**Supplementary materials**

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Supplementary Table 7: All differentially methylated probes (DMP) associated with alcohol consumption identified from nine studies included in the review

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Supplementary Table 9: KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways and GO (Gene Ontology) pathways associated with identified DMP in Dugué et al.

**Word file**

Supplementary Table 10: PRISMA 2020 Checklist

**Supplementary Table 1:**Search strategy in PubMed

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Database/  Consultation date | # | Concept | Search strategy | Results | Combination of concepts | |
| # | Total |
| PubMed/  20201220 | #1 | Outcome = DNA methylation | ("Methylation/etiology"[Mesh] OR "Methylation/genetics"[Mesh] OR "Methylation/immunology"[Mesh] OR "Methylation/physiology"[Mesh] OR "Epigenomics"[Mesh] OR "Epigenesis, Genetic"[Mesh:NoExp] OR "Epigenetic Repression"[Mesh] OR "Gene Silencing"[Mesh] OR "Epigenome"[Mesh]  OR "epigenome"[tiab] OR "epigenetic"[tiab] OR "epigenetics"[tiab] OR "epigenomic"[tiab] OR "epigenomics"[tiab] OR "methylome"[tiab] OR "methylomes"[tiab] OR "methylat\*"[tiab] OR "hypomethylat\*"[tiab] OR "hypermethylat\*"[tiab] OR "EWAS"[tiab]) | 257374 | #1 AND  #2 NOT  #3 | 2394 |
| #2 | Exposure = Alcohol consumption | ("Alcohol-Induced Disorders/genetics"[Mesh] OR "Drinking Behavior/genetics"[Mesh] OR "Alcohol-Related Disorders/genetics"[Mesh] OR "Alcohol Drinking"[Mesh:NoExp] OR "Alcoholic Beverages"[Mesh] OR "Ethanol"[Mesh:NoExp]  OR "drinking behavior"[tiab] OR "alcoholism"[tiab] OR "alcohol"[tiab] OR "liquor"[tiab] OR "beverage\*"[tiab] OR "beer"[tiab] OR "cider"[tiab] OR "wine"[tiab] OR "spirits"[tiab] OR "ethanol"[tiab] OR "absinthe"[tiab] OR "brandy"[tiab] OR "gin"[tiab] OR "vodka"[tiab] OR "rum"[tiab] OR "sake"[tiab] OR "whiskey"[tiab] OR "tequila"[tiab] OR "drinking behavior"[OT] OR "alcoholism"[OT] OR "alcohol"[OT] OR "liquor"[OT] OR "alcoholic beverage\*"[OT] OR "beverage\*"[OT] OR "beer"[OT] OR "cider"[OT] OR "wine"[OT] OR "spirits"[OT] OR "ethanol"[OT] OR "absinthe"[OT] OR "brandy"[OT] OR "gin"[OT] OR "vodka"[OT] OR "rum"[OT] OR "sake"[OT] OR "whiskey"[OT] OR "tequila"[OT]) | 460048 |
| #3 | Population = human | ("Animals"[Mesh] NOT "Humans"[Mesh]) | 4768732 |

**Supplementary Table 2:**Search strategy in Embase

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Database/  Consultation date | # | Concept | Search strategy | Results | Combination of concepts | |
| # | Total |
| Embase/20201220 | #1 | Outcome = DNA methylation | ('methylation'/de OR 'DNA methylation'/de OR 'epigenetics'/de OR 'epigenetic repression'/de OR 'gene silencing'/de OR 'epigenome'/exp  OR 'epigenome':ti,ab,kw OR 'epigenetic':ti,ab,kw OR 'epigenetics':ti,ab,kw OR 'epigenomic':ti,ab,kw OR 'epigenomics':ti,ab,kw OR 'methylome':ti,ab,kw OR 'methylomes':ti,ab,kw OR 'methylat\*':ti,ab,kw OR 'hypomethylat\*':ti,ab,kw OR 'hypermethylat\*':ti,ab,kw OR 'EWAS':ti,ab,kw) | 367309 | #1 AND#2 NOT#3 AND #4 | 3731 |
| #2 | Exposure = Alcohol consumption | ('alcohol'/de OR 'drinking behavior'/de OR 'alcoholism'/de OR 'alcoholic beverage'/exp  OR 'drinking behavior':ti,ab,kw OR 'alcoholism':ti,ab,kw OR 'alcohol':ti,ab,kw OR 'liquor':ti,ab,kw OR 'beverage\*':ti,ab,kw OR 'beer':ti,ab,kw OR 'cider':ti,ab,kw OR 'wine':ti,ab,kw OR 'spirits':ti,ab,kw OR 'ethanol':ti,ab,kw OR 'absinthe':ti,ab,kw OR 'brandy':ti,ab,kw OR 'gin':ti,ab,kw OR 'vodka':ti,ab,kw OR 'rum':ti,ab,kw OR 'sake':ti,ab,kw OR 'whiskey':ti,ab,kw OR 'tequila':ti,ab,kw) | 688910 |
| #3 | Population = human | ('animal'/exp NOT 'human'/exp) | 5537368 |
|  | #4 | Database = Embase | [embase]/lim | 26720391 |

**Supplementary Table 3:**Search strategy in Web of Science

*Indexes “Science Citation Index Expanded (SCI-EXPANDED) –1900-present” and “Emerging Sources Citation Index (ESCI) –2005-present” were selected for the search strategy in Web of Science.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Database/  Consultation date | # | Concept | Search strategy | Results | Combination of concepts | |
| # | Total |
| Web of Science (SCI-EXPANDED AND ESCI)/20201220 | #1 | Outcome = DNA methylation | TS=("epigenome" OR "epigenetic$" OR "epigenomic$" OR "methylome$" OR "methylat\*" OR "hypomethylat\*" OR "hypermethylat\*" OR "EWAS") | 233840 | #1 AND  #2 | 4325 |
| #2 | Exposure = Alcohol consumption | TS=("drinking behavior" OR "alcoholism" OR "alcohol" OR "liquor" OR "beverage$" OR "beer" OR "cider" OR "wine" OR "spirits" OR "ethanol" OR "absinthe" OR "brandy" OR "gin" OR "vodka" OR "rum" OR "sake" OR "whiskey" OR "tequila") | 747731 |

No limitation on humans was applied in the search strategy because there is no human/animal separation in this database. This option is exclusive to biomedical databases (PubMed and Embase).

**Supplementary Table 4:**Search strategy in CINAHL

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Database/  Consultation date | # | Concept | Search strategy | Results | Combination of concepts | |
| # | Total |
| CINAHL/20201220 | #1 | Outcome = DNA methylation | (MH "DNA Methylation") OR (MH "Methylation") OR (MH "Epigenomics")  OR (TI(epigenome OR epigenetic OR epigenetics OR epigenomic OR epigenomics OR methylome OR methylomes OR "methylat\*" OR "hypomethylat\*" OR "hypermethylat\*" OR EWAS) OR AB(epigenome OR epigenetic OR epigenetics OR epigenomic OR epigenomics OR methylome OR methylomes OR "methylat\*" OR "hypomethylat\*" OR "hypermethylat\*" OR EWAS)) | 13477 | #1 AND  #2 | 450 |
| #2 | Exposure = Alcohol consumption | (MH "Alcohol-Related Disorders") OR (MH "Drinking Behavior") OR (MH "Alcohol Drinking") OR (MH "Alcoholic Beverages") OR (MH "Ethanol")  OR (TI("drinking behavior" OR alcoholism OR alcohol OR liquor OR "beverage\*" OR beer OR cider OR wine OR spirits OR ethanol OR absinthe OR brandy OR gin OR vodka OR rum OR sake OR whiskey OR tequila) OR AB("drinking behavior" OR alcoholism OR alcohol OR liquor OR "beverage\*" OR beer OR cider OR wine OR spirits OR ethanol OR absinthe OR brandy OR gin OR vodka OR rum OR sake OR whiskey OR tequila)) | 115157 |

No limitation on humans was applied in the search strategy because the exclusion of animal studies in a non-biomedical database is riskier.

**Supplementary Table 5:**Search strategy in PsycInfo Ovid

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Database/  Consultation date | # | Concept | Search strategy | Results | Combination of concepts | |
| # | Total |
| PsycInfo Ovid/20201220 | #1 | Outcome = DNA methylation | Epigenetics/  OR epigenome.ti,ab,id OR epigenetic.ti,ab,id OR epigenetics.ti,ab,id OR epigenomic.ti,ab,id OR epigenomics.ti,ab,id OR methylome.ti,ab,id OR methylomes.ti,ab,id OR methylat\*.ti,ab,id OR hypomethylat\*.ti,ab,id OR hypermethylat\*.ti,ab,id OR EWAS.ti,ab,id | 8215 | #1 AND  #2 | 466 |
| #2 | Exposure = Alcohol consumption | Drinking Behavior/ OR Alcoholism/ OR Alcohol Abuse/ OR Alcohols/ OR Alcohol Drinking Patterns/ OR Alcoholic Beverages/ OR Ethanol/  OR "drinking behavior".ti,ab,id OR alcoholism.ti,ab,id OR alcohol.ti,ab,id OR liquor.ti,ab,id OR "beverage\*".ti,ab,id OR beer.ti,ab,id OR cider.ti,ab,id OR wine.ti,ab,id OR spirits.ti,ab,id OR ethanol.ti,ab,id OR absinthe.ti,ab,id OR brandy.ti,ab,id OR gin.ti,ab,id OR vodka.ti,ab,id OR rum.ti,ab,id OR sake.ti,ab,id OR whiskey.ti,ab,id OR tequila.ti,ab,id | 147823 |

No limitation on humans was applied in the search strategy because the exclusion of animal studies in a non-biomedical database is riskier.

**Supplementary Table 6:** Risk of bias assessment of cross-sectional studies (n=11) using JBI tool

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Q1** | **Q2** | **Q3** | **Q4** | **Q5** | **Q6** | **Q7** | **Q8** |
| **Cronjé et al.** | Yes | Yes | Yes | Unclear | Yes | Yes | Yes | Yes |
| **Langdon et al.** | Yes | Yes | Unclear | Unclear | Yes | Yes | Yes | Yes |
| **Liang et al.** | Yes | Yes | Yes | Unclear | Yes | Yes | Yes | Yes |
| **Stephenson et al.** | Yes | Unclear | Yes | Unclear | Yes | Yes | Yes | Yes |
| **Dugué et al.** | Yes | Unclear | Yes | Unclear | Yes | Yes | Yes | Yes |
| **Perrier et al.** | Yes | Unclear | Yes | Unclear | No | Yes | Yes | Yes |
| **Wilson et al.** | Yes | Yes | Unclear | Unclear | Yes | Yes | Yes | Yes |
| **Liu et al.** | Yes | Yes | Unclear | Unclear | No | Yes | Yes | Yes |
| **McCartney et al.** | Yes | Unclear | Unclear | Unclear | No | Yes | Yes | Yes |
| **Clark et al.** | Yes | Unclear | Unclear | Unclear | Yes | Yes | Yes | Yes |
| **Flanagan et al.** | Yes | Yes | Unclear | Unclear | Yes | Yes | Yes | Yes |

**Q1:** Were the criteria for inclusion in the sample clearly defined?; **Q2:** Were the study subjects and the setting described in detail?; **Q3:** Was the exposure measured in a valid and reliable way?; **Q4:** Were objective, standard criteria used for measurement of the condition?; **Q5:** Were confounding factors identified?; **Q6:** Were strategies to deal with confounding factors stated?; **Q7:** Were the outcomes measured in a valid and reliable way?; **Q8:** Was appropriate statistical analysis used?

**Supplementary Table 10:** PRISMA 2020 Checklist

| **Section and Topic** | **Item #** | **Checklist item** | **Line number(s)** |
| --- | --- | --- | --- |
| **TITLE** | | |  |
| Title | 1 | Identify the report as a systematic review. | See Title page |
| **ABSTRACT** | | |  |
| Abstract | 2 | See the PRISMA 2020 for Abstracts checklist. | 2-9 |
| **INTRODUCTION** | | |  |
| Rationale | 3 | Describe the rationale for the review in the context of existing knowledge. | 21-62 |
| Objectives | 4 | Provide an explicit statement of the objective(s) or question(s) the review addresses. | 62-63 |
| **METHODS** | | |  |
| Eligibility criteria | 5 | Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses. | 70-94 |
| Information sources | 6 | Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted. | 97-99 |
| Search strategy | 7 | Present the full search strategies for all databases, registers and websites, including any filters and limits used. | 102-107 |
| Selection process | 8 | Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process. | 110-114 |
| Data collection process | 9 | Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process. | 117-118 |
| Data items | 10a | List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect. | 128-132 |
| 10b | List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information. | 121-128 |
| Study risk of bias assessment | 11 | Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process. | 135-138 |
| Effect measures | 12 | Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results. | 141-146 |
| Synthesis methods | 13a | Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)). | NA |
| 13b | Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions. | NA |
| 13c | Describe any methods used to tabulate or visually display results of individual studies and syntheses. | NA |
| 13d | Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used. | 149-158 |
| 13e | Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression). | NA |
| 13f | Describe any sensitivity analyses conducted to assess robustness of the synthesized results. | NA |
| Reporting bias assessment | 14 | Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases). | Not done |
| Certainty assessment | 15 | Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome. | Not done |
| **RESULTS** | | |  |
| Study selection | 16a | Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram. | 162-164 |
| 16b | Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded. | NA |
| Study characteristics | 17 | Cite each included study and present its characteristics. | 167-183 |
| Risk of bias in studies | 18 | Present assessments of risk of bias for each included study. | 186-198 |
| Results of individual studies | 19 | For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots. | 201-219 |
| Results of syntheses | 20a | For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies. | 222-236 |
| 20b | Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect. | NA |
| 20c | Present results of all investigations of possible causes of heterogeneity among study results. | NA |
| 20d | Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results. | NA |
| Reporting biases | 21 | Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed. | Not done |
| Certainty of evidence | 22 | Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed. | Not done |
| **DISCUSSION** | | |  |
| Discussion | 23a | Provide a general interpretation of the results in the context of other evidence. | 239-332 |
| 23b | Discuss any limitations of the evidence included in the review. | 333-356 |
| 23c | Discuss any limitations of the review processes used. | 357-360 |
| 23d | Discuss implications of the results for practice, policy, and future research. | 361-371 |
| **OTHER INFORMATION** | | |  |
| Registration and protocol | 24a | Provide registration information for the review, including register name and registration number, or state that the review was not registered. | NA |
| 24b | Indicate where the review protocol can be accessed, or state that a protocol was not prepared. | NA |
| 24c | Describe and explain any amendments to information provided at registration or in the protocol. | NA |
| Support | 25 | Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review. | See Title page |
| Competing interests | 26 | Declare any competing interests of review authors. | See Title page |
| Availability of data, code and other materials | 27 | Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review. | 416-438 |

*From:*  Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

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