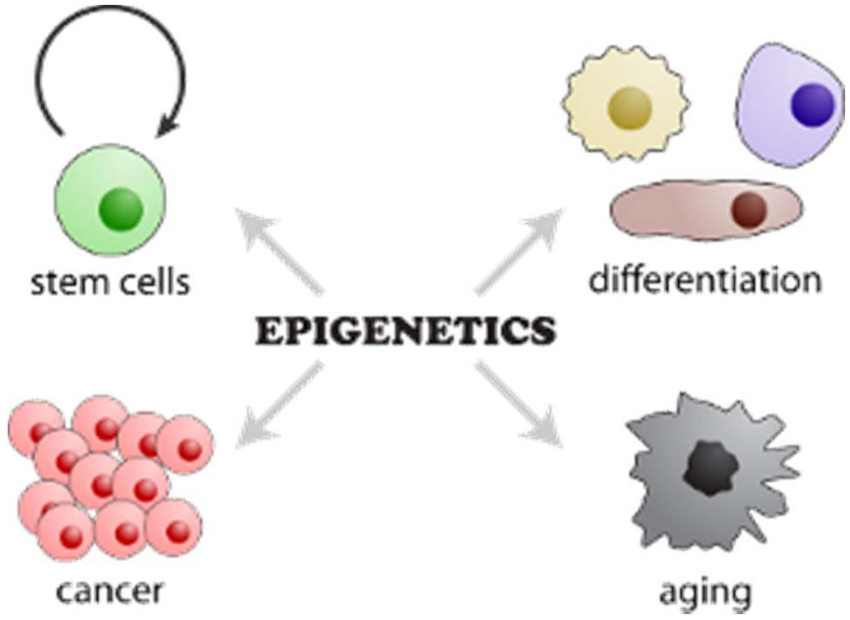
- Genetic causes
  - Heretability of IS: 15 - 40%
  - Heterogeneous disease
  - Different genetic background depending on etiology

  - PITX2, ZFHX3, COL4A1, COL4A2,HDAC9 *(WTCCC2, Nature Genetics 2012)*
    - Massive genotyping (GWAS)
    - Exome sequencing

Genetic + environment = Phenotype

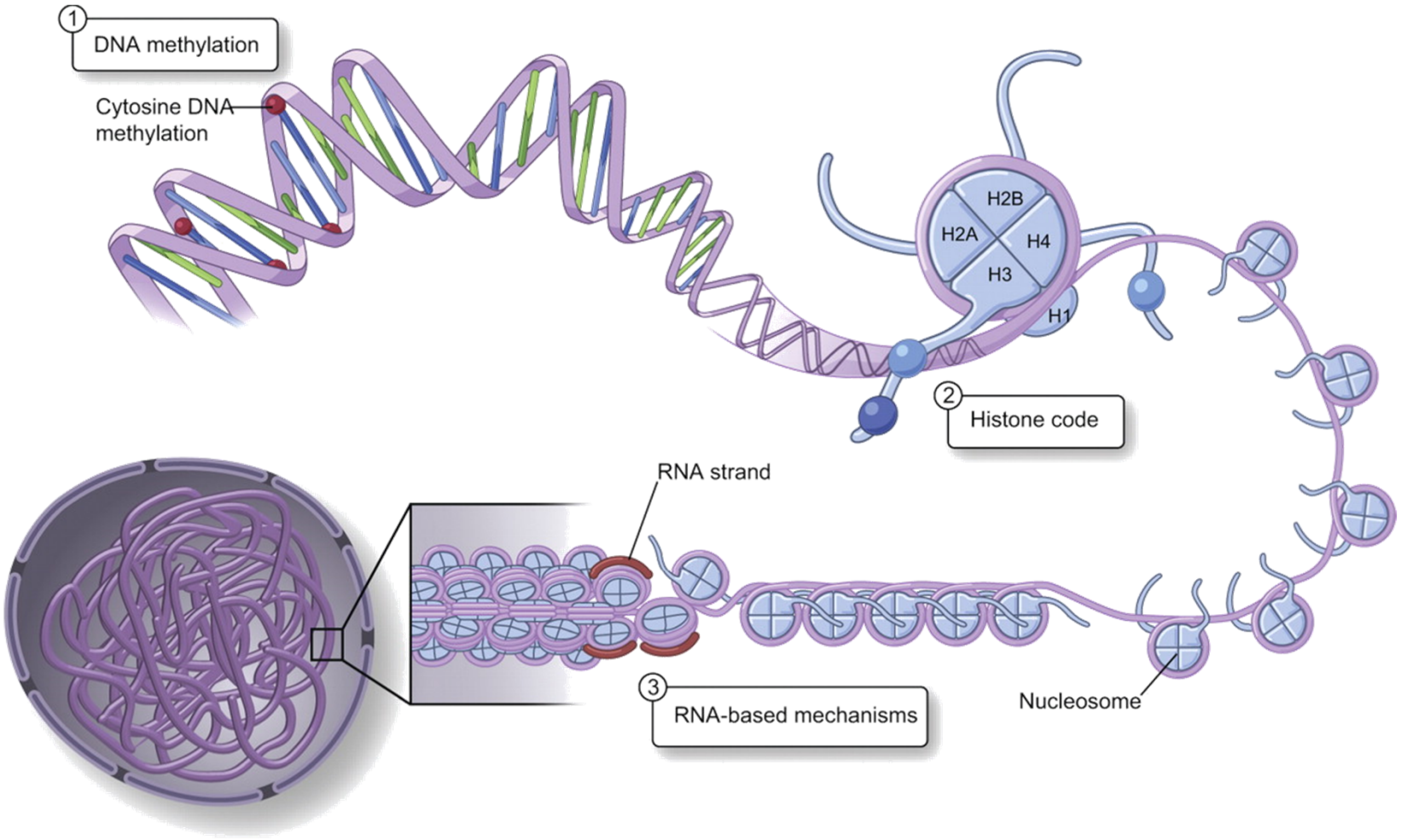
Epigenetics

Heritable changes in gene activity that do not involve a change in the nucleotide sequence (DNA)

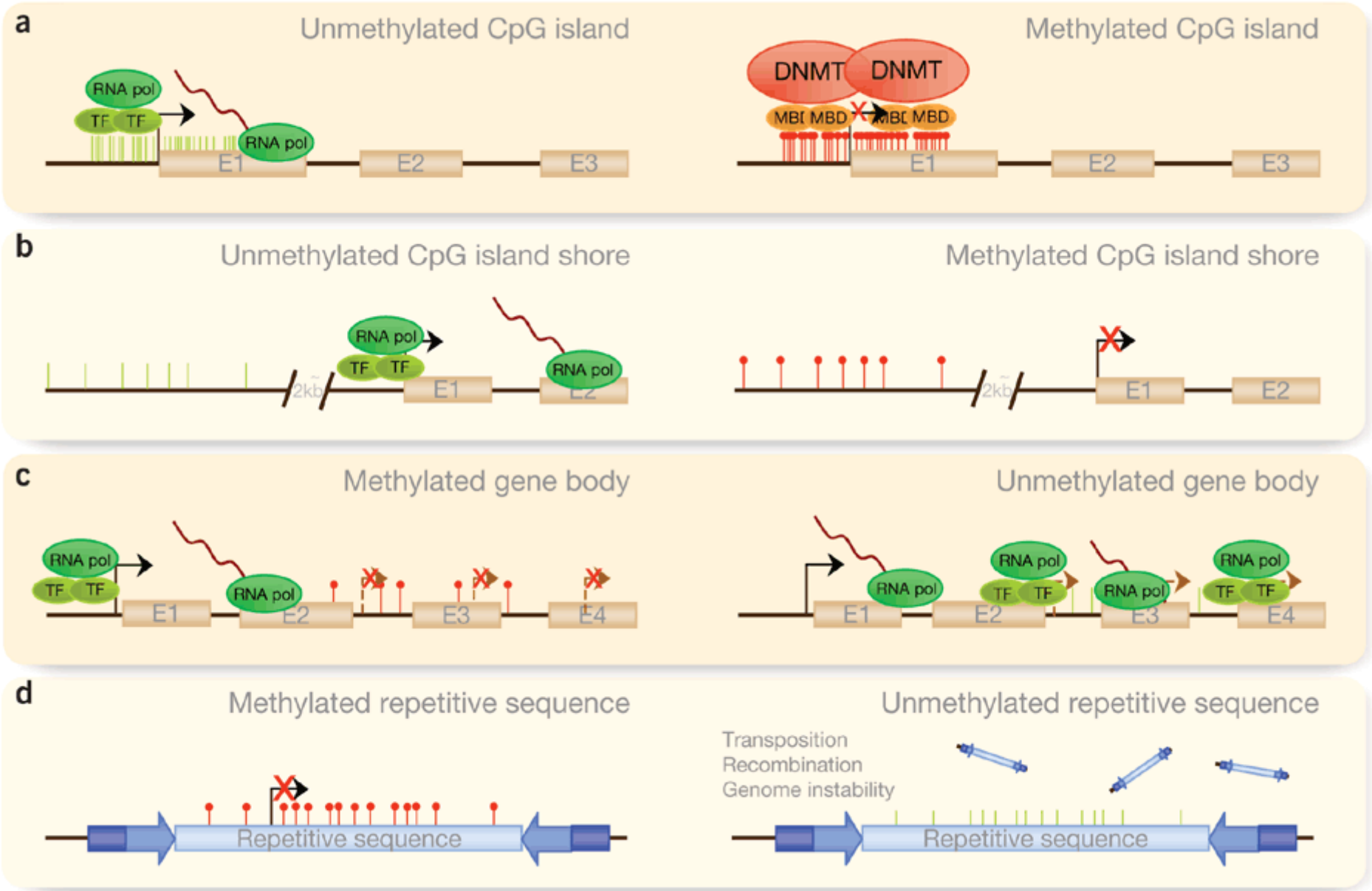


Epigenetics

Three fundamental mechanisms of epigenetic gene regulation

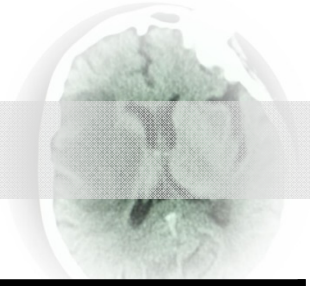


(Yan, M. S. et al. J Appl Physiol 2010)



(Portela & Esteller, Nat Biotech; 2010)

Stroke and Vascular Risk factors



	Global Methylation	Genes	
Stroke	**-/?**	**+**	*(Baccarelli et al., Epidemiology 2010; Qureshi & Mehler, Arch Neurol, 2010)*
Aterosclerosis	**-**	**+**	*(Turunen, et al, Biochimica 2009)*
Myocardial infarction	**-**	**+**	*(Baccarelli et al., Epidemiology 2010; Kim et al.,Plos One 2010; Yan et al., J Appl Physiol 2010)*
Homocysteinemia	**-**	**+**	*(Chang et al., Circ Res, 2008; Jamaluddin et al., Blood 2007; Castro et al., Clin Chem 2003)*
Hypertension	**-**	**+**	*(Millis R, Curr Hypertens Rep 2011)*
Age	**-**	**+**	*(Terry et al, Epigenetics 2011; Koch & Wagner Aging, 2011; Bjornsson et al; JAMA 2008)*
Gender (male)	**+**		*(El-Maarri et al, Plos One 2011; (Zhang et al, Epigenetics 2011)*
Environmental Pollutants	**+/-**		*(Tarantini et al,Environ Health Perspect 2009)* *(Terry et al, Epigenetics 2011)*
Alcohol, smoking, BMI, physical activity	**¿+/-?**		*(Terry et al, Epigenetics 2011; Breitling et al., AJHG 2011; Zhong-Zheng et al,Int J Epidemiol 2010)*

Hypothesis

It has been reported that stroke patients show global DNA hypomethylation compared with healthy individuals. However, stroke was analyzed disregarding its etiology. Taking into account the differences in pathogenesis, some differences in methylation status would exist between IS etiologies.

Goal

Analyze global DNA methylation using LUMA in 3 different ischemic stroke subtypes: large-artery atherosclerosis, small-artery disease, and cardio-aortic embolism.

Methods

Design

Recruited from 2005 to 2012
Hospital del Mar (BASICMAR)
REGICOR
Vall d'Hebrón
Informed consent

IS cases

Inclusion criteria

- First Ischemic stroke (LAA,CE,SVD)
- Brain imaging (TC o RM)
- Clinical Data available

Exclusion criteria

- Intracranial Hemorrhage
- Neoplasms
- Demyelinating and autoimmune disease
- Anticoagulants

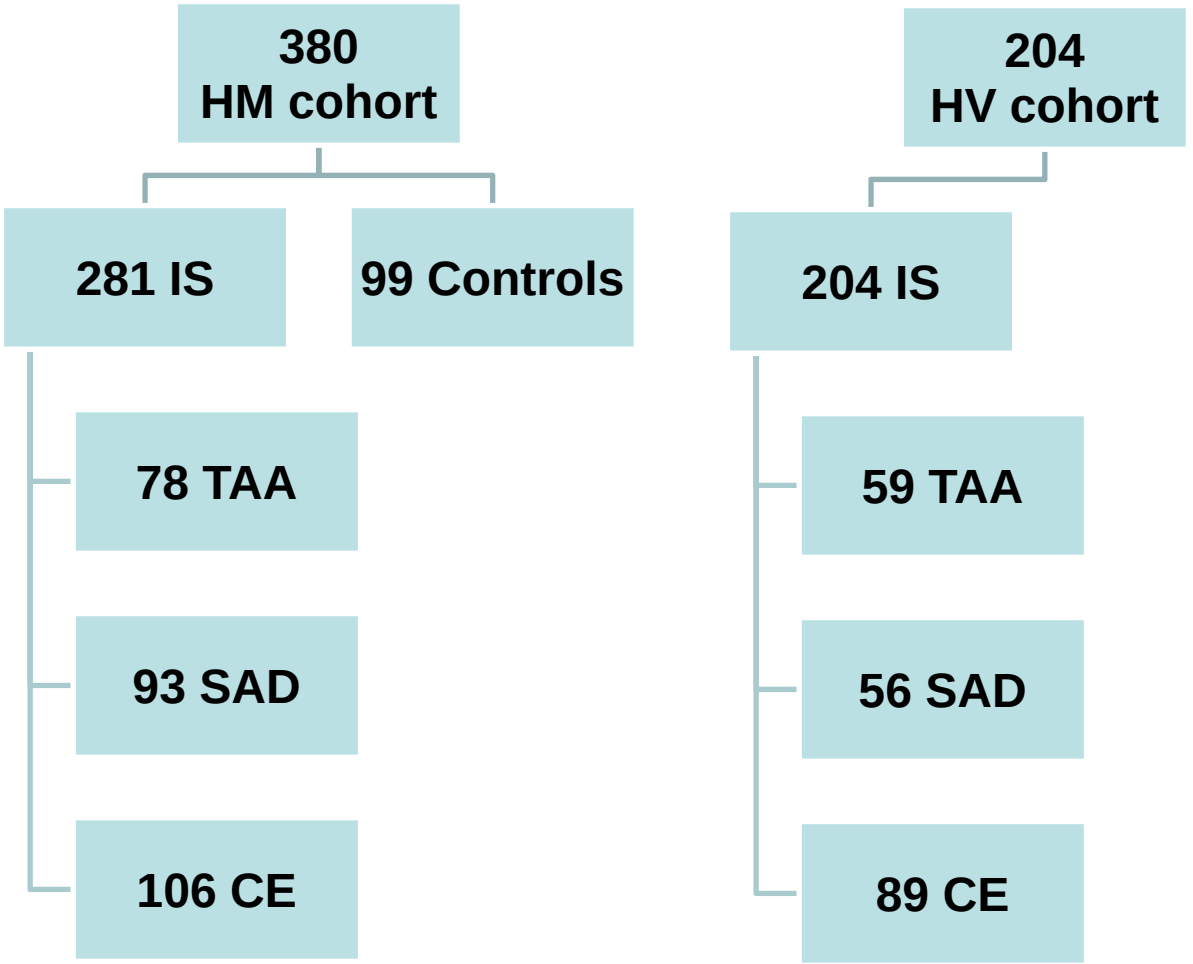
Controls

- No Stroke

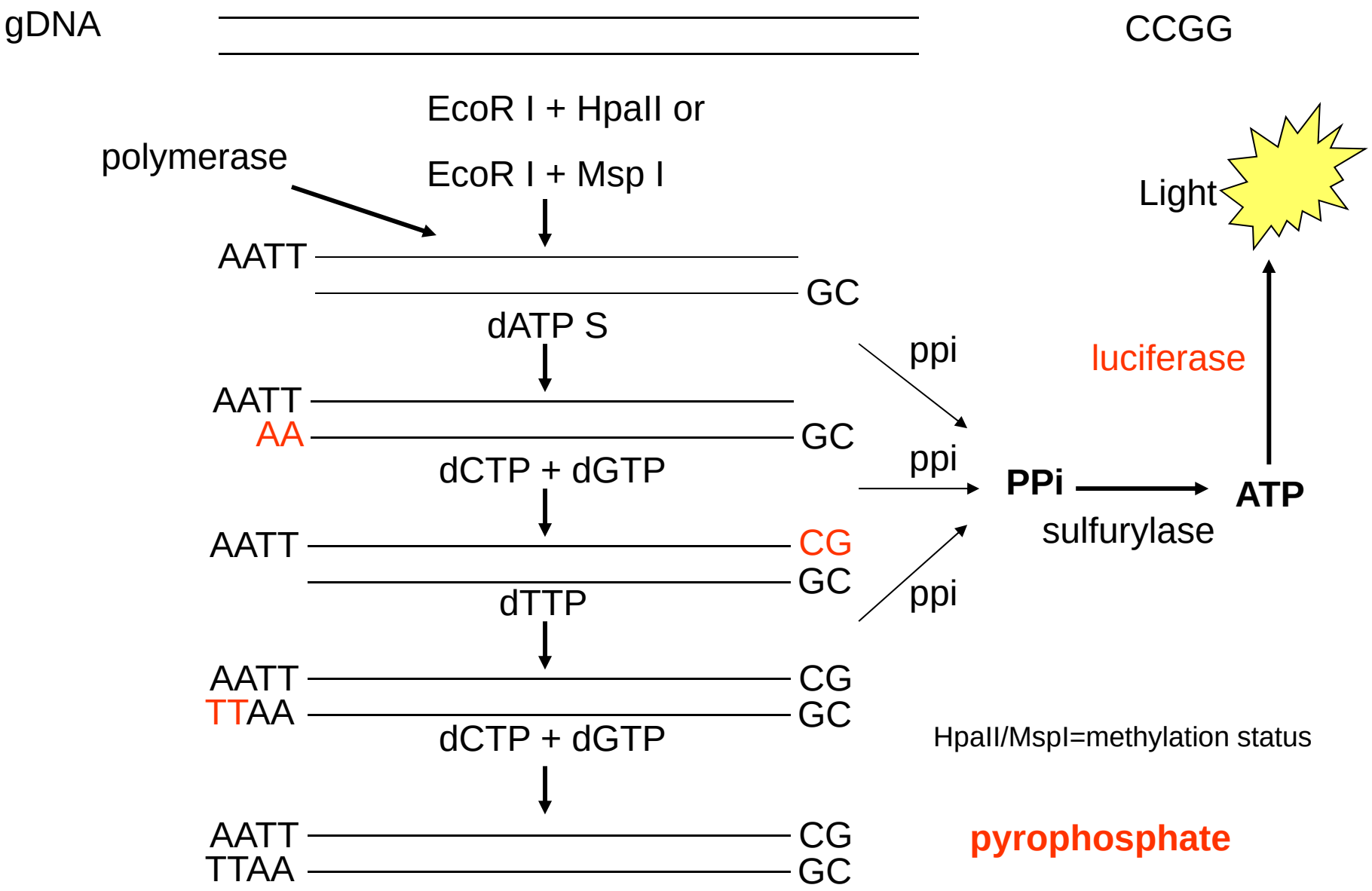
Demographic and Vascular Risk Factor Variable

- Demographic data (age, gender, …)
- Vascular risk factors

Stroke and Vascular Risk factors



LUMA LUminometric Methylation Assay



Results and Discussion

Controls vs Ischemic Stroke

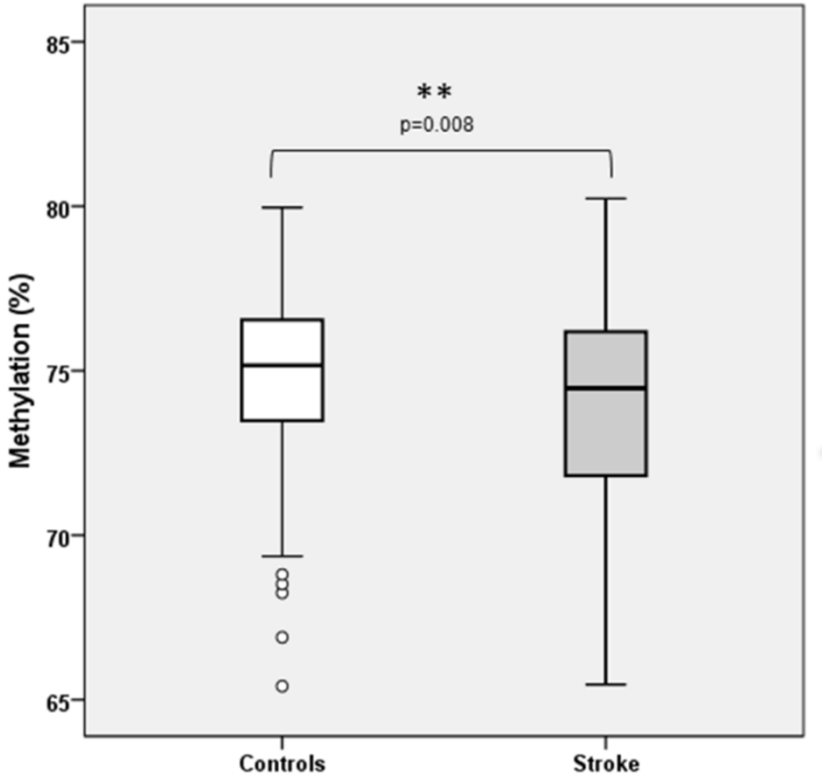


Table 1. Baseline Characteristics of the Study Participants.

Variables	HM Cohort n = 281				HV Cohort n = 204			
	LAA = 78	SAD = 97	CE = 106	p	LAA = 59	SAD = 56	CE = 89	p
Age (mean, SD)	70.5 (9.6)	68.6 (10.4)	75.8 (8.7)	<0.001	69.3 (11.8)	69.8 (11.6)	75 (14.4)	<0.001
Sex, female	23 (29.3%)	29 (29.9)	62 (58.5)	<0.001	21 (35.6)	23 (41.1)	53 (59.6)	0.009
Diabetes Mellitus	34 (43.6%)	33 (34)	37 (34.9)	0.36	19 (32.2)	18 (32.7)	25 (28.1)	0.8
Hyperlipidemia	46 (59%)	37 (38.1)	45 (42.5)	0.016	15 (25.4)	16 (29.1)	21 (23.6)	0.76
Hypertension	52 (66.7%)	65 (67%)	82 (77.4)	0.17	35 (59.3)	27 (49.1)	51 (57.3)	0.50
Current smoking	29 (37.7%)	27 (28.1%)	11 (10.6)	<0.001	12 (20.3)	10 (18.2)	12 (13.5)	0.52
Coronary disease	13 (17.8)	7 (7.7)	20 (19.8%)	0.05	0	0	8 (9)	0.005
Atrial fibrillation	0	0	92 (87%)	<0.001	0	0	45 (51%)	<0.001
Methylation %	74	74.6	74.2	0.68	76.4	77	77.4	0.12
(median, IQR)	(71.8–75.9)	(71.8–76.4)	(71.7–76.2)		(75.5–77.6)	(75.2–77.9)	(76.3–77.9)	
Methylation (n, quartiles)	80.2–75.9 (19, 24.4%)	30 (30.9%)	31 (29.2%)	0.91	81.3–77.6 (15, 25.4%)	19 (36%)	32 (36%)	0.102
	75.9–73.9 (20, 25.6%)	26 (26.8%)	26 (24.5%)		77.6–76.4 (15, 25.4%)	15 (26.8%)	30 (33.7%)	
	73.9–71.7 (20, 25.6%)	17 (17.5%)	23 (21.7%)		76.4–75.5 (15, 25.4%)	5 (8.9%)	12 (13.5%)	
	71.7–65.5 (19, 24.4%)	97 (24.7%)	26 (24.5%)		75.5–68.6 (14, 23.7%)	17 (30.4%)	15 (16.9%)	

Univariate analysis of LUMA methylation as continuous variable and quartile categories. Ischemic stroke (IS), large-artery atherosclerosis (LAA), small-artery disease (SAD), and cardio-aortic embolism (CE), in HM Cohort and HV Cohort. LAA was taken as reference in methylation quartiles.

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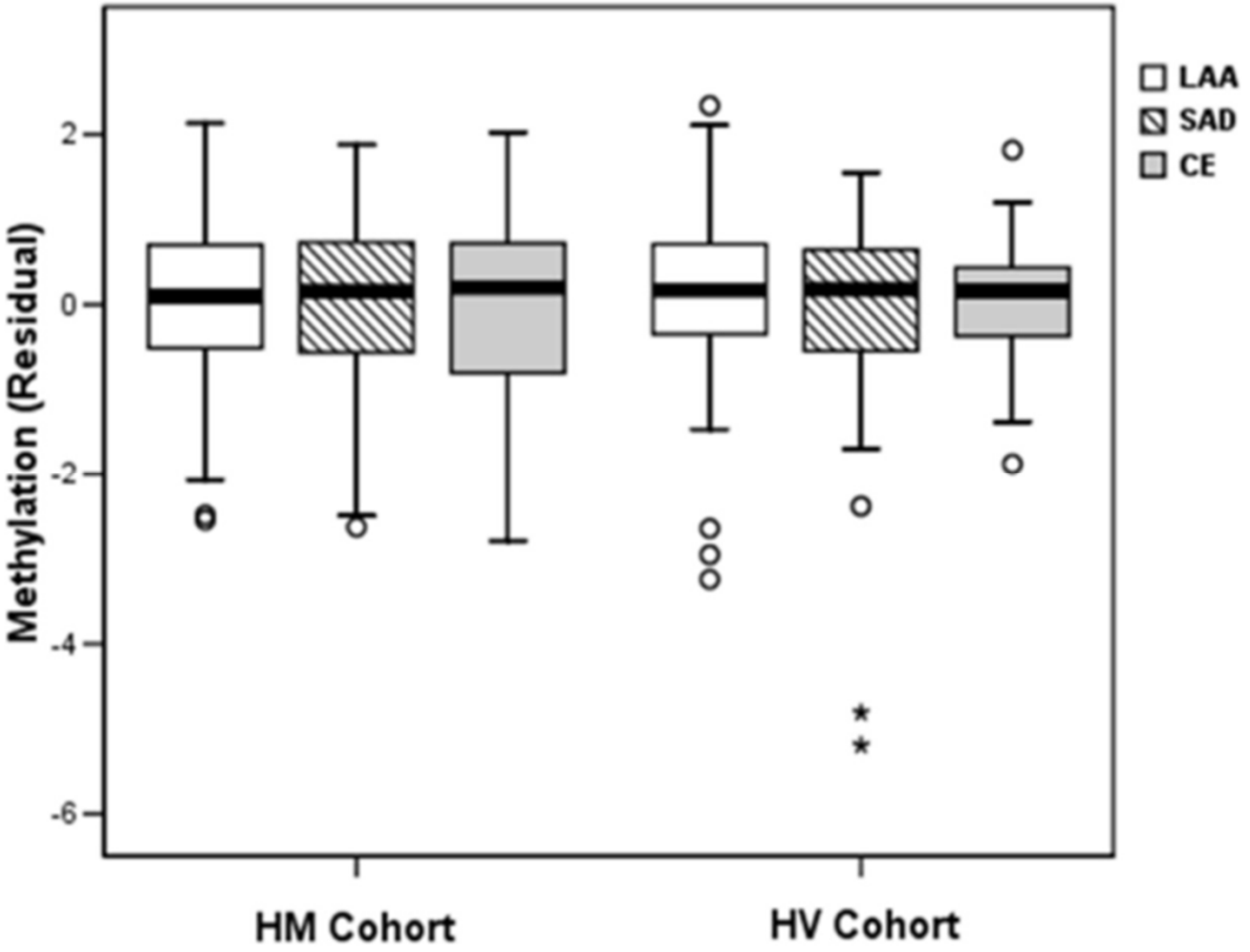
Multivariate analysis

		HM Cohort							HV Cohort						
		LAA	SAD			CE			LAA	SAD			CE		
		OR	OR	95% CI	p	OR	95% CI	p	OR	OR	95% CI	p	OR	95% CI	p
Age		1	0.97	0.94–1.00	0.06	1.03	0.99–1.07	0.08	1	1.00	0.97–1.03	0.98	1.03	1.00–1.06	0.03
Sex		1	0.94	0.47–1.89	0.86	0.37	0.19–0.74	0.004	1	0.83	0.37–1.87	0.66	0.42	0.20–0.89	0.02
HL		1	0.38	0.20–0.71	0.003	0.46	0.24–0.88	0.02	1	1.17	0.50–2.71	0.72	1.01	0.45–2.26	0.98
SMK		1	0.46	0.22–0.96	0.04	0.33	0.14–0.77	0.01	1	0.93	0.33–2.68	0.90	1.38	0.50–3.83	0.53
Methylation (%)		1	1.01	0.92–1.12	0.81	1.02	0.92–1.13	0.74	1	0.97	0.83–1.13	0.69	1.17	0.98–1.40	0.08
Methylation	**Q4**	1	1.05	0.44–2.51	0.91	1.19	0.49–2.90	0.71	1	0.98	0.36–2.65	0.97	1.76	0.66–4.74	0.26
(quartiles)	**Q3**	1	0.86	0.36–2.10	0.74	1.33	0.53–3.31	0.54	1	0.81	0.29–2.28	0.70	1.44	0.53–3.93	0.47
	Q2	1	0.71	0.28–1.76	0.46	1.04	0.41–2.61	0.94	1	0.26	0.08–0.92	0.04	0.58	0.19–1.72	0.32
	Q1	1	1		.	1		.	1	1		.	1		.

Large-artery atherosclerosis (LAA), small-artery disease (SAD), and cardio-aortic embolism (CE), in HM and HV cohorts. LAA was taken as reference in the multinomial regression. SMK = Smoking habit; OR = odds ratio; p = one-side p value; HL = hyperlipidemia.

doi:10.1371/journal.pone.0096543.t002


LAA
SAD
CE
Methylation (Residual)
2
0
-2
-4
-6
HM Cohort
HV Cohort

Limitations

The limitations of the study are related,

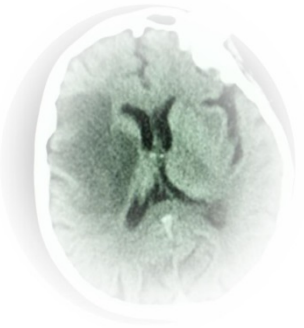
First, retrospective design.

Second, we were unable to adjust for alcohol consumption because of an excess of missing data across one of the cohorts. However, in the other cohort we were able to include alcohol in the analyses but this adjustment did not make any difference.

Conclusions

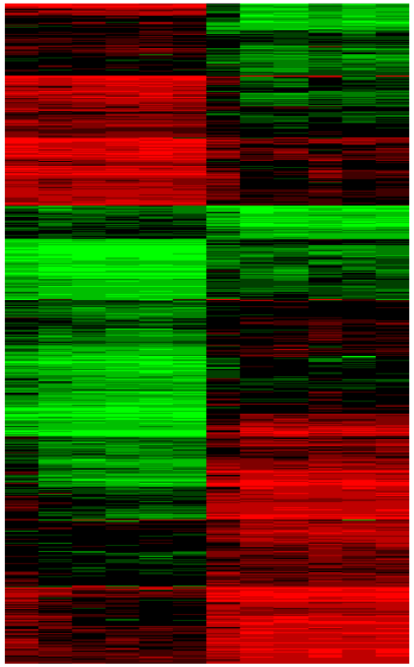
- Global DNA hypomethylation of stroke patients has been replicated compared with healthy individuals
- Despite differences in pathogenesis, our results showed no global methylation differences between LAA, SAD, and CE subtypes of IS
- Further work is required to establish whether the epigenetic mechanism of methylation might play a role in this complex disease.

EWAStroke



Epigenome Wide Association Study

HumanMethylation450 DNA Analysis BeadChip

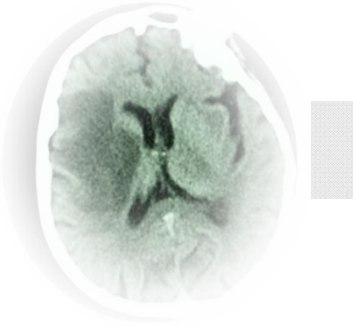


EWAS_1= 404
EWAS_2= 185

N=589

FONDO DE INVESTIGACIÓNSANITARIA. ISCIII (PI12/01238)
RECERCAIXA 2013 (JJ086116)

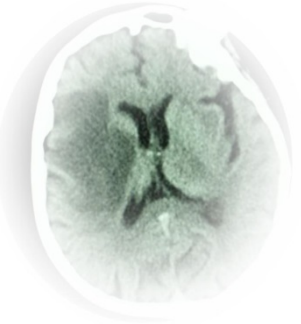
Base line characteristics of study participants

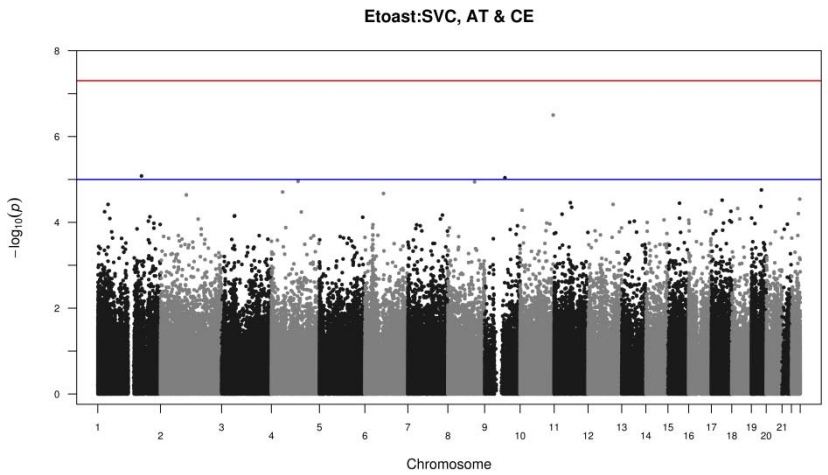


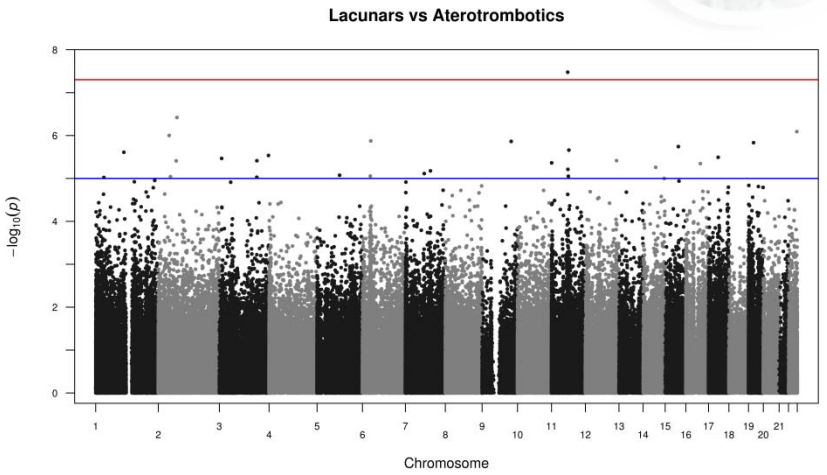
BASICMAR cohort	Ischemic Stroke etiology			
	Cardioembolic (CE)	Large-artery atherosclerosis (LAA)	Small-artery disease (SAD)	**p-value**
N=400	N=127	N=132	N=141	
Age, years*	78.4 (8.8)	72.7 (10.9)	72.5 (11.0)	<0.001
Gender, female, n (%)	82 (64.6)	46 (34.8)	45 (31.9)	<0.001
Hypertension, n (%)	93 (73.2)	92 (69.7)	101(71.6)	0.82
HTA treatment*	94 (74.0)	89 (67.4)	88 (62.4)	0.127
Smoking habit, n (%)	13 (10.7)	50 (38.2)	36 (25.5)	<0.001
BMI, kg/m^2 *	27.8 (4.9)	27.3 (4.9)	27.4 (4.3)	0.177
Diabetes mellitus, n (%)	54 (42.5)	63 (47.7)	59 (41.8)	0.57
Hyperlipidemia, n (%)	48 (37.8)	70 (53.0)	67 (47.5)	0.045
Statins*	35 (37.2)	43 (41.7)	39 (34.2)	0.52
DM treatment*	37 (29.1)	40 (30.3)	42 (29.8)	0.98
Atrial fibrillation, n (%)	117 (92.1)	1 (0.8)	1 (0.7)	<0.001
Ischemic heart disease, n (%)	26 (20.5)	19 (14.4)	14 (9.9)	0.05
Anticoagulants*	23 (19.2)	2 (1.6)	1 (0.7)	<0.001
NIH†	12 (5-18)	5 (3-10)	3 (2-5)	<0.001

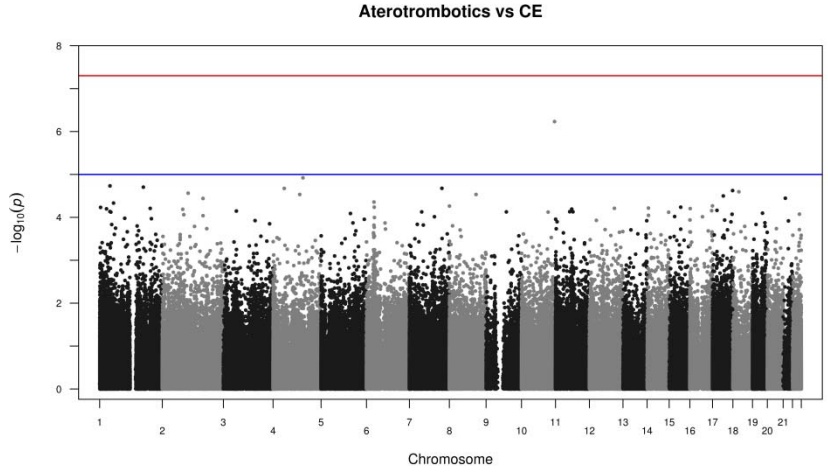
*Mean (Standard deviation)
†Median (Interquartile range)

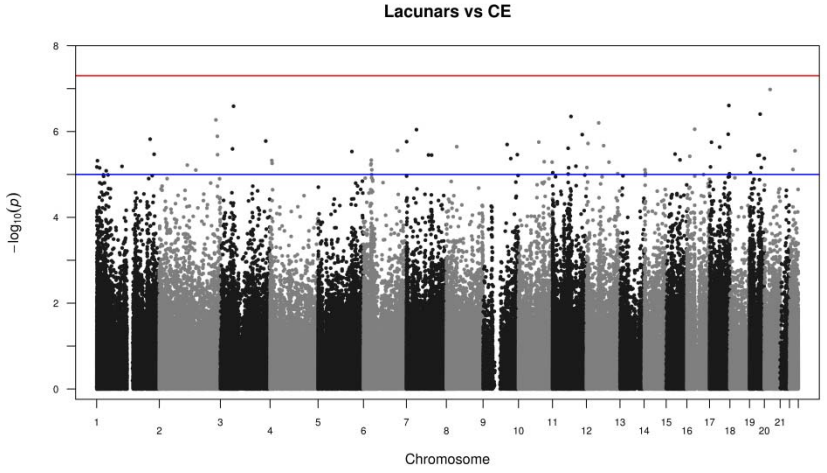
(1) Preliminary Results



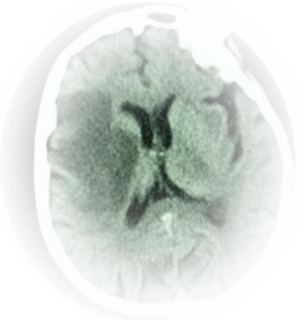




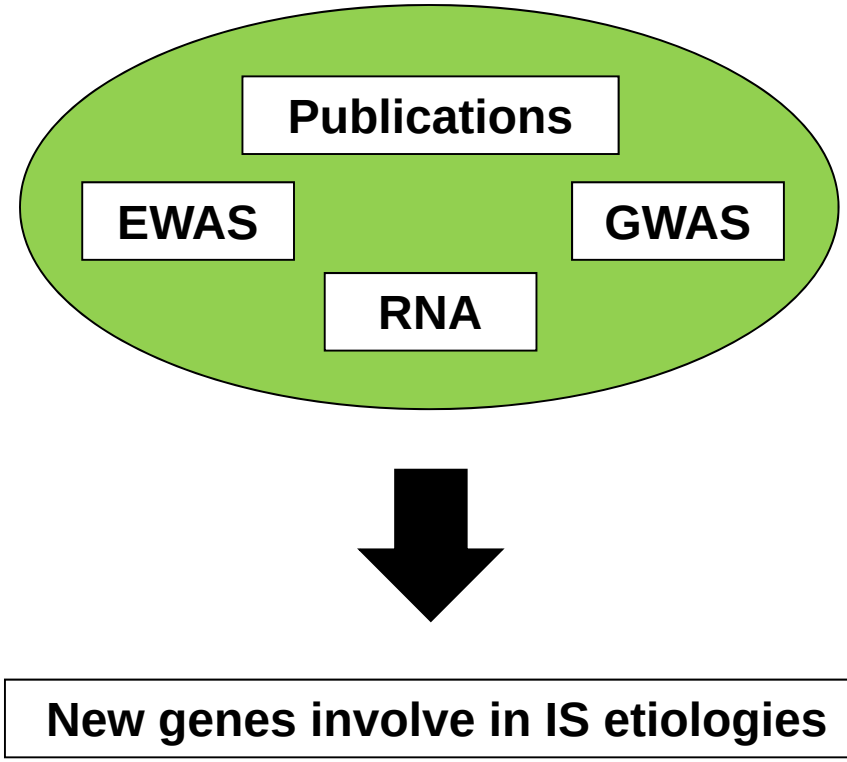




(2) Preliminary Results



Genomic Convergence: integrative approaches combining multiple data sources to identify susceptible genes in complex disorders



Thanks

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