Genome-Wide Association Study of Postoperative Cognitive Dysfunction in Older Surgical Patients

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Abstract

Background: Postoperative cognitive dysfunction (POCD) is a common neurocognitive complication after surgery and anesthesia, particularly in elderly patients. Various studies have suggested genetic risk factors for POCD. The study aimed to detect genome-wide associations of POCD in older patients.

Methods: In this prospective observational cohort study, participants aged ≥65 years completed a set of neuropsychological tests before, at 1 week, and 3 months after major noncardiac surgery. Test variables were converted into standard scores (z-scores) based on demographic characteristics. POCD was diagnosed if the decline was >1 standard deviation in ≥2 of the 15 variables in the assessment battery. A genome-wide association study (GWAS) was performed to determine potential alleles that are linked to the POCD phenotype. In addition, candidate genes for POCD were identified in a literature search for further analysis.

Results: Sixty-three patients with blood samples were included in the study. POCD was diagnosed in 47.6% of patients at 1 week and in 34.2% of patients at 3 months after surgery. Insufficient sample quality led to exclusion of 26 patients. In the remaining 37 patients, a GWAS was performed, but no association ($P < 5 \times 10^{-8}$) with POCD was found. The subsequent gene set enrichment analysis of 34 candidate genes did not reveal any significant associations.

Conclusion: In this patient cohort, a GWAS did not reveal an association between specific genetic alleles and POCD at 1 week and 3 months after surgery. Future genetic analysis should focus on specific candidate genes for POCD.

Trial registration: ClinicalTrials.gov (NCT02864173)

Background

Postoperative cognitive dysfunction (POCD) is characterized as a new onset of transient cognitive impairment after surgical intervention. POCD presents as a decline in cognitive performance such as lack of concentration and attention, disturbance of memory and learning function, and an inability to complete intellectual tasks. It affects up to 41% of older patients (≥60 years) on hospital discharge and 13%, 3 months after major noncardiac surgery [1]. Besides increasing age, risk factors for POCD include preexisting cognitive impairment [2], a history of stroke, lower level of education [1, 3], and alcohol abuse [4]. Although POCD is considered to be a transient impairment of cognitive functions, previous studies have shown that it may worsen quality of life [5, 6], leads to prolonged hospitalization and increased health care costs, and that it is associated with 1-year mortality [1, 7].

To date, the pathogenic mechanisms leading to POCD are not completely understood. Various etiologies have been discussed, and there is preliminary evidence for a genetic influence on the risk of developing POCD. Previous reports have focused on the analysis of the apolipoprotein E (APOEε4) genotype as a predisposing factor for POCD [8–11], and results were pooled in a recent meta-analysis [12]. Other studies have investigated polymorphisms of complement [13, 14], platelet glycoprotein IIIa (GPIIIa) [15], phosphodiesterase 4D (PDE4D) [16], P-selectin (SELP) [17], C-reactive protein (CRP) [17], inducible nitric oxide synthase promoter (iNOS) [18], and the brain-derived neurotrophic factor (BDNF) [19].
The objective of this secondary analysis of prospective cohort data was to investigate common genetic variations and single nucleotide polymorphisms (SNPs) systematically across the whole genome and to identify SNP genotypes that predispose older patients undergoing major noncardiac surgery to POCD.

Methods

This two-center prospective cohort study was approved by the Ethics Committee EKBB Basel (N° 75/07 and 340/08) and the Ethics Committee CER Lausanne (N° 247/09) in Switzerland. All participants provided written informed consent. Genetic sequencing of stored blood samples was authorized by amendment to the original study protocol (Ethics Committee EKNZ, N° PB_2016–02097). The study was retrospectively registered on ClinicalTrials.gov (NCT02864173) and conducted according to the STrengthening the REporting of OBservational studies in Epidemiology (STROBE) (An additional pdf file shows this in more detail [see Additional file 1]) guideline with STrengthening the REporting of Genetic Association studies (STREGA) extension. The work presented has been performed in accordance with the most recent version of the Helsinki Declaration.

Participants and setting

We included patients aged ≥ 65 years, American Society of Anesthesiologists (ASA) physical status I–IV, who were scheduled for major noncardiac surgery under standardized general anesthesia. Patients were eligible, if they were native German or French speakers and were physically able to participate in neuropsychological testing. Exclusion criteria were cardiac or neurosurgery, surgery within the past 12 months, patients with a history of intracranial or cerebrovascular pathology or psychiatric disease, preoperative Mini-Mental State Examination (MMSE) score < 24, and long-term psychopharmacological treatment. Study participants were followed-up until 3 months after surgery.

Management of anesthesia

All patients received standardized general anesthesia according to a predefined protocol using thiopental (3–5 mg kg\(^{-1}\) iv), fentanyl (1–3 µg kg\(^{-1}\) iv) and neuromuscular blockade with atracurium (0.5 mg kg\(^{-1}\)) for tracheal intubation. Maintenance of anesthesia was achieved with sevoflurane at approximately one minimal alveolar concentration (MAC). Vital signs were recorded using routine monitors.

Genetic analyses

Venous blood was collected into EDTA or serum tubes preoperatively. DNA was extracted using the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. After quality control with spectrophotometric measurement and Qubit (ThermoFisher Scientific, Zug, Switzerland), genotyping was performed using the Infinium Global Screening Array (GSA) 1.0 with multi-disease drop in (MD) on the fully automated iScan System (Illumina, San Diego, CA, USA). The Infinium GSA 1.0 + MD has a total of 700K markers including 590K markers tagging SNPs for the genome-wide backbone, 60K markers from the National Human Genome Research Institute genome-wide association study (NHGRI-GWAS), clinical and pharmacogenomics catalogs, and 50K markers from a large-scale meta-analysis. Subsequent quality control
procedures were implemented using PLINK (version 1.9) [20, 21]. Samples with low genotyping call rates (< 90%) were excluded. This resulted in 506,929 SNPs available for downstream analysis.

**Selection of candidate genes for gene set enrichment analysis**

The candidate genes were obtained by a literature search on the POCD phenotype in the PubMed and Cochrane Library databases using the following key words: “postoperative cognitive dysfunction AND polymorphism”, “postoperative cognitive dysfunction AND genotype”, “postoperative cognition AND polymorphism”, “cognitive impairment AND polymorphism” and “postoperative delirium AND genetic polymorphism”. The publications used for the gene set enrichment analysis are those, in the authors’ view, which might make a substantial contribution to the genotype of patients affected by POCD. Gene names were checked against the list in VEGAS2 [22], and were corrected by checking for synonymous names in the National Center for Biotechnology Information (NCBI) gene database, if necessary.

**Neuropsychological assessment**

The cognitive performance of study participants was tested using a sequence of neuropsychological assessments, namely the German and French versions of the Consortium to Establish a Registry for Alzheimer's Disease-Neuropsychological Assessment Battery (CERAD-NAB), the Trail Making Tests Part A and B, and the Phonemic Fluency Test (S-words), covering a wide range of cognitive functions. Tests were administered by trained investigators under the supervision of a neuropsychologist at baseline, and at 1 week and 3 months after surgery. Detailed content of the neuropsychological assessment and associated procedures have been described elsewhere [23]. After converting test variables into standard scores (z-scores) based on demographic variables (age, sex, and level of education) derived from a normative sample of cognitively healthy individuals [24], differences from baseline were calculated in patients who completed postoperative cognitive testing at 1 week and/or 3 months. POCD was diagnosed if the decline was > 1 standard deviation of z-scores ≥ 2 of the 15 variables in the test battery [25].

**Statistical analysis**

Descriptive statistics were used to describe the included patients and the quality of genotyping data. Continuous data were summarized using mean (SD), and categorical data were summarized using number (percentage). Differences between groups were calculated in SPSS (version 22, IBM, Armonk, NY, USA) using Pearson's $\chi^2$ test or the Mann-Whitney U test with a 2-sided significance level of $P < 0.05$.

A GWAS was performed based on the quality-controlled genotypes for the two postoperative time points (1 week and 3 months after surgery). We applied moderate quality parameters for genotype with a sample call rate of 90%, a minimum allele frequency of 1%, and a Hardy-Weinberg equilibrium of $10^{-6}$. Thirty-seven samples remained after quality control (18 cases with POCD and 19 cases without POCD at 1 week after surgery, and 12 cases with POCD and 25 cases without POCD at 3 months after surgery, respectively). We used the PLINK [20, 21] tool to perform an association analysis with age and baseline CERAD total score as covariates. The genome-wide significance threshold and the suggestive threshold were set at $P < 5 \times 10^{-8}$ and $P < 1 \times 10^{-5}$, respectively. Manhattan plots of the significance levels in the GWAS were generated using R (version
3.5.1) [26]. An additional candidate gene analysis was performed using VEGAS2 (version 2.0) [22] in order to address the cumulative impact of several markers in a gene that have moderate effects on the phenotype.

Given the hypothesis-generating nature of this investigation, we did not perform a statistical power analysis or sample size calculation.

**Results**

Eighty-six patients were enrolled at University Hospital Basel and Lausanne University Hospital, Switzerland, between August 2007 and October 2011. A study flowchart is shown in Fig. 1. Blood samples were missing in 23 patients. The remaining 63 patients all completed the neuropsychological assessment at 1 week after surgery; however, 25 patients did not complete testing at 3 months. Out of the 63 analyzed datasets, blood sample quality was insufficient to perform a GWAS in 26 cases. These samples were predominantly serum aliquots. Ultimately, a GWAS was performed in 37 subjects.

Thirty patients (47.6%) and 13 patients (34.2%) were diagnosed with POCD at 1 week and 3 months after surgery, respectively. There were no differences between patients with and without POCD in baseline demographics, medical comorbidities, and health risks (see Table 1). Patients underwent abdominal, gynecologic, urologic, vascular, orthopedic, or reconstructive procedures. However, there was no association between the type of surgery and the incidence of POCD.

No genome-wide significant associations ($P<5*10^{-8}$) or suggestive associations ($P<1*10^{-5}$) were attained in the GWAS, neither in patients affected by POCD at 1 week nor in those affected by POCD at 3 months after surgery. A Manhattan plot of the results is shown in Fig. 2. The literature search on the POCD phenotype yielded 34 genes of interest, which entered the candidate gene set enrichment analysis based on the VEGAS2 [22] toolkit. However, no significant enrichment could be detected (see Table 2).
| Baseline demographics | All patients (n = 63) | No POCD\(^a\) (n = 33) | POCD\(^a\) (n = 30) | \(P\) value |
|-----------------------|-----------------------|-------------------------|----------------------|-------------|
| Age; years            | 73.4 (7.0)            | 71.8 (5.9)              | 75.2 (7.8)           | 0.12        |
| Male sex; n (%)       | 40 (63.5)             | 20 (60.6)               | 20 (66.7)            | 0.62        |
| Education; years      | 12.3 (2.8)            | 12.9 (2.7)              | 11.6 (2.6)           | 0.07        |
| BMI; kg/m\(^2\)       | 26.2 (4.3)            | 26.5 (4.3)              | 26.0 (4.3)           | 0.38        |
| Medical comorbidities; n (%) |        |                        |                      |             |
| Diabetes mellitus     | 8 (12.7)              | 3 (9.1)                 | 5 (16.7)             | 0.37        |
| Arterial hypertension | 36 (57.1)             | 17 (51.5)               | 19 (63.3)            | 0.34        |
| History of cardiac disease | 10 (15.9)     | 6 (18.2)                | 4 (13.3)             | 0.60        |
| Atrial fibrillation   | 5 (7.9)               | 3 (9.1)                 | 2 (6.7)              | 0.72        |
| COPD                  | 11 (17.5)             | 5 (15.2)                | 6 (20.0)             | 0.61        |
| Peripheral artery disease | 6 (9.5)       | 4 (12.1)                | 2 (6.7)              | 0.46        |
| Health risks          |                       |                        |                      |             |
| Smoking; pack years   | 21.4 (31.6)           | 16.7 (24.0)             | 27.2 (38.7)          | 0.47        |
| Alcohol; units/week   | 11.0 (17.2)           | 10.3 (15.7)             | 11.8 (19.1)          | 0.79        |
| Neuropsychological tests; points |   |                        |                      |             |
| MMSE total score at baseline | 28.3 (1.4) | 28.6 (1.2)              | 28.0 (1.6)           | 0.14        |
| MMSE total score at 1 week | 27.9 (2.1) | 28.6 (1.4)              | 27.2 (2.5)           | 0.012       |
| MMSE total score at 3 months | 28.6 (1.3) | 28.9 (1.2)              | 28.3 (1.5)           | 0.23        |
| CERAD-NAB total score at baseline | 75.0 (10.9) | 76.9 (11.5)              | 72.9 (9.9)           | 0.08        |
| CERAD-NAB total score at 1 week | 78.7 (11.9) | 82.8 (9.7)              | 74.1 (12.5)          | 0.005       |
| CERAD-NAB total score at 3 months | 82.1 (9.7) | 82.9 (8.7)              | 81.0 (11.2)          | 0.78        |
| IADL scale at baseline | 7.6 (1.0)            | 7.8 (1.0)               | 7.5 (1.1)            | 0.16        |
| GDS-15 score at baseline | 1.3 (2.0)            | 1.0 (1.3)               | 1.6 (2.7)            | 0.40        |
|                          | All patients (n = 63) | No POCD<sup>a</sup> (n = 33) | POCD<sup>a</sup> (n = 30) | P value |
|--------------------------|-----------------------|-------------------------------|----------------------------|---------|

Data are presented as mean (SD) or number (percentage).

<sup>a</sup> at 1 week after surgery.

BMI indicates body mass index; CERAD-NAB, Consortium to Establish a Registry for Alzheimer’s Disease-Neuropsychological Assessment Battery; COPD, chronic obstructive pulmonary disease; GDS-15, Geriatric Depression Scale (15-item); IADL, Instrumental Activities of Daily Living; MMSE, Mini-Mental State Examination; POCD, postoperative cognitive dysfunction.
Table 2
Gene set enrichment analysis (VEGAS2) for 34 candidate genes

| Gene       | Markers in gene (n) | Corrected $P$ value | Top SNP          | Top SNP          | Corrected $P$ value | Top SNP          | Top SNP          |
|------------|---------------------|---------------------|------------------|------------------|---------------------|------------------|------------------|
| POCD at 1 week |            |                     |                  |                  |                      |                  |                  |
| APOE       | n/a*              |                     |                  |                  | 2                    | 0.4885           | rs7412           | 0.267           |
| BDNF       | 15                 | 0.557               | rs66866077       | 0.161            | 15                  | 0.6603           | rs7124442        | 0.220           |
| BDNF-AS    | 21                 | 0.934               | rs7127273        | 0.304            | 24                  | 0.8242           | rs10767646       | 0.156           |
| C3         | 11                 | 0.828               | rs3745567        | 0.201            | 10                  | 0.5604           | rs2250656        | 0.099           |
| C5         | 7                  | 0.629               | rs17216529       | 0.164            | 6                   | 0.9940           | rs79554848       | 0.618           |
| CFH        | 17                 | 0.711               | rs1061170        | 0.168            | 18                  | 0.6663           | rs1329423        | 0.137           |
| COMT       | 18                 | 0.585               | rs5993883        | 0.093            | 17                  | 0.4176           | rs174699         | 0.058           |
| CRP        | 2                  | 0.069               | rs1205           | 0.043            | 2                   | 0.3427           | rs1417938        | 0.210           |
| DBH        | 10                 | 0.720               | rs1108581        | 0.168            | 14                  | 0.6533           | rs3025416        | 0.105           |
| DRD1       | 5                  | 0.892               | rs5326           | 0.550            | 5                   | 0.0495           | rs5326           | 0.016           |
| DRD2       | 24                 | 0.123               | rs17529477       | 0.010            | 23                  | 0.3307           | rs4460839        | 0.038           |
| DRD3       | 14                 | 0.484               | rs17605608       | 0.081            | 15                  | 0.2048           | rs167770         | 0.022           |
| GRIN2B     | 74                 | 0.539               | rs75830871       | 0.017            | 68                  | 0.3217           | rs10772713       | 0.009           |
| GRIN3A     | 22                 | 0.571               | rs10989591       | 0.064            | 17                  | 0.3876           | rs1323423        | 0.042           |
| HAMP       | n/a*              |                     |                  |                  |                     |                  |                  |
| HFE        | 3                  | 0.191               | rs1799945        | 0.073            | 2                   | 0.9750           | rs1799945        | 0.845           |
| POCD at 3 months |            |                     |                  |                  |                      |                  |                  |

*Missing data for the APOE and HAMP gene set analysis for POCD at 1 week and POCD at 1 week and 3 months, respectively. This is due to the sample to group assignment and an insufficient number of SNPs for gene set analysis after applying quality thresholds.

APOE indicates Apolipoprotein E; BDNF, Brain Derived Neurotrophic Factor; BDNF-AS, BDNF Antisense RNA; C3, Complement C3; C5, Complement C5; CFH, Complement Factor H; COMT, Catechol-O-Methyltransferase; CRP, C-Reactive Protein; DBH, Dopamine Beta-Hydroxylase; DRD1, Dopamine Receptor D1; DRD2, Dopamine Receptor D2; DRD3, Dopamine Receptor D3; GRIN2B, Glutamate Ionotropic Receptor NMDA Type Subunit 2B; GRIN3A, Glutamate Ionotropic Receptor NMDA Type Subunit 3A; HAMP, Haptocorrin; HFE, Homocysteic Iron Regulator; HTR2A, 5-Hydroxtryptamine Receptor 2A; HTR2A-AS1, HTR2A Antisense RNA 1; IL6, Interleukin 6; IL6R, Interleukin 6 Receptor; ITGB3, Integrin Subunit Beta 3; MBL2, Mannose Binding Lectin 2; n/a, not applicable; NOS2, Nitric Oxide Synthase 2; NQO2, N-Ribosylhydroxycinnamide:Quinone Reductase 2; NR3C1, Nuclear Receptor Subfamily 3 Group C Member 1; PDE4D, Phosphodiesterase 4D; PER3, Period Circadian Regulator 3; POCD, postoperative cognitive dysfunction; SELP, Selectin P; SNP, single nucleotide polymorphism; SLC40A1, Solute Carrier Family 40 Member 1; SLC6A3, Solute Carrier Family 6 Member 3; SLC6A4, Solute Carrier Family 6 Member 4; SOAT1, Sterol O-Acytransferase 1; TF, Transferrin; WWC1, WW And C2 Domain Containing 1.
|                        | POCD at 1 week |            |            | POCD at 3 months |            |            |
|-----------------------|----------------|------------|------------|------------------|------------|------------|
| HTR2A                 | 27             | 0.462      | rs2224721  | 0.030            | 26         | 0.9780     | rs1002513  | 0.215          |
| HTR2A-AS1             | 3              | 0.969      | rs74970393 | 0.706            | 3          | 0.9590     | rs7984966  | 0.667          |
| IL6                   | 2              | 0.087      | rs2069837  | 0.041            | 2          | 0.7572     | rs2069837  | 0.500          |
| IL6R                  | 11             | 0.537      | rs12083537 | 0.128            | 11         | 0.4775     | rs4133213  | 0.103          |
| ITGB3                 | 12             | 0.796      | rs11868894 | 0.191            | 15         | 0.9540     | rs55989631 | 0.304          |
| MBL2                  | 5              | 0.275      | rs5030737  | 0.074            | 5          | 0.2148     | rs1800450  | 0.059          |
| NOS2                  | 9              | 0.531      | rs2297516  | 0.141            | 9          | 0.7183     | rs3794764  | 0.226          |
| NQO2                  | 8              | 0.860      | rs2071002  | 0.336            | 8          | 0.7532     | rs2071002  | 0.250          |
| NR3C1                 | 10             | 0.862      | rs10482633 | 0.219            | 10         | 0.9500     | rs10482672 | 0.316          |
| PDE4D                 | 179            | 0.984      | rs6869149  | 0.046            | 158        | 0.7682     | rs6869149  | 0.015          |
| PER3                  | 12             | 0.107      | rs228729   | 0.015            | 13         | 0.7193     | rs707467   | 0.171          |
| SELP                  | 12             | 0.512      | rs3917688  | 0.072            | 11         | 0.3636     | rs3766122  | 0.047          |
| SLC40A1               | 6              | 0.912      | rs34206448 | 0.486            | 6          | 0.8611     | rs35623329 | 0.424          |
| SLC6A3                | 8              | 0.902      | rs27072    | 0.317            | 7          | 0.8252     | rs2617605  | 0.274          |
| SLC6A4                | 7              | 0.407      | rs2066713  | 0.120            | 8          | 0.9391     | rs2066713  | 0.434          |
| SOAT1                 | 5              | 0.987      | rs13306729 | 0.596            | 6          | 0.7333     | rs2265932  | 0.206          |
| TF                    | 14             | 0.779      | rs8177197  | 0.216            | 14         | 0.9680     | rs1799899  | 0.432          |
| WWC1                  | 41             | 0.382      | rs10051783 | 0.014            | 36         | 0.9590     | rs17551608 | 0.121          |

*Missing data for the APOE and HAMP gene set analysis for POCD at 1 week and POCD at 1 week and 3 months, respectively. This is due to the sample to group assignment and an insufficient number of SNPs for gene set analysis after applying quality thresholds.

APOE indicates Apolipoprotein E; BDNF, Brain Derived Neurotrophic Factor; BDNF-AS, BDNF Antisense RNA; C3, Complement C3; C5, Complement C5; CFH, Complement Factor H; COMT, Catechol-O-Methyltransferase; CRP, C-Reactive Protein; DBH, Dopamine Beta-Hydroxylase; DRD1, Dopamine Receptor D1; DRD2, Dopamine Receptor D2; DRD3, Dopamine Receptor D3; GRIN2B, Glutamate Ionotropic Receptor NMDA Type Subunit 2B; GRIN3A, Glutamate Ionotropic Receptor NMDA Type Subunit 3A; HAMP, Hepcidin Antimicrobial Peptide; HFE, Homeostatic Iron Regulator; HTR2A, 5-Hydroxytryptamine Receptor 2A; HTR2A-AS1, HTR2A Antisense RNA 1; IL6, Interleukin 6; IL6R, Interleukin 6 Receptor; ITGB3, Integrin Subunit Beta 3; MBL2, Mannose Binding Lectin 2; n/a, not applicable; NOS2, Nitric Oxide Synthase 2; NQO2, N-Ribosyldihydronicotinamide:Quinone Reductase 2; NR3C1, Nuclear Receptor Subfamily 3 Group C Member 1; PDE4D, Phosphodiesterase 4D; PER3, Period Circadian Regulator 3; POCD, postoperative cognitive dysfunction; SELP, Selectin P; SNP, single nucleotide polymorphism; SLC40A1, Solute Carrier Family 40 Member 1; SLC6A3, Solute Carrier Family 6 Member 3; SLC6A4, Solute Carrier Family 6 Member 4; SOAT1, Sterol O-Acyltransferase 1; TF, Transferrin; WWC1, WW And C2 Domain Containing 1.

**Discussion**
To our knowledge, this is the first study that has systematically investigated the potential association between common variations across the genome and POCD using a GWAS. Due to the limited sample size, the main objective of this secondary analysis of existing cohort data was to generate hypotheses, which may subsequently be followed-up by independent research in patients with POCD. In our cohort, 47.6% of the patients were affected by POCD 1 week after surgery. This rate is comparable with findings from the International Study of Post-Operative Cognitive Dysfunction (ISPOCD) study [3]. At 3 months, POCD was present in 34.2% of patients; however, Monk et al. [1] and Moller et al. [3] reported a lower incidence (12.7% and 9.9%, respectively). Our findings also confirm that older and less educated patients are more likely affected by POCD.

Our investigation did not identify any genome-wide significant association ($P < 5 \times 10^{-8}$) between specific SNPs and POCD. This is probably due to the small number of samples, which prohibits the analysis of a complex phenotype such as POCD. These findings suggest that a single common SNP genotype for POCD is unlikely to exist. Further research with a larger number of samples is necessary to substantiate this assumption. However, it is still possible that specific combinations of SNP genotypes (or a burden of risk genotypes) can be a trigger for POCD. Depending on the precise genetic architecture, much larger sample sizes would be needed to identify these SNPs with high statistical confidence.

Specific candidate genes that are involved in cognitive impairment due to Alzheimer’s disease, dementia, and delirium have been studied in the context of POCD. Several studies suggest an association with the APOEε4 genetic variant and POCD [8, 9]. However, this finding is inconsistent [10, 11]. In a recent meta-analysis, Cao and coworkers found a significant link between the genetic polymorphism of APOEε4 and POCD, but concluded that the influence of a single large study made the association questionable [12]. Other candidate genes showing an association with POCD have been investigated [13–19]. In the light of these inconsistencies, the GWAS offers an exploratory study methodology.

**Strengths and limitations**

In 2018, a new nomenclature for the cognitive change associated with anesthesia and surgery was recommended by an expert panel [27]. Our study was launched prior to the nomenclature change; thus, we chose the terminology in use at the time. However, the diagnostic criteria for POCD used in this study are identical to those proposed for delayed neurocognitive recovery and mild neurocognitive disorder (decline of > 1 standard deviation in cognitive test performance).

We chose to correct for the covariables patient age and baseline CERAD-NAB total score in the GWAS in order to eliminate confounding. This is reasonable because advanced age and preexisting cognitive impairment are recognized as important independent non-genetic risk factors for POCD.

Our study has several limitations. Symptoms of POCD may include very subtle changes in cognition, which are newly diagnosed after surgery, and include impairments in memory and learning, planning and organizing, attention, speed of information processing, and speech. Assessment for POCD is complex because there is no single test that allows the identification of these symptoms. Therefore, a combination of neurocognitive tests is usually used. The prevalence of POCD depends on the assessment batteries used, the timing of tests, and the statistical methodology, making comparison between studies difficult [25, 28]. In addition, patients who are
lost to follow-up are an important issue in POCD research. In our study, only 38 out of 63 patients (60%) completed the neuropsychological assessment at 3 months after surgery. Despite the clinical relevance of POCD and the excellent characterization of patients, the small sample size is a limiting factor in this study. This is relevant if the phenotype is oligo- or even polygenic. Yet, there was a realistic chance to find hypothetical variants of high penetrance. Limited sample size is a frequent limitation in POCD research because this outcome measure requires repeated pre- and postoperative testing and is resource intensive. Another issue limiting the outreach of this study was the availability of biomaterial in studied subjects since a large proportion of blood samples were serum aliquots remaining from a previous investigation. Despite efforts to amplify genetic material in these samples, the yield and quality of the DNA extracted from serum aliquots were low, so samples had to be excluded from the analysis. The loss of information from these samples may not have occurred at random.

Conclusion

In conclusion, our findings confirm the incidence rate of POCD in older surgical patients that is reported in the literature. The GWAS and subanalysis of 34 candidate genes did not identify SNPs that are associated with POCD. Further research in large patient populations is needed to investigate the role of genetic polymorphisms and specific candidate genes in the etiology of POCD.

Abbreviations

ASA: American Society of Anesthesiologists; APOEε4: Apolipoprotein E; BDNF: Brain-derived neurotrophic factor; CERAD-NAB: Consortium to Establish a Registry for Alzheimer's Disease-Neuropsychological Assessment Battery; CRP: C-reactive protein; GPIIIa: Platelet glycoprotein IIIa; DNA: Deoxyribonucleic acid; EDTA: Ethylenediaminetetraacetic acid; GSA: Innium Global Screening Array; GWAS: Genome-wide association study; iNOS: Inducible nitric oxide synthase promoter; ISPOCD: International Study of Post-Operative Cognitive Dysfunction; iv: intravenous; MAC: minimal alveolar concentration; MD: Multi-disease drop; MMSE: Mini-Mental State Examination; NCBI: National Center for Biotechnology Information; NHGRI-GWAS: National Human Genome Research Institute genome-wide association study; PDE4D: Phosphodiesterase 4D; POCD: Postoperative cognitive dysfunction; SELP: P-selectin; SNPs: Single nucleotide polymorphisms; STREGA: STrengthening the REporting of Genetic Association studies; STROBE: STrengthening the REporting of Observational studies in Epidemiology

Declarations

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Authors’ contributions

Study concept and design: MR, CSR, SH, PH, ASW, AUM, LAS, NG. Acquisition of data: LAS, NG. Analysis and interpretation of data: MR, CSR, SH, PH, NG. Drafting of the manuscript: MR, ASW, SH, NG. Critical revision of the manuscript for important intellectual content: All authors. Final approval of the version to be published: All authors. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: All authors.

Availability of data and materials

The datasets generated and/or analyzed in the current study are not publicly available due the data security guidelines but are available from the corresponding author on reasonable request.

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Ethics approval and consent to participate

This two-center prospective cohort study was approved by the Ethics Committee EKBB Basel (N° 75/07 and 340/08) and the Ethics Committee CER Lausanne (N° 247/09) in Switzerland. All participants provided written informed consent.

Consent for publication

Not applicable.

Competing interests

LAS has received speaker honoraria from Medtronic Schweiz (Münchenbuchsee, Switzerland), Covidien (Neuhausen am Rheinfall, Switzerland) MSD, (Luzern, Switzerland), Hamilton Medical (Bonaduz, Switzerland), Lilly (Vernier, Switzerland), and Orion Pharma (Zug, Switzerland). NG has received consultancy fees from PIPRA AG (Zug, Switzerland). None of these fees were related to this study. The remaining authors have no competing interest to disclose.

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**Figures**
Figure 1

Study flowchart.
Manhattan plots of genome-wide associations in genotypes that passed quality control. Age and baseline CERAD total score were used as covariates. In both analyses, none of the markers reached the genome-wide significance level of $P < 5 \times 10^{-8}$ (red line) or the suggestive level of $P < 1 \times 10^{-5}$ (blue line). (A) Evaluation at 1 week after surgery (18 cases, 19 controls). (B) Evaluation at 3 months after surgery (12 cases, 25 controls). POCD indicates postoperative cognitive dysfunction.

**Supplementary Files**
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- Additionalfile1STROBESatementchecklist.pdf