

## Online Resource 1

### STROBE checklist for cohort studies with the STREGA additions for genetic association studies, mapped to the manuscript

Article: *PITX2* rs67249485 and age, not the postoperative inflammatory response, predict new-onset atrial fibrillation after cardiac surgery with cardiopulmonary bypass: a prospective genotyped cohort study (INFLACOR). Journal of Applied Genetics.

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Sources: von Elm E et al (2007) Lancet 370:1453–1457, [https://doi.org/10.1016/S0140-6736\(07\)61602-X](https://doi.org/10.1016/S0140-6736(07)61602-X); Little J et al (2009) PLoS Med 6:e1000022, <https://doi.org/10.1371/journal.pmed.1000022>. Item wording is paraphrased; locations refer to section headings of the submitted manuscript.

| Item | Topic                                   | STROBE recommendation (paraphrased)                                                                      | STREGA addition (paraphrased)                                                                                                                                                  | Where addressed in the manuscript                                                                                                                                |
|------|-----------------------------------------|----------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1a   | Title and abstract: design in the title | Indicate the study design with a commonly used term in the title or abstract                             |                                                                                                                                                                                | Title ('prospective genotyped cohort study'); Abstract                                                                                                           |
| 1b   | Title and abstract: summary             | Provide in the abstract an informative and balanced summary of what was done and found                   |                                                                                                                                                                                | Abstract                                                                                                                                                         |
| 2    | Background/rationale                    | Explain the scientific background and rationale                                                          |                                                                                                                                                                                | Introduction, paragraphs 1 to 3                                                                                                                                  |
| 3    | Objectives                              | State specific objectives and any prespecified hypotheses                                                | State whether the study is the first report of an association, a replication effort, or both                                                                                   | Introduction, last paragraph (question, aim, and statement that the analysis is a replication study with a different 4q25 marker)                                |
| 4    | Study design                            | Present key elements of study design early in the paper                                                  |                                                                                                                                                                                | Material and methods, Study design, ethics and population                                                                                                        |
| 5    | Setting                                 | Describe the setting, locations and relevant dates, including recruitment, follow-up and data collection |                                                                                                                                                                                | Study design (single tertiary centre; 22 October 2009 to 16 March 2011; follow-up to five years)                                                                 |
| 6a   | Participants: eligibility and selection | Give eligibility criteria and the sources and methods of selection and follow-up                         | Give information on the criteria and methods for selection of subsets of participants from a larger study or cohort                                                            | Study design (inclusion and exclusion criteria; exclusion of 158 patients with pre-existing atrial fibrillation and 3 with incomplete data; 364 of 525 analysed) |
| 6b   | Participants: matching                  | For matched studies, give matching criteria                                                              |                                                                                                                                                                                | Not applicable (unmatched cohort)                                                                                                                                |
| 7    | Variables                               | Define all outcomes, exposures, predictors, confounders and effect modifiers; give diagnostic criteria   | Define genetic exposures with widely used nomenclature; identify variables likely to be associated with population stratification                                              | Endpoints and candidate variables; Genotyping (rs numbers, risk and other alleles, Table 1); Study design (Polish patients of European ancestry)                 |
| 8    | Data sources and measurement            | For each variable give sources of data and methods of assessment; describe comparability of methods      | Describe laboratory methods, including source and storage of DNA, genotyping method and platform, error and call rates; state whether genotypes were assigned blind to outcome | Genotyping (blood sampling, DNA extraction, SNaPshot single-base extension, capillary electrophoresis, Sanger confirmation, call rate 98.4%, laboratory staff    |

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|      |                                  |                                                                                   |                                                                                                                                                                                                                                     | unaware of outcome); Endpoints (rhythm monitoring, cytokine assays)                                                                                                                                                                                                                                                                                                                              |
| 9    | Bias                             | Describe any efforts to address potential sources of bias                         | For quantitative outcomes, state whether potential bias from pharmacotherapy was investigated                                                                                                                                       | Limitations (observation window and treatment threshold bias associations towards the null; genotype unknown to treating clinicians; assay dropout considered). STREGA addition not applicable: binary outcome                                                                                                                                                                                   |
| 10   | Study size                       | Explain how the study size was arrived at                                         |                                                                                                                                                                                                                                     | Study design (Kelsey formula, 80% power for an odds ratio of at least 1.65, n = 492 rounded to 525)                                                                                                                                                                                                                                                                                              |
| 11   | Quantitative variables           | Explain how quantitative variables were handled and any groupings chosen          | If applicable, describe how effects of treatment were dealt with                                                                                                                                                                    | Statistical analysis (age per 10-year increment; Youden threshold of 64 years; continuous versus categorical testing). STREGA addition not applicable                                                                                                                                                                                                                                            |
| 12a  | Statistical methods: all methods | Describe all statistical methods, including those used to control for confounding | State software version and options; describe how Hardy-Weinberg equilibrium was tested; state the genetic model; describe correction for multiple comparisons and handling of population stratification, haplotypes and relatedness | Statistical analysis (Epi Info 7, Statistica 12; $\chi^2$ goodness of fit for Hardy-Weinberg equilibrium; dominant model for <i>PITX2</i> because no risk-allele homozygote was observed; Bonferroni correction; cross-tabulation for confounding). Population stratification: not formally tested, ethnically homogeneous Polish cohort. Haplotypes and relatedness: not assessed (Limitations) |
| 12b  | Subgroups and interactions       | Describe methods used to examine subgroups and interactions                       |                                                                                                                                                                                                                                     | Statistical analysis and Results (age strata; weighted risk ratio across strata)                                                                                                                                                                                                                                                                                                                 |
| 12c  | Missing data                     | Explain how missing data were addressed                                           |                                                                                                                                                                                                                                     | Genotyping (358 of 364 genotyped); Statistical analysis (mortality in 363 with vital status); Limitations (left-atrial diameter incompletely recorded)                                                                                                                                                                                                                                           |
| 12d  | Loss to follow-up                | Cohort study: explain how loss to follow-up was addressed                         |                                                                                                                                                                                                                                     | Statistical analysis (mortality analysed in patients with vital status at all three time points)                                                                                                                                                                                                                                                                                                 |
| 12e  | Sensitivity analyses             | Describe any sensitivity analyses                                                 |                                                                                                                                                                                                                                     | None performed beyond the age-stratified analysis (Results, multivariable model)                                                                                                                                                                                                                                                                                                                 |
| 13a  | Participants: numbers            | Report numbers at each stage (eligible, examined, confirmed                       | Report numbers of individuals in whom genotyping                                                                                                                                                                                    | Study design and Results (525 enrolled; 364                                                                                                                                                                                                                                                                                                                                                      |

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|      |                                   | eligible, included, followed up, analysed)                                                                                          | was attempted and in whom it was successful                                                                                                                   | analysed; genotyping successful in 358, 98.4%; mortality in 363)                                                                                                                                                                              |
| 13b  | Non-participation                 | Give reasons for non-participation at each stage                                                                                    |                                                                                                                                                               | Study design (exclusions: pre-existing atrial fibrillation, incomplete data)                                                                                                                                                                  |
| 13c  | Flow diagram                      | Consider use of a flow diagram                                                                                                      |                                                                                                                                                               | Not included; the numbers are given in the text. A flow diagram of the parent cohort is published in Kowalik et al. 2020                                                                                                                      |
| 14a  | Descriptive data: characteristics | Give characteristics of participants and information on exposures and potential confounders                                         | Consider giving genotype frequencies by outcome status                                                                                                        | Results, first paragraph (parent-cohort characteristics by reference to Kowalik et al. 2018 and 2026); Online Resource 2 (clinical characteristics of the analytical sample by outcome); Table 1 (genotype counts and frequencies by outcome) |
| 14b  | Descriptive data: missing         | Indicate number of participants with missing data for each variable of interest                                                     |                                                                                                                                                               | Genotyping (6 without genotype); Limitations (left-atrial diameter)                                                                                                                                                                           |
| 14c  | Follow-up time                    | Cohort study: summarise follow-up time                                                                                              | Report the results of any Hardy-Weinberg equilibrium test                                                                                                     | Endpoints (30-day, one-year and five-year mortality). Hardy-Weinberg results: Results, Genetic risk factors, and Table 1 footnote                                                                                                             |
| 15   | Outcome data                      | Cohort study: report numbers of outcome events or summary measures over time                                                        | Report outcomes by genotype categories                                                                                                                        | Results (115 of 364 with the outcome; Table 3); Fig. 2 and Table 1 (outcome by genotype)                                                                                                                                                      |
| 16a  | Main results: estimates           | Give unadjusted and, if applicable, confounder-adjusted estimates with precision; state which confounders were adjusted for and why | Report results of any adjustments for multiple comparisons                                                                                                    | Tables 1 and 2; Results, multivariable model (candidate variables and collinear terms explained; Bonferroni-corrected p values)                                                                                                               |
| 16b  | Category boundaries               | Report category boundaries when continuous variables were categorised                                                               |                                                                                                                                                               | Results (age per 10 years; at-risk age 64 years or older)                                                                                                                                                                                     |
| 16c  | Absolute risk                     | If relevant, translate relative risk into absolute risk                                                                             |                                                                                                                                                               | Results and Fig. 2 (incidence 40.8% in heterozygous carriers versus 26.3% in non-carriers)                                                                                                                                                    |
| 17   | Other analyses                    | Report other analyses done (subgroups, interactions, sensitivity analyses)                                                          | If numerous genetic exposures were examined, summarise results from all analyses; if detailed results are available elsewhere, state how they can be accessed | Results (age strata; cross-tabulation; ROC-AUC; the ten inflammation-related variants of the original protocol summarised as null, detailed in Kowalik et al. 2018 and 2020)                                                                  |
| 18   | Key results                       | Summarise key results with reference to study objectives                                                                            |                                                                                                                                                               | Discussion, first paragraph                                                                                                                                                                                                                   |
| 19   | Limitations                       | Discuss limitations, including sources of bias or imprecision,                                                                      |                                                                                                                                                               | Discussion, Limitations (endpoint definition,                                                                                                                                                                                                 |

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|------|------------------|------------------------------------------------------------------------------------------------------------------------------|-------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
|      |                  | and their direction and magnitude                                                                                            |                               | Hardy-Weinberg departure and possible allele dropout, incomplete left-atrial data, restricted panel, era, single centre)         |
| 20   | Interpretation   | Give a cautious overall interpretation considering objectives, limitations, multiplicity, similar studies and other evidence |                               | Discussion, sections on the 4q25/ <i>PITX2</i> locus, prediction, the negative inflammatory result and early outcome; Conclusion |
| 21   | Generalisability | Discuss the generalisability (external validity) of the results                                                              |                               | Limitations, final paragraph (single centre, Polish population of European ancestry, replication required)                       |
| 22   | Funding          | Give the source of funding and the role of the funders                                                                       |                               | Statements and Declarations, Funding; title page, Acknowledgements                                                               |