

Online-only section – Supplemental data

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I. eTables

eTable1. Overview of the scored cardio- and cerebrovascular risk factors. BMI: body mass index, HDL: high-density lipoprotein, LDL: low-density lipoprotein.

	Used Cut-off values	References
Hypertension	>140/90 mmHg	(5)
Excessive alcohol use	- >3/4 units/day or - > 7/14 units/week (female/male)	(4,13)
Tobacco use	- currently smoking - quit smoking less than 10 years ago - never smoked	(2)
Weight	- BMI >25: overweight - BMI>30: obesity	(12)
Diabetes mellitus	glycemia >126mg/dL	(1,6)
Hypercholesterolemia	>190 mg/dL	(8)
Hypertriglyceridemia	laboratory dependent	(8)
Hypercholesterolemia HDL	laboratory dependent	(8)
Hypercholesterolemia LDL	laboratory dependent	(8)

eTable 2. Overview of the additional clinical investigations in carriers of 1 pathogenic *ABCC6* variant and PXE patients in the multigenerational family. Calcification of the carotids and arteries of the lower limbs did not have a functional impact in affected patients. *ABCC6*: ATP-binding cassette subfamily C member 6, AS: angioid streaks, F: female, M: male, NA: not applicable, Pd'O: peau d'orange.

Patient code	III-3	IV-2	IV-17	IV-19	IV-22	IV-23	IV-24	IV-30	IV-11	IV-12
Age (years)	69	50	62	59	53	52	47	45	34	35
Sex	M	F	M	F	F	M	M	M	F	M
Echocardiography	abnormal	abnormal	abnormal	abnormal	abnormal	abnormal	normal	abnormal	normal	normal
Left ventricle morphology	sigmoid septum (bottom)	normal	concentric remodeling	decreased relative thickness ventricle wall	normal	normal	normal	dilated (eutrophic)	normal	normal
Left ventricle - diastolic function	dysfunction	dysfunction	normal	normal	normal	dysfunction	normal	dysfunction	normal	normal
Left atrium morphology	normal	normal	normal	normal	normal	normal	normal	dilated atrium	normal	normal
Valvopathy – mitral valve	posterior annulus calcification	normal	normal	degeneration without dysfunction	bulging posterior leaflet	normal	normal	prolapse	normal	normal
Valvopathy – aortic valve	normal	normal	sclerosis	normal	normal	normal	normal	normal	normal	normal
Aortic anomalies	no	no	no	no	no	dilated sinus aorta (z-score 2.95)	no	no	no	no
Internal calcifications	no	no	liver	no	kidney	no	no	gall bladder	no	kidney
Intestinal cysts	no	gall bladder	no	liver	no	no	no	no	no	no

Patient code	III-3	IV-2	IV-17	IV-19	IV-22	IV-23	IV-24	IV-30	IV-11	IV-12
Testicular microlithiasis	no	NA	no	NA	NA	no	no	no	NA	yes
Carotid calcification	yes	no	no	no	no	yes	no	no	no	no
Calcification arteries lower limbs	yes	no	yes	no	no	no	yes	no	yes	yes
Retinopathy	no	no	no	comets	comets	no	comets	no	bilateral AS and Pd'O	bilateral AS and Pd'O
Cutaneous signs	absent	absent	absent	absent	absent	absent	absent	absent	Yellowish skin papules/ plaques	Yellowish skin papules/ plaques

eTable3. Variant classification data. For each of the mentioned variants, variant classification was performed, taking into account Clinvar and literature data on pathogenicity of the variant, the frequency of the variant in the non-Finnish European population on the gnomAD database, *in silico* pathogenicity prediction tools Align GVGD, SIFT, Mutation taster and Polyphen-2, and conservation of the variant in 5 vertebrate species (Homo sapiens, Pan troglodytes, Mus musculus, Rattus norvegicus, Danio rerio). B: benign, Cons: conservation, D: deleterious, DC: disease causing, E: exon, id: identifier, MT: mutation taster, P: polymorphism, PoD: possibly damaging, PrD: probably damaging, T: tolerated.

gDNA	cDNA	Protein	E	Variant id	Clinvar	Gnomad	Align GVGD	SIFT	MT	Polyphen-2	Cons	Pathogenic in literature
16295863	c.1171A>G	p.(Arg391Gly)	9	rs72653762	likely pathogenic ^a	0.79%	C15	D	DC	PrD	5/5	(2)
16282828	c.1639G>A	p.(Ala547Thr)	13	rs56877937	/	0.017%	C0	D	DC	PoD	5/5	/
16251531	c.3871G>A	p.(Ala1291Thr)	27	rs58694313	conflicting ^b	0.029%	C0	T	P	B	4/5 ^f	(9)
16248792	c.3979G>A	p.(Gly1327Arg)	28	rs79536709	/	0.025%	C0	D	DC	PrD	5/5	/
16248737	c.4034T>C	p.(Ile1345Thr)	28	/	/	/	C65	D	DC	PrD	5/5	/
16248611	c.4082A>G	p.(Asp1361Gly)	29	/	/	/	C65	D	DC	PrD	5/5	other variant same position (10,11)
16248589	c.4104delC	p.Asp1368Glu fs*35)	29	rs72664237	pathogenic ^c	0.0040%	/	/	/	/	5/5	(7)
16248566	c.4127A>C	p.(Glu1376Ala)	29	/	/	/	C35	D	DC	PoD	5/5	/
16248566	c.4127A>G	p.(Glu1376Gly)	29	/	/	/	C35	D	DC	PrD	5/5	/
16248525	c.4168C>G	p.(Gln1390Glu)	29	/	/	/	C0	T	DC	PrD	4/5 ^f	/
16248495	c.4198G>A	p.(Glu1400Lys)	29	rs63751241	pathogenic ^d	0.001%	C0	T	DC	PrD	3/5 ^g	(2)
16248476	c.4208+9G>A	p.?	29	rs58760581	pathogenic ^e	1.62%	splice site prediction: no significant alterations (eFigure 1)					/

a: clinvar record RCV000256117.1; b: clinvar records SCV000589081.1, SCV000515882.2; c: clinvar records SCV000589109.1, SCV000598672.1; d: clinvar record SCV000589112.1; e: clinvar record SCV000589158.1; f: not Danio rerio; g: not Mus musculus, Rattus norvegicus.

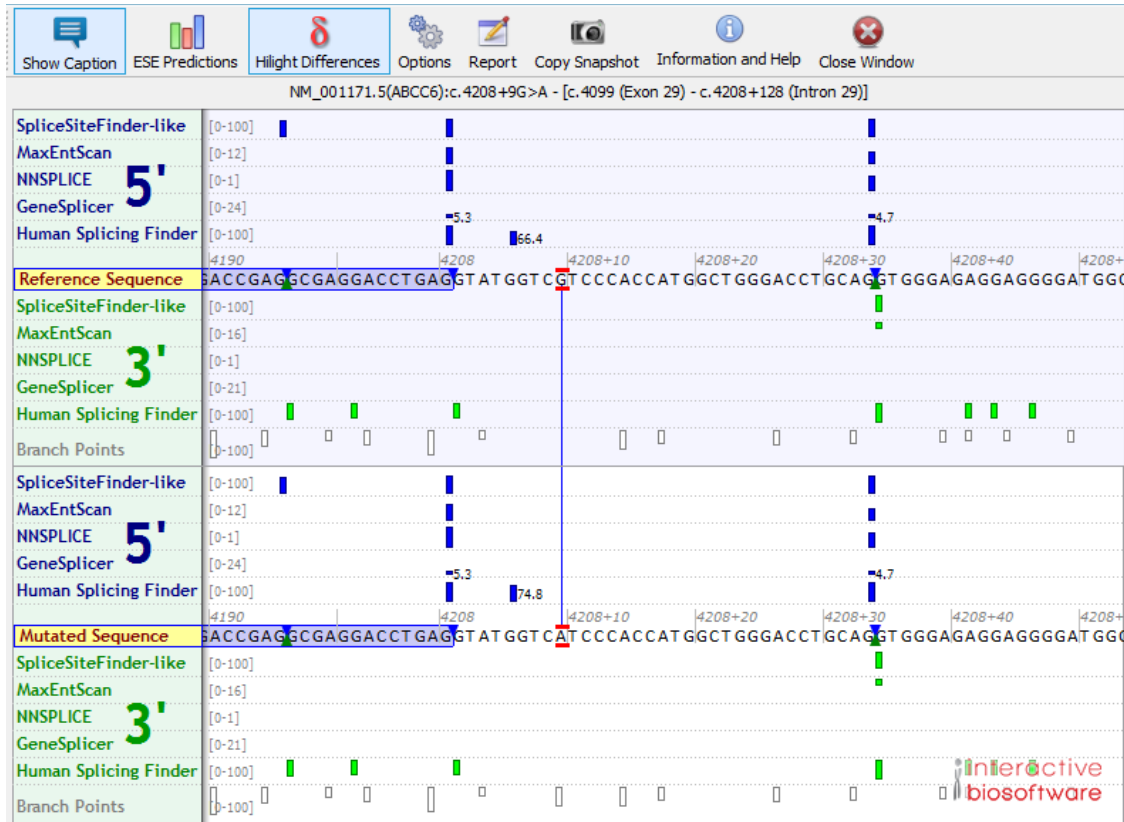
eTable 4. Logistic regression analysis for cardiovascular risk factors as modifiers for *ABCC6* variant-stroke interaction. CI: confidence interval, OR: odds ratio.

	Chi square	Regression coefficient	Standard error	p-value	OR (95% CI)
Tobacco use	0.689	0.701	0.782	0.370	2.016 (0.436-9.330)
Hypertension	3.650	-1.005	0.548	0.067	0.3660 (0.125-1.072)
Diabetes mellitus	0.104	0.215	0.655	0.742	1.242 (0.344-4.476)
Dyslipidemia	0.099	-0.172	0.549	0.755	0.842 (0.287-2.472)

eTable 5. Associated anomalies on cerebral imaging in the 16 stroke patients of the independent patient cohort carrying a pathogenic *ABCC6* variant. CP: Choroid plexus; FC: Falx cerebri; GP: Glandula pinealis; Id: patient identifier, PVL: Periventricular leukomalacia; SOC: Semioval center.

Id	White matter lesions	Intracerebral calcifications	Vascular malformations	Vascular calcifications
006	PVL	GP, CP	/	/
026	PVL, bilateral in SOC	GP, CP, FC	/	/
035	Bilateral frontoparietal	/	/	/
037	PVL, SOC	/	/	/
065	/	/	/	/
088	1 lesion left parietal lobe	/	/	/
098	PVL	GP, CP	/	/
135	/	/	/	/
143	/	/	/	/
146	/	/	/	Carotid artery siphon
166	/	/	/	/
193	/	/	/	/
236	/	/	/	/
249	/	/	/	/
305	Bilateral frontoparietal	/	/	/
382	/	/	/	/

II. eFigures
 eFigure 1. Splice site effect prediction for *ABCC6* variant c.4208+9G>A.



III. eReferences

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