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Towards the genetic basis of cerebral venous thrombosis—the BEAST Consortium: a study protocol

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ABSTRACT

Introduction: Cerebral venous thrombosis (CVT) is a rare cerebrovascular condition accounting for <1% of all stroke cases and mainly affects young adults. Its genetic aetiology is not clearly elucidated.

Methods and analysis: To better understand the genetic basis of CVT, we have established an international biobank of CVT cases, Biorepository to Establish the Aetiology of Sinovenous Thrombosis (BEAST) which aims to recruit highly phenotyped cases initially of European descent and later from other populations. To date we have recruited 745 CVT cases from 12 research centres. As an initial step, the consortium plans to undertake a genome-wide association analysis of CVT using the Illumina Infinium HumanCoreExome BeadChip to assess the association and impact of common and low-frequency genetic variants on CVT risk by using a case–control study design. Replication will be performed to confirm putative findings. Furthermore, we aim to identify interactions of genetic variants with several environmental and comorbidity factors which will likely contribute to improve the understanding of the biological mechanisms underlying this complex disease.

Ethics and dissemination: BEAST meets all ethical standards set by local institutional review boards for each of the participating sites. The research outcomes will be published in international peer-reviewed open-access journals with high impact and visibility. The results will be presented at national and international meetings to highlight the contributions into improving the understanding of the mechanisms underlying this uncommon but important disease. This international DNA repository will become an important resource for investigators in the field of haematological and vascular disorders.

Strengths and limitations of this study

- This study is the largest collaboration on cerebral venous thrombosis (CVT) conducted to-date and has the advantage that it includes highly phenotyped individuals.
- This is the first study that aims to perform a genome-wide association analysis to assess the association and impact of common and low-frequency genetic variants on CVT risk.
- Identifying genetic variants associated with CVT risk will likely contribute to improving our understanding of the biological mechanisms underlying this disease and may lead to the discovery of novel therapeutic targets.
- A potential limitation of the study is the difficulty of recruiting a large number of cases due to the very low incidence and prevalence of this condition. Major efforts are being made to include as many research centres able to investigate this disease across Europe and beyond.

BACKGROUND

Cerebral venous thrombosis (CVT) is a rare cerebrovascular condition that accounts for <1% of all strokes,[1] with an overall annual incidence estimated at 1.32 per 100 000 person-years.[2] CVT commonly affects young

adults and is more prevalent in women, accounting for ~75% of the adult affected patients.[3] It can lead to mortality or severe morbidity but generally has a good clinical outcome particularly following early identification of less severe cases using advanced imaging.[4]

The condition has two broadly different aetiological mechanisms: thrombosis of cerebral veins with local effects caused by venous obstruction and thrombosis of the dural sinuses which may cause intracranial hypertension.

However, both processes usually occur simultaneously in most patients with thrombosis often present in more than one sinus.[1 5 6] Compared with arterial thrombosis, CVT is less frequent in terms of incidence and more variable in its clinical presentation and neuroimaging.[7]

The condition has multiple risk factors (box 1) and presents as a diagnostic and therapeutic challenge given the diversity of symptomatic presentation and variety of putative aetiological factors.

CVT is a rare manifestation of venous thromboembolism (VTE). Compared with CVT, traditional venous thrombosis manifestations such as deep vein thrombosis (DVT) and pulmonary embolism (PE) are much more common and are diseases of ageing.[8]

There is a lack of data evaluating the risk of CVT recurrence, as well as whether the risk factors for CVT are similar to those for DVT and PE. One recent study has found that after a 10-year follow-up on patients with DVT and PE only 5.2% developed CVT,[9] while for patients with CVT only 5.8% developed DVT/PE later on.[10] Therefore, no significant link between CVT and DVT/PE has been found so far.

Interestingly, one study has found no differences in thrombophilia markers between CVT and patients with DVT/PE; however, the frequency of other risk factors, such as oral contraceptive (OC) use, pregnancy or puerperium was significantly different.[11] CVT showed to be more frequent in women, secondary to hormonal factors and less often secondary to trauma, immobilisation or surgery compared with patients with DVT/PE.[11]

Therefore, it is not clear why CVT occurs less often than DVT/PE, and age-dependent differences in the risk profile between CVT and DVT/PE, as well as genetic factors may play a role in the pathogenesis. Thus, due to its rarity and risk profile, CVT represents a particular form of VTE.

Neither the genetic component of CVT nor its heritability has been widely assessed mainly because of its low incidence and lack of large number of cases. However, there is reasonable evidence to support a genetic predisposition to CVT.

A significant proportion of cases (~13–25%) have no risk factors identified[1 7] suggesting that undetermined genetic factors may at least partly account for this unexplained risk. Although it is a more rare condition, it does not usually cluster in families and there is no evidence to suggest a Mendelian inheritance.

The genetic component of CVT has so far been assessed mainly by candidate gene studies. As CVT is

Box 1 Risk factors associated with cerebral venous thrombosis.[3 7]

Genetic prothrombotic conditions
- Antithrombin deficiency
- Protein C and S deficiency
- Factor V Leiden mutation
- Prothrombin G20120A mutation
- Hyperhomocysteinaemia caused by MTHFR C677 T polymorphism

Acquired prothrombotic states
- Nephrotic syndrome
- Antiphospholipid antibodies
- Pregnancy
- Puerperium

Systemic inflammatory disease
- Systemic lupus erythematosus
- Inflammatory bowel disease
- Wegener's granulomatosis
- Behcet's syndrome
- Sarcoidosis
- Thyroid disease

Systemic infectious disease
- Bacterial: septicaemia, endocarditis, typhoid, tuberculosis
- Viral: measles, hepatitis, encephalitis, herpes, HIV, cytomegalovirus
- Parasitic: malaria, trichinosis
- Fungal: aspergillosis

Head and neck infections
- Extradural: mastoiditis, sinusitis, otitis, facial cellulitis, osteomyelitis, tonsillitis
- Intradural/parenchymal: abscess, empyema, meningitis

Haematological disorders
- Polycythaemia (primary and secondary)
- Thrombocythaemia
- Anaemia (including paroxysmal nocturnal haemoglobinuria)
- Sickle cell disease

Drugs
- Oral contraceptives
- L-asparaginase therapy
- Hormone supplement therapy

Systemic malignancies
- Visceral carcinomas
- Lymphomas
- Leukaemia
- Myeloproliferative disease

Central nervous system tumours
- Meningioma, metastases, carcinomatous infiltration

Gastrointestinal disease
- Ulcerative colitis, Crohn's disease

Cardiac disease
- Congenital heart disease, cardiac insufficiency

Mechanical causes and trauma
- Head injury, injury to sinuses or jugular vein, neurosurgical procedures, jugular vein catheterisation, lumbar puncture

Others
- Cerebral infarcts and haemorrhage
- Arteriovenous malformations
- Dural arteriovenous malformation
- Arachnoid cyst
- Internal jugular compression
- Severe exfoliative dermatitis
- Severe dehydration of any cause

Idiopathic

known to be associated with inherited thrombophilia,[1] most candidate gene studies have assessed mutations associated with this condition such as factor V Leiden and prothrombin G20120A mutation.[12] Other mutations investigated by candidate gene studies have included the MTHFR C677T polymorphism (risk factor for hyperhomocysteinaemia),[13] the plasminogen activator inhibitor-1 4G/5G polymorphism (risk factor for thrombosis),[14] protein Z G79A polymorphism (involved in formation of blood clots)[15] and Janus Kinase-2 V617F mutation (involved in making haematopoietic cells more sensitive to growth factors).[16] However, the results from such individual candidate gene studies have been conflicting mainly because of lack of sufficient power due to the low number of cases. One large meta-analysis on 1183 CVT cases and 5189 controls that pooled together results from 26 candidate gene studies highlighted significant associations of factor V Leiden G1691A mutation (OR=2.40; 95% CI 1.75 to 3.30; $p<10^{-5}$) and prothrombin G20120A mutation (OR=5.48; 95% CI 3.88 to 7.74; $p<10^{-5}$) in adult populations.[17] Interestingly, this study also found that genes involved in the clotting cascade provide a greater level of thrombosis risk in the cerebral venous circulation compared with its arterial circulation implying a larger genetic liability for CVT compared with sporadic ischaemic stroke (IS).[17] Moreover, previous studies suggested a stronger genetic component in younger patients who had stroke compared with older case of stroke providing additional evidence to support a strong genetic susceptibility to CVT.[18–20]

Other thrombophilic factors involved in the coagulation pathway that are associated with an increased risk of CVT are: protein C, protein S and antithrombin deficiencies.[21] These prothrombotic factors are also associated with an increased risk of DVT and PE[22 23] suggesting that all these venous thrombosis conditions may have a common genetic component.

An important characteristic of the disease is the higher prevalence in women. Large epidemiological studies have confirmed that OC users, particularly users of third-generation OCs, are at increased risk of VTE.[24–26] Although contraceptive drugs are an important factor in explaining this gender distribution, genetic factors interacting with pharmacological or environmental determinants may also play a significant role. In addition, very little is known about why the rate of CVT is relatively low given widespread environmental exposures on a population level (eg, OCs, sinus infections, etc), suggesting that an underlying background genetic risk may contribute to increasing the incidence of CVT in those with common exposures.

To better understand the genetic basis of CVT, we have established an international biorepository of highly characterised CVT cases, Biorepository to Establish the Aetiology of Sinovenous Thrombosis (BEAST). The BEAST Consortium includes CVT cases recruited currently from 10 centres across seven countries in Europe, and one each from the USA and Mexico.

Our study aims first to assess the association and impact of common and low-frequency genetic variants on CVT risk by using a case–control study design and second, to identify interactions of genetic variants with several environmental and comorbidity factors which collectively will likely contribute to a better understanding of the biological mechanisms underlying this complex disease.

METHODS

Study participants

Cases

The ongoing international BEAST Consortium has to-date recruited DNA and clinical data from 745 patients with CVT (aged ≥18 years) from 12 research centres located in the following countries: Belgium, Finland, Greece, Italy, the Netherlands, Portugal, UK, USA and Mexico.

In all cases, CVT is confirmed by CT or MRI of the brain and dedicated venography (CT angiography, MR angiography, or conventional angiogram). The inclusion criteria for cases are presented in table 1. Detailed phenotypic data is provided by each participating centre (box 2).

Owing to differences in the genetic structure between the different populations participating in the study,[27] cases will be split for genetic association analysis into four groups: West European, South European (Italian and Portuguese), Finnish and Mexican cases, to obtain homogeneous populations. The US population is all European origin (non-Hispanic white). The results will be presented per ancestral population and then subjected to a pooled meta-analysis of all populations.

Controls

The inclusion criteria for the control population are presented in table 1.

For the West European CVT cohort, the BEAST study will use data from previously genotyped control samples, namely 2469 British controls from the 1958 British Birth Cohort part of the Wellcome Trust Case Control Consortium (WTCCC).[28 29]

In addition, we have recruited healthy age-matched and sex-matched controls numbering 300 Italians for

Table 1 Inclusion criteria for CVT cases and controls

Inclusion criteria for CVT cases	Inclusion criteria for controls
Age ≥18 years at the time of enrolment	Age ≥18 years at the time of enrolment
CVT determined using: ▶ CT or MRI brain ▶ Dedicated venography (CTA, MRA, or conventional angiogram)	No history of CVT/stroke or any other thrombotic or chronic condition
Patient or relative provision of informed written consent	Provision of informed written consent

CTA, CT angiography; CVT, cerebral venous thrombosis; MRA, MR angiography.

Box 2 Phenotypic data provided by each participating centre

Demographic data (age, sex, ethnicity).
Date of cerebral venous thrombosis diagnosis.
Clinical presentation and symptoms.
Neuroimaging information including sinus/vein involved and extent of oedema, haemorrhage.
Family history of thrombotic or cerebrovascular event.
Thrombophilia screening information:
- Protein C and S deficiencies,
- Genetic polymorphisms (factor V G1691A mutation, prothrombin G20210A mutation),
- Antiphospholipid antibodies,
- Lupus anticoagulant,
- Hyperhomocysteinaemia.

Risk factors and associated conditions:
- Other venous thrombosis,
- Transient risk factors,
- Pregnancy,
- Puerperium,
- Systemic or brain infections,
- Systemic inflammatory disease,
- Haematological disorders,
- Drugs (oral contraceptives, L-asparaginase therapy, hormone replacement therapy),
- Malignancies,
- Bowel disease,
- Cardiac disease,
- Mechanical causes and trauma (head injury, surgery, etc),
- Severe dehydration of any cause.

Modified Rankin scale at last follow-up.

the South European cohort, 230 Finnish for the Finnish cohort and 100 Mexicans for the Mexican cohort.

Ethical considerations

BEAST meets all ethical standards set by local institutional review boards for each of the participating sites. Written informed consent is obtained for all patients with CVT and controls at each participating research centre. Patient confidentiality is protected and patient details are encrypted.

Biological samples

Peripheral blood samples from all participants are collected in EDTA-coated phials or sodium citrate vacutainers using venipuncture. Genomic DNA is extracted from peripheral blood using commercially available DNA isolation kits and stored at −80°C.

Genotyping

Cases

DNA samples for all CVT cases will be processed on the HumanCoreExome BeadChip v1.0 (Illumina, San Diego, California, USA) using standard protocols at the Genetic and Molecular Epidemiology Laboratory, McMaster University, Canada.

The Illumina Infinium HumanCoreExome BeadChip contains ~240 000 exome focused markers, as well as ~240 000 common tagSNP markers. The functional exonic markers include non-synonymous variants, stop altering variants, splice coding variants and variants located in promoter regions.

Controls

The WTCCC British control sample was genotyped using the HumanExome BeadChip v1.0 (Illumina, San Diego, California, USA). The Illumina HumanExome Beadchip includes 247 870 markers focused on protein-altering variants selected from >12 000 exome and genome sequences representing multiple ethnicities and complex traits.

The Finnish controls have already been genotyped using the Illumina Infinium HumanCoreExome BeadChip, while other control samples (Italian and Mexican) will be genotyped with the same array.

Data analysis

We will perform case–control analysis using logistic regression assuming an additive genetic model to assess the association of the genotyped markers with CVT risk. Rigorous quality control procedures will be applied according to the recommended exome chip processing protocol.[30]

Population stratification analysis and testing for relatedness will be conducted, and outliers will be removed from analysis. To investigate residual population stratification, genomic inflation factors will be calculated. Quantile-Quantile plots will be constructed to assess the quality of the association results. Meta-analysis of the association results for the participating cohorts will be performed using a fixed effect model and inverse variance method of weighted β coefficients and SEs from each study. Furthermore, the putative positive findings will be confirmed by replication in independent samples to exclude spurious associations. We are currently collaborating with additional centres to recruit a replication sample.

We will conduct a reciprocal look up in genome-wide association studies (GWAS) of other venous thrombosis conditions (DVT/PE) and potentially pooling of analyses from these studies if available.

We will undertake a subgroup analysis of CVT cases with and without history of other venous thrombosis conditions (DVT/PE). We will also undertake a subgroup analysis of CVT cases with and without inherited thrombophilia.

We will assess the interactions of significant polymorphisms with environmental and comorbidity risk factors, severity of clinical presentation and outcome.

The power for gene-environment interactions depends on the magnitude of the environmental exposure frequency. Therefore, the power is higher if the exposure frequency is low and is lower if the exposure is high.[31]

We will perform sex stratified analysis, adjusting for age, and conduct several comparisons (eg, between OC users and female non-users, cases with factor V Leiden mutation and cases without the mutation), to highlight the influence of genetic factors between different patient groups. We will also stratify the data by IS status (cases with IS vs cases without IS).

Sample size and power

Power calculations were performed using the genetic power calculator CaTS.[32] With the current BEAST repository of 745 CVT cases and a total of ~3000 controls, the study has 80% power to detect a relative risk of 1.6 at a significant p value $<10^{-7}$ with a population allele frequency of 30%. However, the likely genetic liability of this condition[17] suggests that this power calculation may be conservative.

DISCUSSION

The BEAST Consortium is the largest DNA repository of highly characterised CVT cases established to-date. The study aims to improve our understanding of the genetics of CVT by first investigating the influence of common and low-frequency genetic variants on CVT risk and, second, by identifying interactions of genetic variants with environmental and comorbidity risk factors. Comprehensive investigation into the genetics of CVT holds the potential to allow at-risk groups to be identified, as well as disease severity and prognosis to be determined.

In the past several years, the genome-wide association (GWA) approach facilitated by technological developments of high-density genome-wide genotyping arrays has been applied for many complex diseases and has been successful in identifying thousands of novel common genetic variants associated with disease risk.[33] However, for IS GWA has not been as successful with few genetic variants identified[34–39] likely due to the paucity of power in detecting common genetic variants with small effects which require very large cohorts.[40] Another likely reason for the limited positive results is the clinical heterogeneity of IS which is known to be influenced by a heterogeneous collection of disease pathways. Considering that CVT is a rare form of stroke affecting a much younger population and a more clinically homogenous form of stroke, we hypothesise that it is likely to be influenced by rare genetic variants with potentially larger effects compared with sporadic stroke.

The use of the Illumina Infinium HumanCoreExome BeadChip, which includes a significant number of exonic markers, will increase the probability of identifying functional genetic markers with potential large effects. The exome contains a large amount of rare protein-altering variants (missense, nonsense single-base substitutions, insertion–deletions) that are predicted to have functional roles and/or to be deleterious[41 42] which probably account for a considerable amount of the disease-causing mutations.[43] Thus, although the initial sample size of our CVT cohort is small due to the low prevalence/incidence of this disease, this highly phenotyped clinical and DNA repository of CVT cases has the potential of identifying novel coding functional variants associated with CVT with potential large effects. Increasing the sample size with more CVT cases and replicating any initial findings is clearly necessary and is being directly addressed by the BEAST Consortium.

Currently, the main limitation of our study is the insufficient power to detect genetic variants with small effects using the genome-wide approach due to the sample size of our study but continuous efforts are being made to enhance enrolment. An important advantage of our study is the thorough phenotyping using stringent inclusion and exclusion criteria and collection of large amount of clinical variables enabling not just genetic analysis but also allowing differences of associated risk factors or outcomes to be evaluated.

Establishing a large DNA repository of CVT cases worldwide will help elucidate its genetics leading to an improvement in our understanding of the pathophysiological mechanisms underlying this disease, identifying groups at risk and potentially facilitating the identification of novel therapeutic targets.

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Contributors IC was involved in study design, recruitment, contributed to developing the final protocol and drafted the manuscript. TM was involved in study design, recruitment, contributed to developing the final protocol and revising the manuscript. MSK, TP, AH and RD were involved in laboratory analysis and management of samples. SH, EH, TMM, JP, SMZ, MCB, SMP, PB, EP, PC, MC, PC, AT, RS, GF and DC were involved in recruitment and laboratory analysis. MM, EG, MZ, KS, AA, SD, GP, JMF, VT, AP, JJM, IM, JMC and TT are senior investigators who contributed with recruitment and sample collection. PS conceived the idea and is the principal investigator of BEAST who developed the final protocol and drafted the manuscript. All authors contributed intellectually to the protocol and draft versions of the manuscript and approved the final manuscript.

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REFERENCES

1. Ferro JM, Canhao P, Stam J, *et al*. Prognosis of cerebral vein and dural sinus thrombosis: results of the International Study on Cerebral Vein and Dural Sinus Thrombosis (ISCVT). *Stroke* 2004;35:664–70.
2. Coutinho JM, Zuurbier SM, Aramideh M, *et al*. The incidence of cerebral venous thrombosis: a cross-sectional study. *Stroke* 2012;43:3375–7.
3. Stam J. Thrombosis of the cerebral veins and sinuses. *N Engl J Med* 2005;352:1791–8.
4. Coutinho JM, Zuurbier SM, Stam J. Declining mortality in cerebral venous thrombosis: a systematic review. *Stroke* 2014;45:1338–41.
5. Corvol JC, Oppenheim C, Manai R, *et al*. Diffusion-weighted magnetic resonance imaging in a case of cerebral venous thrombosis. *Stroke* 1998;29:2649–52.
6. Yoshikawa T, Abe O, Tsuchiya K, *et al*. Diffusion-weighted magnetic resonance imaging of dural sinus thrombosis. *Neuroradiology* 2002;44:481–8.
7. Ehtisham A, Stern B. Cerebral venous thrombosis: a review. *Neurologist* 2006;12:32–8.
8. Spencer FA, Gore JM, Lessard D, *et al*. Venous thromboembolism in the elderly: a community-based perspective. *Thromb Haemostasis* 2008;100:780–8.
9. Lim HY, Ng C, Donnan G, *et al*. Ten years of cerebral venous thrombosis: male gender and myeloproliferative neoplasm is associated with thrombotic recurrence in unprovoked events. *J Thromb Thrombolysis* 2016;42:423–31.
10. Miranda B, Ferro JM, Canhão P, *et al*. Venous thromboembolic events after cerebral vein thrombosis. *Stroke* 2010;41:1901–6.
11. Koopman K, Uyttenboogaart M, Vroomen PC, *et al*. Risk factors for cerebral venous thrombosis and deep venous thrombosis in patients aged between 15 and 50 years. *Thromb Haemostasis* 2009;102:611–798.
12. Martinelli I, Sacchi E, Landi G, *et al*. High risk of cerebral-vein thrombosis in carriers of a prothrombin-gene mutation and in users of oral contraceptives. *N Engl J Med* 1998;338:1793–7.
13. Martinelli I, Battaglioli T, Pedotti P, *et al*. Hyperhomocysteinemia in cerebral vein thrombosis. *Blood* 2003;102:1363–6.
14. Junker R, Nabavi DG, Wolff E, *et al*. Plasminogen activator inhibitor-1 4G/4G-genotype is associated with cerebral sinus thrombosis in factor V Leiden carriers. *Thromb Haemostasis* 1998;80:706–7.
15. Le Cam-Duchez V, Bagan-Triquenot A, Barbay V, *et al*. The G79A polymorphism of protein Z gene is an independent risk factor for cerebral venous thrombosis. *J Neurol* 2008;255:1521–5.
16. Passamonti SM, Biguzzi E, Cazzola M, *et al*. The JAK2 V617F mutation in patients with cerebral venous thrombosis. *J Thromb Haemost* 2012;10:998–1003.
17. Marjot T, Yadav S, Hasan N, *et al*. Genes associated with adult cerebral venous thrombosis. *Stroke* 2010;42:913–18.
18. Cheng YC, Cole JW, Kittner SJ, *et al*. Genetics of ischemic stroke in young adults. *Circulation* 2014;7:383–92.
19. Cheng YC, O'Connell JR, Cole JW, *et al*. Genome-wide association analysis of ischemic stroke in young adults. *G3 (Bethesda)* 2011;1:505–14.
20. Schulz UGR, Flossmann E, Rothwell PM. Heritability of ischemic stroke in relation to age, vascular risk factors, and subtypes of incident stroke in population-based studies. *Stroke* 2004;35:819–24.
21. Deschiens MA, Conard J, Horellou MH, *et al*. Coagulation studies, factor V Leiden, and anticardiolipin antibodies in 40 cases of cerebral venous thrombosis. *Stroke* 1996;27:1724–30.
22. Rodeghiero F, Tosetto A. Activated protein C resistance and factor V Leiden mutation are independent risk factors for venous thromboembolism. *Ann Intern Med* 1999;130:643–50.
23. Salomon O, Steinberg DM, Zivelin A, *et al*. Single and combined prothrombotic factors in patients with idiopathic venous thromboembolism: prevalence and risk assessment. *Arterioscler Thromb Vasc Biol* 1999;19:511–18.
24. Dentali F, Crowther M, Ageno W. Thrombophilic abnormalities, oral contraceptives, and risk of cerebral vein thrombosis: a meta-analysis. *Blood* 2006;107:2766–73.
25. Venous thromboembolic disease and combined oral contraceptives: results of international multicentre case-control study. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. *Lancet* 1995;346:1575–82.
26. Coutinho JM, Ferro JM, Canhão P, *et al*. Cerebral venous and sinus thrombosis in women. *Stroke* 2009;40:2356–61.
27. Lao O, Lu TT, Nothnagel M, *et al*. Correlation between genetic and geographic structure in Europe. *Curr Biol* 2008;18:1241–8.
28. Power C, Elliott J. Cohort profile: 1958 British birth cohort (National Child Development Study). *Int J Epidemiol* 2006;35:34–41.
29. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–78.
30. Guo Y, He J, Zhao S, *et al*. Illumina human exome genotyping array clustering and quality control. *Nat Protocols* 2014;9:2643–62.
31. Selinger-Leneman H, Genin E, Norris JM, *et al*. Does accounting for gene-environment (GxE) interaction increase the power to detect the effect of a gene in a multifactorial disease? *Genet Epidemiol* 2003;24:200–7.
32. Skol AD, Scott LJ, Abecasis GR, *et al*. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet* 2006;38:209–13.
33. McCarthy MI, Abecasis GR, Cardon LR, *et al*. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genet* 2008;9:356–69.
34. Ikram MA, Seshadri S, Bis JC, *et al*. Genomewide Association Studies of Stroke. *N Engl J Med* 2009;360:1718–28.
35. International Stroke Genetics Consortium; Wellcome Trust Case-Control Consortium 2. Failure to validate association between 12p13 variants and ischemic stroke. *N Engl J Med* 2010;362:1547–50.
36. Olsson S, Melander O, Jood K, *et al*. Genetic variant on chromosome 12p13 does not show association to ischemic stroke in 3 Swedish case-control studies. *Stroke* 2011;42:214–16.
37. Bellenguez C, Bevan S, Gschwendtner A, *et al*. Genome-wide association study identifies a variant in HDAC9 associated with large vessel ischemic stroke. *Nat Genet* 2012;44:328–33.
38. Traylor M, Farrall M, Holliday EG, *et al*. Genetic risk factors for ischaemic stroke and its subtypes (the METASTROKE Collaboration): a meta-analysis of genome-wide association studies. *Lancet Neurol* 2012;11:951–62.
39. Kilarski LL, Achterberg S, Devan WJ, *et al*. Meta-analysis in more than 17,900 cases of ischemic stroke reveals a novel association at 12q24.12. *Neurology* 2014;83:678–85.
40. Meschia JF. Stroke genome-wide association studies: the large numbers imperative. *Stroke* 2010;41:579–80.
41. Kryukov GV, Pennacchio LA, Sunyaev SR. Most rare missense alleles are deleterious in humans: implications for complex disease and association studies. *Am J Hum Genet* 2007;80:727–39.
42. Bamshad MJ, Ng SB, Bigham AW, *et al*. Exome sequencing as a tool for Mendelian disease gene discovery. *Nat Rev Genet* 2011;12:745–55.
43. Ku CS, Cooper DN, Polychronakos C, *et al*. Exome sequencing: dual role as a discovery and diagnostic tool. *Ann Neurol* 2012;71:5–14.