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SOFTWARE FOCUS

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Easy-Amanida: An R Shiny application for the meta-analysis of aggregate results in clinical metabolomics using Amanida and Webchem

Maria Llambrich[1,2] | Pau Satorra[3] | Eudald Correig[4] | Josep Gumà[5] | Jesús Brezmes[1,2] | Cristian Tebé[3] | Raquel Cumeras[1,2,5]

[1]Department of Electrical Electronic Engineering and Automation, Universitat Rovira i Virgili, IISPV, Tarragona, Spain

[2]Metabolomics Interdisciplinary Laboratory, Department of Nutrition and Metabolism, Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus, Spain

[3]Biostatistics Unit, Bellvitge Institute for Biomedical Research (IDIBELL), Hospitalet de Llobregat, Spain

[4]Department of Biostatistics, Universitat Rovira i Virgili, Reus, Spain

[5]Oncology Department, Hospital Universitari Sant Joan de Reus, Institut d'Investigació Sanitària Pere Virgili, Universitat Rovira i Virgili, Reus, Spain

Correspondence
Raquel Cumeras, Department of Electrical Electronic Engineering and Automation, Universitat Rovira i Virgili, IISPV, Tarragona 43007, Spain.
Email: raquel.cumeras@iispv.cat

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Abstract

Meta-analysis is a useful tool in clinical research, as it combines the results of multiple clinical studies to improve precision when answering a particular scientific question. While there has been a substantial increase in publications using meta-analysis in various clinical research topics, the number of published meta-analyses in metabolomics is significantly lower compared to other omics disciplines. Metabolomics is the study of small chemical compounds in living organisms, which provides important insights into an organism's phenotype. However, the wide variety of compounds and the different experimental methods used in metabolomics make it challenging to perform a thorough meta-analysis. Additionally, there is a lack of consensus on reporting statistical estimates, and the high number of compound naming synonyms further complicates the process. Easy-Amanida is a new tool that combines two R packages, "amanida" and "webchem", to enable meta-analysis of aggregate statistical data, like *p*-value and fold-change, while ensuring the compounds naming harmonization. The Easy-Amanida app is implemented in Shiny, an R package add-on for interactive web apps, and provides a workflow to optimize the naming combination. This article describes all the steps to perform the meta-analysis using Easy-Amanida, including an illustrative example for interpreting the results. The use of aggregate statistics metrics extends the use of Easy-Amanida beyond the metabolomics field.

KEYWORDS
aggregate statistics, meta-analysis, metabolomics, name harmonization, Shiny app

Highlights

What is already known

- Naming harmonization is needed in metabolomics.
- Mostly reported aggregate statistical data in metabolomics.

What is new

- Integration of Amanida and Webchem packages to perform a harmonized naming meta-analysis of aggregate statistical data.

Potential impact for Research Synthesis Methods readers outside the authors' field

- Easy-Amanida is easy to use and reachable worldwide as a Shiny web app.
- Other omics data can be used with Easy-Amanida.

1 | INTRODUCTION

Meta-analysis is a highly valuable tool in clinical research, as it combines the results of multiple clinical studies answering the same question on the scientific topic studied.[1] In recent years, there has been a substantial increase in publications using meta-analysis across a wide range of clinical research topics, with over 40,000 meta-analysis reports now available on PubMed (search using keyword: meta-analysis).[2] Meta-analyses are generally considered the highest level of evidence synthesis in biomedical research. Obtaining a quantitative estimate clarifies the heterogeneity of different studies and increases the statistical power.[3] In that sense, being able to combine results from different sources needs to follow strict guidelines, as reported by Cochrane.[4]

However, in the field of metabolomics, the number of published meta-analyses is significantly lower (~400) compared to other omics disciplines (e.g., 10,000 for genomics). Metabolomics is the study of the small chemical compounds present in living organisms. These compounds are influenced by both internal and external factors, and therefore they can provide important insights into an organism's phenotype. The study of the metabolome in clinical applications allows the detection of changes caused by diseases faster than with other omic approaches. Consequently, metabolomics is increasing its popularity as a diagnosis tool.[5–7] The power of metabolomics relies on high throughput instrumentation, allowing to detect in a single analysis a large number of compounds simultaneously. The wide number of compounds in the human metabolome, with more than 200,000 entities,[8] creates a significant amount of variability in the findings between studies. Additionally, the selection of the experimental methodology used can affect the fraction of compounds captured, further complicating the process of combining the results from different multiple studies.

Unfortunately, the guidelines used for other omic disciplines are hard to apply in metabolomics due to the nature of the data. In metabolomics, different experimental methods imply the identification of a vast number of compounds, which will differ from one study to another, making it challenging to perform a thorough meta-analysis, where the aim is to have the same elements evaluated in all studies. Moreover, the metrics disclosed in most studies are not self-explanatory enough. For example, non-numeric values are the only ones disclosed in many reports. Due to the lack of consensus about reporting statistical estimates,[9–11] it is almost impossible to apply the existing meta-analysis methods to metabolomics studies. The toolboxes for meta-analysis available,[12–14] require the complete statistical information (mean and standard deviations) or the raw data.

However, certain meta-analysis methods have not yet been widely applied to metabolomics data. The combination of *p*-values is a well-established approach employed in genome-wide association studies (GWAS) and gene expression studies.[15,16] This method facilitates the identification of consistently significant results across diverse datasets, while the computation cost is small. Additionally, this method is also valuable in addressing study heterogeneity heterogeneity and the controlling Type I Error through adjustments for multiple testing. In our method, to complement the *p*-value, we incorporated fold-change to account for factors such as differences on equipment sensibility. To date, the Amanida meta-analysis has been used successfully employed in five meta-analysis, encompassing biomarker discovery for colorectal cancer data,[17] pregnancy,[18] hepatocellular carcinoma,[19] urogenital cancer,[20] and Parkinson's disease.[21] Conversely, in metabolomics when raw data are not available is usual to conduct analysis at higher levels such as pathways[22] or ontologies.[23] These approaches involves identifying compounds that exhibit over-representation and potentially correlate with specific phenotypes or diseases. Notably, widely-used tools in metabolomics research, such as MetaboAnalyst, MetExplore, or Reactome,[24] primarily facilitate the identification of metabolite groups or pathways. Despite the utility of these tools in initial analyses, they are limited in their capacity to delve into deeper clinical insights. They excel in pointing associations at a broader scale but fall short in providing the detailed analysis required for comprehensive clinical applications.

Another problem the metabolomics community faces is the high number of compounds naming synonyms.[25] There are plenty of public and private databases for metabolites, where each one chooses their own identifier following different rules or criteria. The different identifiers lead to finding the same metabolite with different names, such as acetic acid and acetate, which are conjugates of the same compound. Moreover, the researchers sometimes use abbreviations of the chemical name, impeding the duplicates detection. Several initiatives to use unique identifiers have been reported, such as CAS,[26] InChIKey,[27] or PubChem CID[2] but the implementation on published papers is scarce.[28] While other omics have reached the standardized data format, in metabolomics has not been achieved.

The reuse of published metabolomics data through meta-analysis holds significant importance in advancing metabolomics research. But to extract the maximum knowledge from the wealth of metabolomics available is needed the certainty of the compounds name and the use of aggregate statistics. Easy-Amanida is designed to overcome the metabolomics limitations for meta-analysis by the harmonization of the compounds name and the use of aggregated data, all automatized. The "Easy-Amanida" is an user friendly app bringing possibilities also for researchers without strong software skills. Easy-Amanida is implemented in Shiny (an R package for interactive web apps) and it is easily accessible using common internet browsers (https://ubidi.shinyapps.io/easy-amanida/). In this article, we will describe the functionalities of Easy-Amanida meta-analysis together with a proposed workflow to optimize the retrieval of the data. We additionally provide an illustration example of the tool.

2 | THE EASY-AMANIDA TOOL

The Easy-Amanida web-app (Figure 1) relies on two R packages: (1) the Amanida meta-analysis[29] which is designed to be able to perform a meta-analysis study on reports with minimal data disclosed; and (2) the Webchem[30] used for naming harmonization. The users can upload their own datasets and proceed with the meta-analysis. All results generated can be downloaded separately or included on a full report. The tool is designed to follow the proposed pipeline to ensure the inclusion of the maximum number of results for the meta-analysis, and at the same time automatically check for possible naming mismatch issues (Figure 2).

2.1 | Input data

Easy-Amanida users are required to submit a list of compounds. Hence, prior to using the tool, users must have already determined the studies to be included and obtained the necessary information for conducting the meta-analysis (as shown in Table 1), whether it involves quantitative or qualitative data. The variables to be

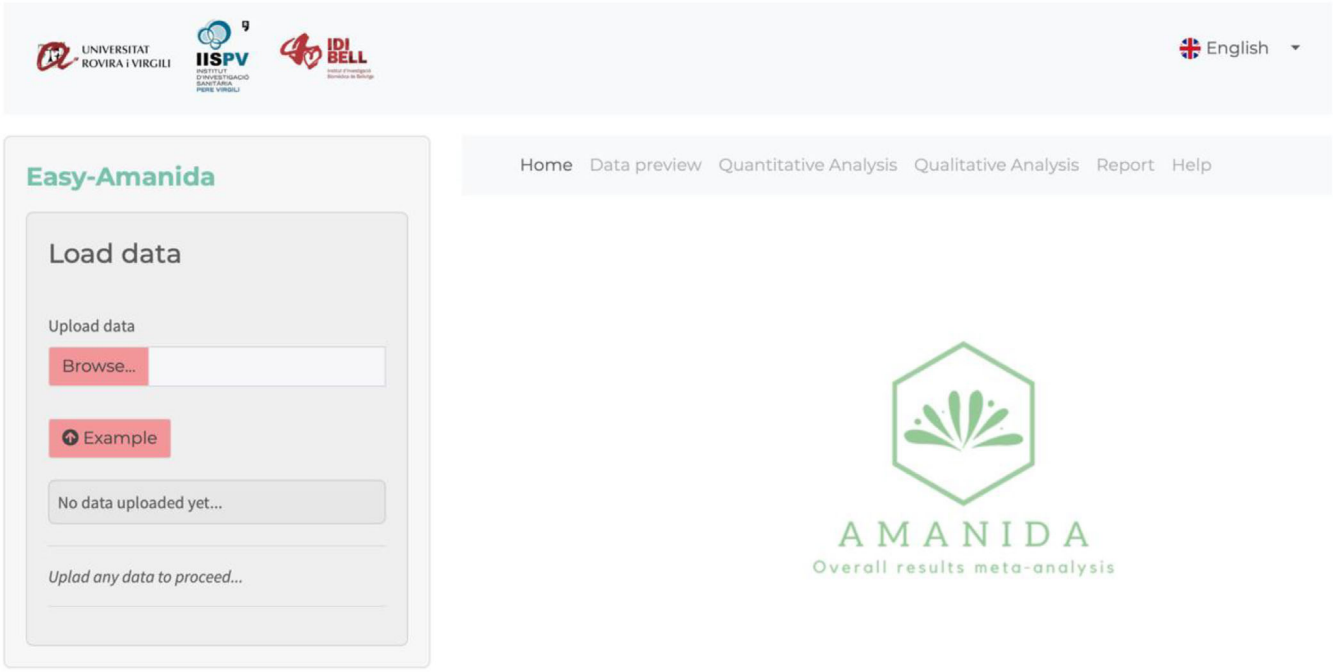

FIGURE 1 Easy-Amanida web app home page.

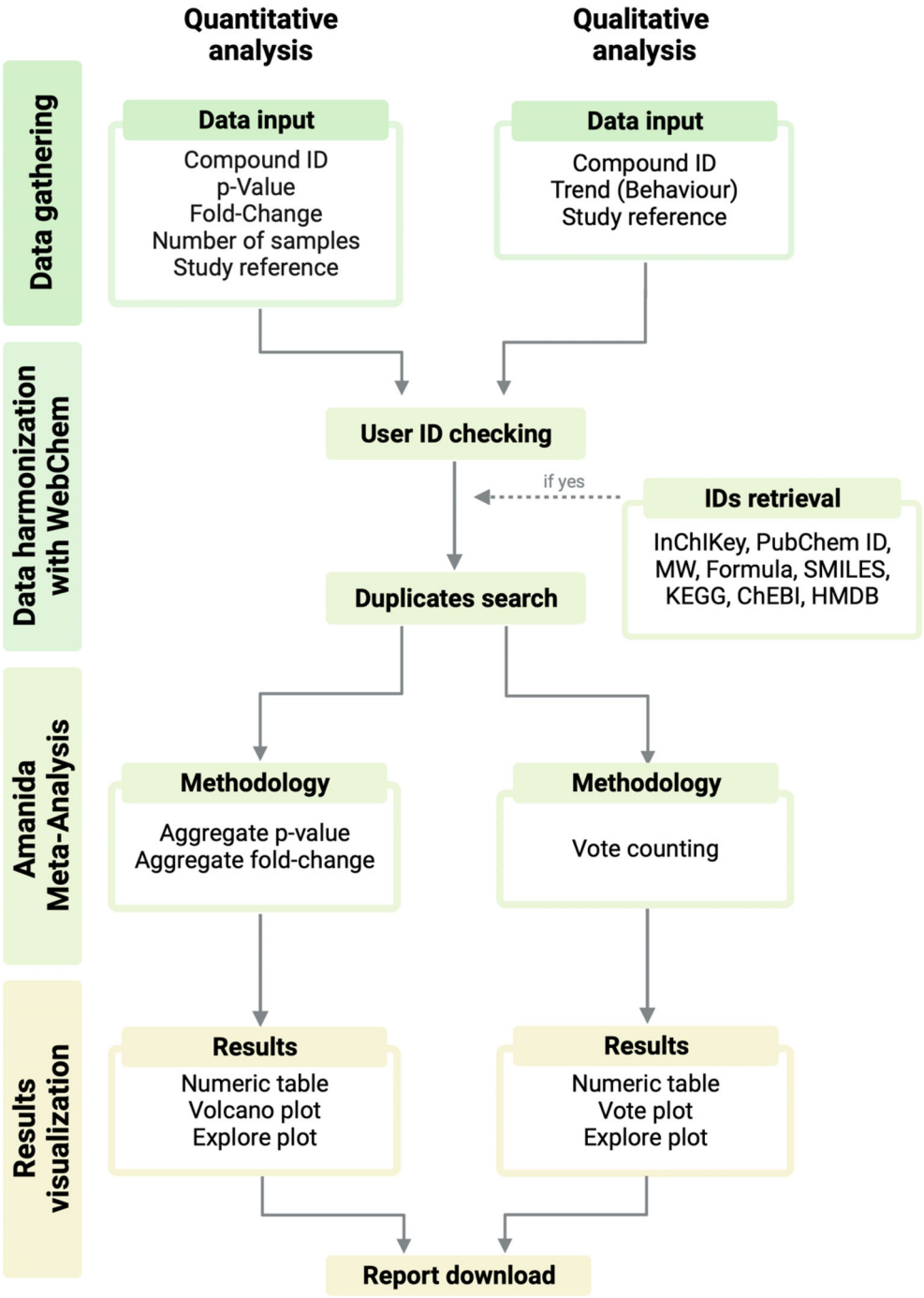

FIGURE 2 Workflow chart describing the functionalities of the Easy-Amanida meta-analysis tool and data harmonization with WebChem.

TABLE 1 Needed information for each of the meta-analysis the Easy-Amanida can perform.

Quantitative analysis	Qualitative analysis
Compound ID	Compound ID
p-value	
Fold-change	Trend (behavior)
Number of samples	
Study references	Study reference

included for the quantitative analysis are: compound name (ID), its *p*-value statistic, its fold-change, sample size (*N*), and the study reference from where this specific compound is retrieved. On the other hand, there is a qualitative option of meta-analysis based on the vote-counting approach.[31] In this case the variables to be included are: compound name (ID), trend (behavior), and study reference. By default, the quantitative mode encompasses the qualitative analysis as the trend is obtained from the fold-change.

The app Easy-Amanida includes the same example dataset as the amanida R package[18] (referred to as "Example" in the app). This dataset was used to investigate the metabolome and volatilome of colorectal cancer in urine samples through a meta-analysis.[17] The dataset includes 143 metabolites from 5 studies where 15 metabolites are reported in more than one study. It also includes the numerical metrics: *p*-value, fold-change, and sample size, and a non-numerical metric, the behavior or trend.

2.2 | Data harmonization with Webchem R package

Once the researcher has selected the data to be analyzed, data harmonization is crucial. The tool offers the option of converting the names of the metabolites to a unique identifier, the InChIKey. The webchem package ($v >= 1.1.0$)[30] is used, since it extracts chemical information from around the web, including: Alan Wood's Compendium of Pesticide Common Names, Chemical Identifier Resolver, ChEBI, Chemical Translation Service, ChemIDplus, ChemSpider, ETOX, Flavornet, NIST Chemistry WebBook, OPSIN, PAN Pesticide Database, PubChem, SRS, and Wikidata. From the webchem options, we have implemented the naming harmonization via PubChem, as it is the largest public database of chemical compounds. So, the name of the compound is searched in PubChem to obtain the CID and the InChIKey. Then the IDs are used to harmonize the names, and finally to retrieve several chemical properties and other database identifiers. The final table will include, the compound name or id, PubChem CID, Molecular Formula, Molecular Weight, Canonical SMILES, InChIKey, KEGG, ChEBI, HMDB, and Drugbank. Even though users have the option to harmonize data automatically, they can choose to skip this step.

For a successful metabolites combination, all identifiers should be any (or a combination) of the following ones: chemical name including synonyms, PubChem CID, InChI, InChIKey, and canonical SMILES.

2.3 | Meta-analysis with Amanida R package

The Amanida aggregate statistical meta-analysis can be found in Llambrich et al.[29] Briefly, the quantitative approach uses the *p*-values and fold-changes of the compounds found in the different studies to combine them. The p-values are combined using a weighted Fisher's method, and a gamma distribution is used to assign non-integral weights to each *p*-value proportional to study size. The fold-changes are logarithmically transformed (base 2) to reduce skewness due to methodology and are averaged with weighting by study size. The number of participants involved in each study is required as a weighting value for the estimate's combination. Studies with larger number of participants are given more reliability than small studies which have a higher standard error. Note that for all compounds present in the list the *p*-value and fold-change are required for the quantitative analysis. If instead, the user has been unlucky to retrieve fold-changes, and they have trends of behavior, a qualitative analysis can be performed.

2.4 | Visualization of the results

For the quantitative results, users can download the combined *p*-values and fold-changes, the harmonized IDs, and a volcano plot. For the qualitative analysis, users can download the vote-counting plot ($a +1/-1$ is assigned to up/downregulated, respectively and they are summed for each repeated compound), and an explore plot (a mixture of a vote-counting plot with the number of references with which each compound is found). By default is always performed the quantitative analysis plus de qualitative one to include the explore plot in the results interpretation. An example dataset is illustrated in Section 3.

2.5 | Report and help

Results of Easy-Amanida can be downloaded as an html report, that allows the user to select the cut-offs of the different analysis plots (Figure 3). A Help section is also included at the end, which how using the Easy-Amanida app is detailed, and also contains an About section in which the authors and how to cite the Easy-Amanida tool are indicated.

3 | AN ILLUSTRATIVE EXAMPLE

The Easy-Amanida meta-analysis was applied on a meta-analysis with public data,[32] for which they analyzed metabolomics data of pancreatic ductal adenocarcinoma (PDAC) from 24 clinical studies. Data are provided in the Supplementary Table 1.[17]

For the PDAC data, we upload it as CSV file in the "Upload Data" panel. Subsequently, we designate the type of analysis by opting for "Quantitative analysis", and confirm the accuracy column names with their respective tags. The used dataset has metabolites names that have been manually curated, thereby obviating the need to

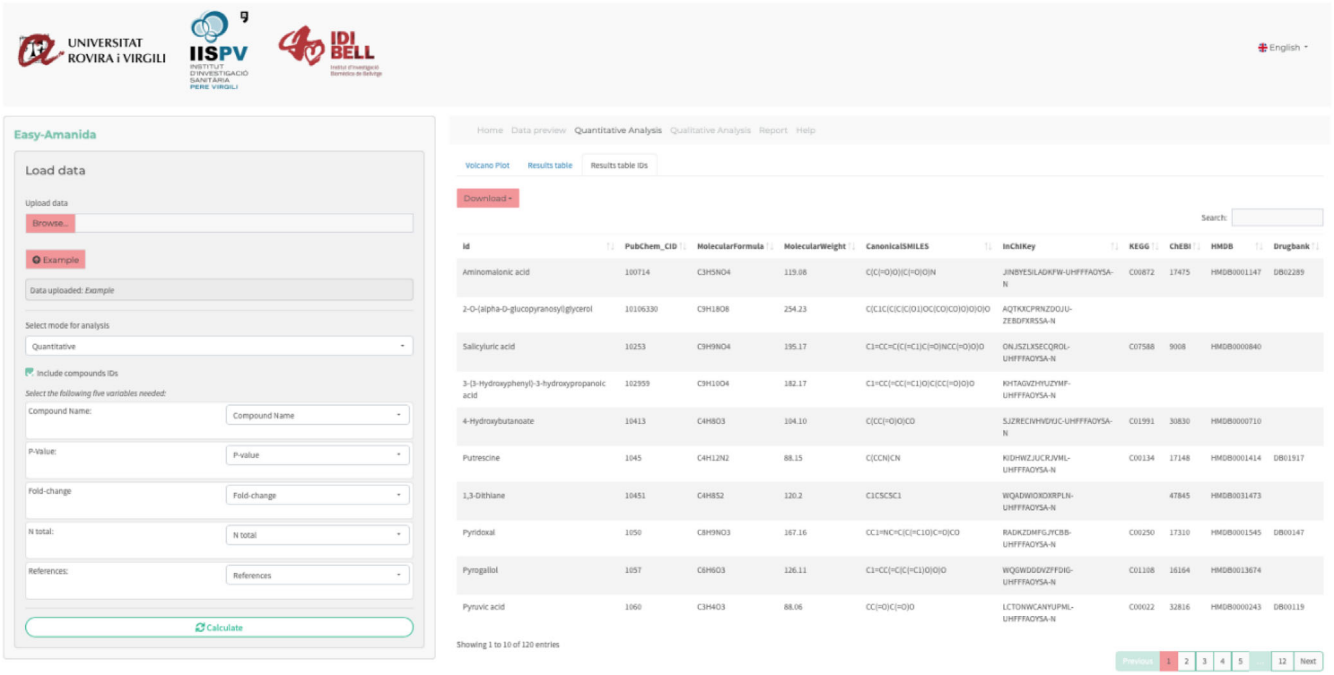

FIGURE 3 Easy-Amanida quantitative results section, showing the results table IDs.

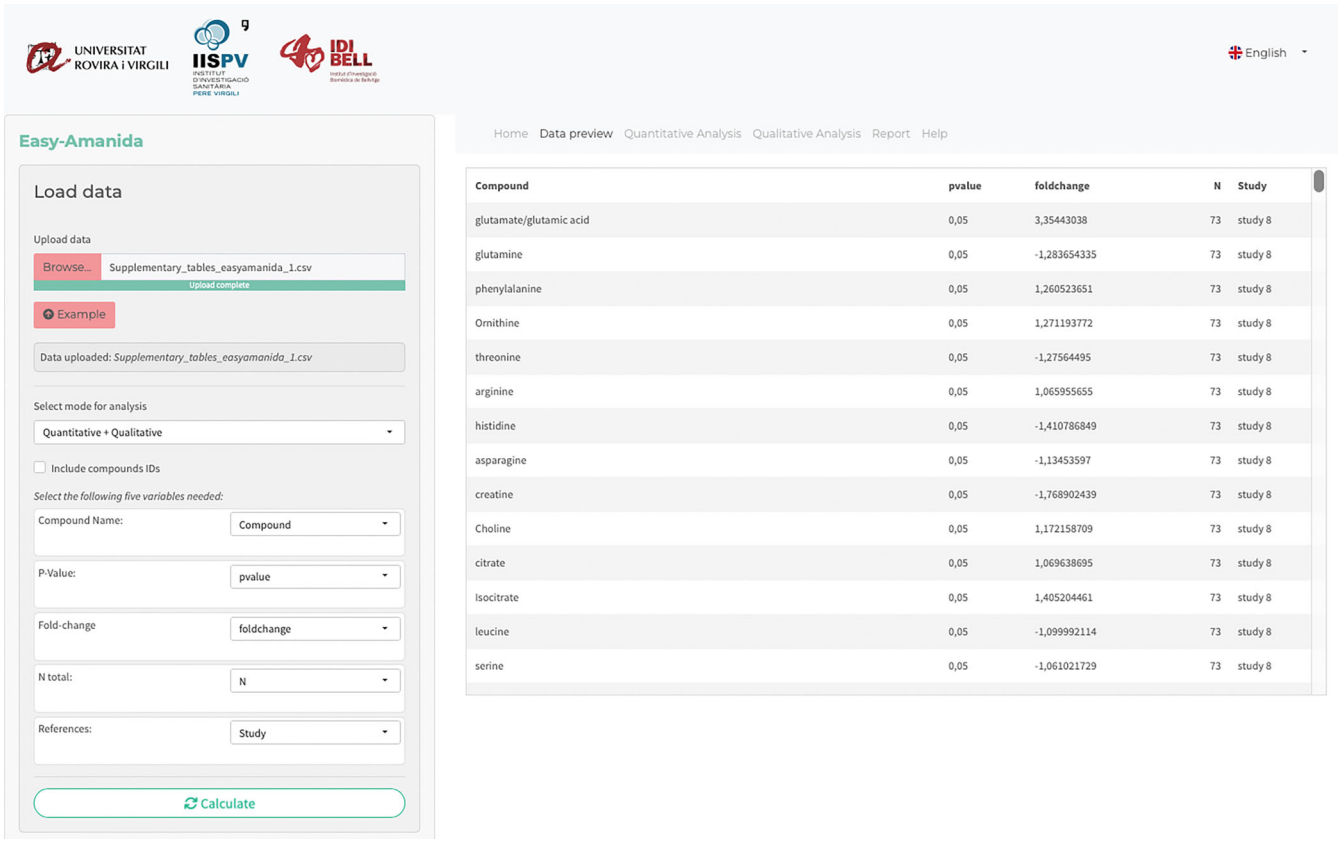

FIGURE 4 Loaded example quantitative dataset.

select the "Include compounds IDs" option. While uploading the data, we observe that there are no rows containing missing values (Figure 4).

Then, we initiate the meta-analysis by clicking the "Calculate" button. The Amanida meta-analysis simultaneously integrates p-values and fold-changes while

TABLE 2 Results from combining PDAC metabolites. The metabolites in blood with a higher total *N*, found over the 12 studies included.

Compound	*P*-value combined	Fold-change combined[b]	*N* combined
Histidine	0.023	0.0017	9500
Proline	0.024	0.0016	9400
Glutamine	0.029	0.0033	9100
Arginine[a]	0.038	0.0095	9000
Asparagine	0.039	0.0037	9000
Phenylalanine[a]	0.033	0.0072	9000
Lysine[a]	0.038	0.0040	9000
Threonine	0.039	0.0035	9000
Alanine[a]	0.048	0.0062	8900
Ornithine[a]	0.042	0.0078	8900
Valine[a]	0.049	160	8900
Glycine[a]	0.050	0.0053	8900
Tryptophan[a]	0.049	150	8900
Serine[a]	0.048	0.0050	8900
Leucine	0.049	0.0047	8800
Methionine	0.050	0.0046	8800
Citrulline	0.050	0.0047	8800
Isoleucine	0.051	0.0053	8800
Tyrosine	0.050	0.0043	8800

[a]Inconsistency in trend between studies.
[b]Fold-change <1 indicates that the metabolite is down-regulated and therefore, lower in concentration in the PDAC group. A FC of 0.004 for example, indicates that controls have 250 times higher concentrations (250 = 1/0.004) than the PDAC.

assessing the trends of the original studies. Results, both numerical and graphical, are obtained under the "Quantitative Analysis" tab. Combining the significant metabolites of the 12 studies used by Roth et Powers, we obtain a total of 311 metabolites, with only 38 compounds appearing in more than one study.

One objective of meta-analysis is to increase the sample size (N). Initially, we examine compounds with the highest N combined (Table 2). Among these, 19 compounds exhibit an *N* greater than 8800 participants, all belonging to the amino acids class. While 80% of them yield a significant *p*-value (*p*-value <0.05) and a significant fold-change (FC >2). Only Valine and Tryptophan were found up-regulated for PDAC patients.

After combining the estimates, we identified 35 metabolites out of 311 with significant results (*p*-value <0.05 and fold-change >2) across multiple studies (Figure 5). Glutamate yielded the smallest combined *p*-value and it was also one of the most frequently reported compounds. Among the significant compounds, 13 of them were up-regulated in PDAC patients, while 22 were down-regulated (see Supplementary Report 1).

The Easy-Amanida meta-analysis also incorporates a qualitative meta-analysis based in the vote-counting approach (Figure 6) when performing the quantitative analysis. The qualitative meta-analysis allows to evaluate the trend of the compounds, the explore plot is designed to indicate the consistency or inconsistency in trends across all studies. In the PDAC data the highest number of occurrences was observed for Glutamine, identified in 7 studies, followed by Histidine (found in 5 studies), with both being consistently down-regulated. In the up-regulated category, three bile acids were recurrent in 3 studies: Tauroursodeoxycholic acid, taurocholic acid and glycocholic acid. Additionally, Glutamate showed a vote-counting of 3; however, the explore plot reveals that while 5 studies report glutamate as up-regulated, 2 indicate it as down-regulated in PDAC patients.

To assess the consistency of these findings, we examined the trends of the compounds across all studies. Among the significant compounds, 15 metabolites exhibited varying trends in the studies. So, removing the inconsistent metabolites we obtained as significant 8 compounds up-regulated, and 12 down-regulated (see Table 3).

4 | META-ANALYSIS CONSISTENCY

To validate the accuracy of the amanida meta-analysis estimations, we compared the results with those obtained

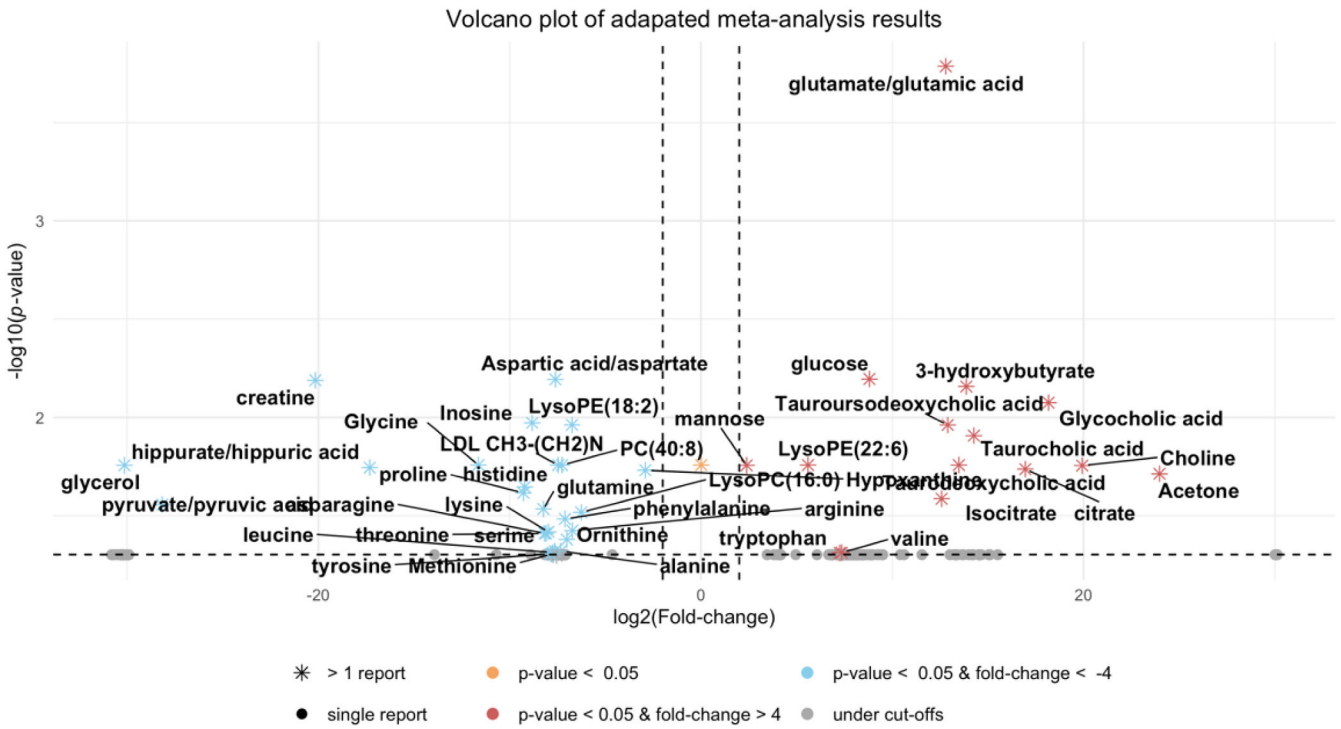

FIGURE 5 Easy-Amanida quantitative results section, showing the volcano plot graphical visualization.

from Metaboanalyst example data for meta-analysis, that are part of a study of lung cancer in blood available at Metabolomics Workbench (PR000293). Data are provided in the Supplementary Table 2, we followed the same processing as metaboanalyst (data are logarithmically transformed and autoscaled). Remarkably, three metabolites were found consistently significant in both analyses (Figure 7 and Supplementary Report 2). Salicylic acid was also found significant in Easy-Amanida meta-analysis, however, it showed inconsistency in the trends.

5 | DATA HARMONIZATION VALIDATION

To demonstrate the process of data harmonization, we employed data from a systematic review and meta-analysis focused on metabolomics and volatilomics in colorectal cancer.[17] This dataset serves as the illustrative example within our R Shiny web app, data with multiple IDs is provided in the Supplementary Table 3. Within this dataset, we identified seven studies with three types of identifiers (common name, InChIKey, and SMILES) were intermixed.

Upon activating the "Include compounds IDs" option, we examined 133 unique identifiers. Standardizing these identifiers to a common ID revealed 120 unique IDs and facilitated the identification of additional duplicates. Notably, when duplicates were not considered, maximum votes were assigned to hippuric acid and indole-3-acetic acid with a count of 2. However, with IDs checked, these counts increased to 3 (Figure 8, Supplementary Report 3 without harmonization, and Supplementary Report 4 with harmonization).

Utilizing the webchem package for the retrieval of public IDs proved to be a robust approach, significantly diminishing the need for manual curation of tables. This not only enhanced the accuracy of the results but also expedited the overall process.

6 | DISCUSSION

In this article, we present the Easy-Amanida meta-analysis tool, an accessible and lasting web app, that combines the new quantitative estimate for aggregate statistical data using the Amanida package, with name harmonization using Webchem package.[30] The use of aggregate results (*p*-values and fold-changes) is a solution when no other resources can be used.

Naming harmonization is becoming a major obstacle for metabolomics-based research. Although new guidelines have been published,[9] they are being incorporated very slowly, and they are not yet implemented in thousands of already published studies. The Easy-Amanida solution incorporates the Webchem package for name

FIGURE 6 Easy-Amanida qualitative results: (a) Vote plot; and (b) Explore plot. Showing the 15 more relevant compounds.

harmonization, and the name of the compounds are transformed to the common chemical name when the "Include compounds IDs" option is selected. However, if the researcher follows another strategy the accuracy of the Easy-Amanida meta-analysis could decrease.

The results of the Easy-Amanida meta-analysis approach should be taken carefully, as one of the limitations is the lack of combined mean values and combined standard deviations. Nevertheless, the inclusion of not significant results from the different studies is recommended to avoid false positives. The Easy-Amanida is not able to overcome the heterogeneity of the data, however the Amanida package[29] uses weighted *p*-values and fold-changes, to give more reliability to the studies with a bigger sample size. Also, the use of qualitative plots, will allow the user to identify discrepancies between studies easily. If discrepancies are detected in the data, one should question which is the source of variation to address the understanding of the findings. Furthermore, the selection of which studies must be included in the meta-analysis is determined by the criteria of the user. We recommend using the PRISMA method[33] for systematic reviews, although finding studies that reach all criteria in PRISMA sometimes is challenging. One measure of consistency is through the use of the explore plot, which shows the consistency of the trend between different studies. Thus, the inclusion of studies with different questions, such as different sample types or species used, will invalidate the results obtained.

A similar strategy was performed for PDAC data in Ketavarapu et al. Based in an enrichment analysis, they found the most altered pathway for glycerophospholipid

metabolism,[34] which we observe in our results with the up-regulation of glycocholic acid, taurocholic acid, taurodeoxycholic acid, and tauroursodeoxycholic acid. An increase in ketogenesis is also observed by the increase of acetone and 3-hydroxybutyrate. PDAC is a severe wasting disease, associated with high levels of protein catabolism as we can see in the down-regulation of almost all aminoacids.[35] As a cancer characteristic the increase of TCA cycle is observed by the increase of demand for citrate and isocitrate. Consistent results were observed from a targeted analysis where glutamine is found down-regulated and glutamate is found up-regulated,[36] same trends as in the meta-analysis results.

For the lung cancer data it has been reported an increase of glucose via lactic fermentation,[37] which we observe with an increase of pyruvic acid in blood. Together with an increase of ATP via oxidative phosphorylation, leading an increase of pyrophosphate, metabolite from the pathway, and the adenosine-5-phosphate (AMP).[37] Salicylic acid has demonstrated an apoptotic effect into lung cancer cells,[38] thus can be the reason of the decrease found in blood.

The effectiveness of harmonizing identifiers through the integration of information from public databases has been demonstrated. The ability to combine various identifiers not only streamlines the process of conducting a meta-analysis but also enriches the dataset, offering additional information crucial for the complex interpretation of the results.

TABLE 3 Significant compounds obtained from Easy-Amanida meta-analysis for PDAC serum and plasma samples.

	Up-regulated	Down-regulated
Ketones	Acetone	
Lipids	Choline, glycocholic acid, taurocholic acid, taurodeoxycholic acid, tauroursodeoxycholic acid, LysoPE (22:6)	Glycerol, LDL, PC(40:8)
TCA-Carbohydrates	Isocitrate	Pyruvate, creatinine
Amino acids		Proline, histidine, glutamine, asparagine, threonine
Nucleosides		Inosine
Others		Hippurate

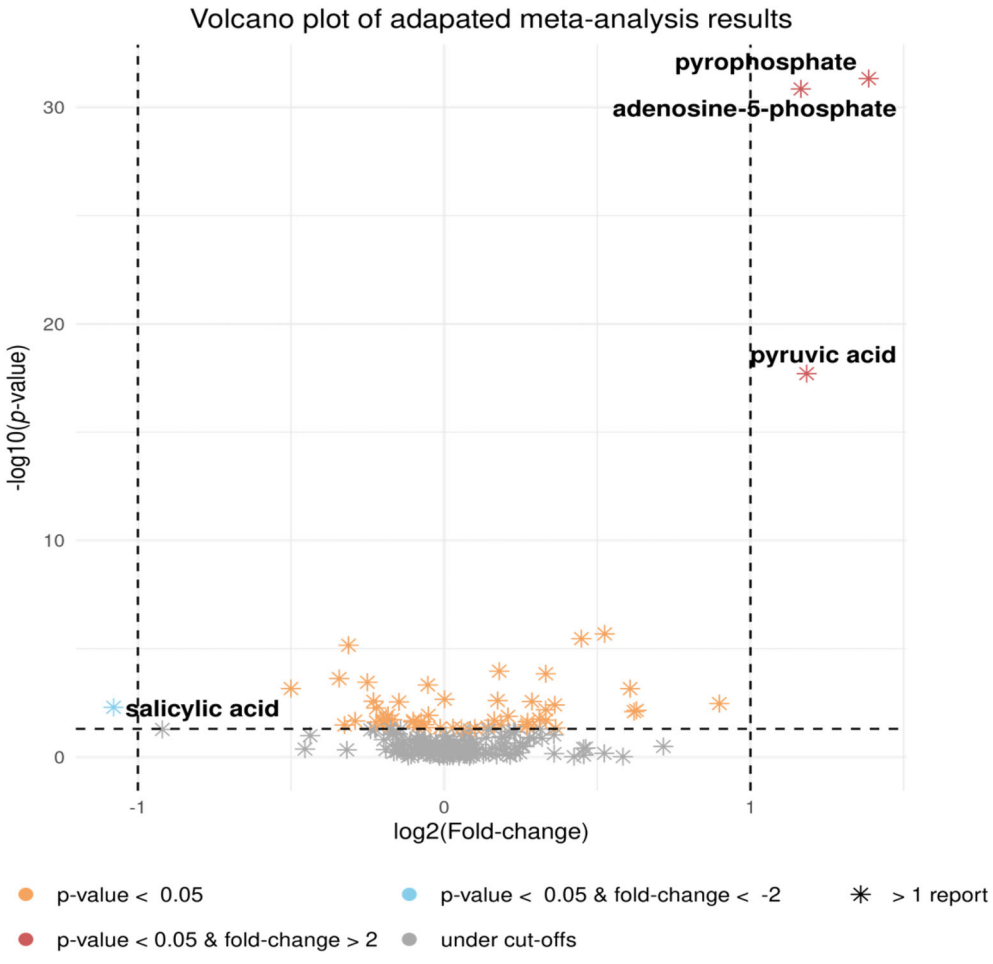

FIGURE 7 Easy-Amanida quantitative results section for Metaboanalyst data, showing the volcano plot graphical visualization.

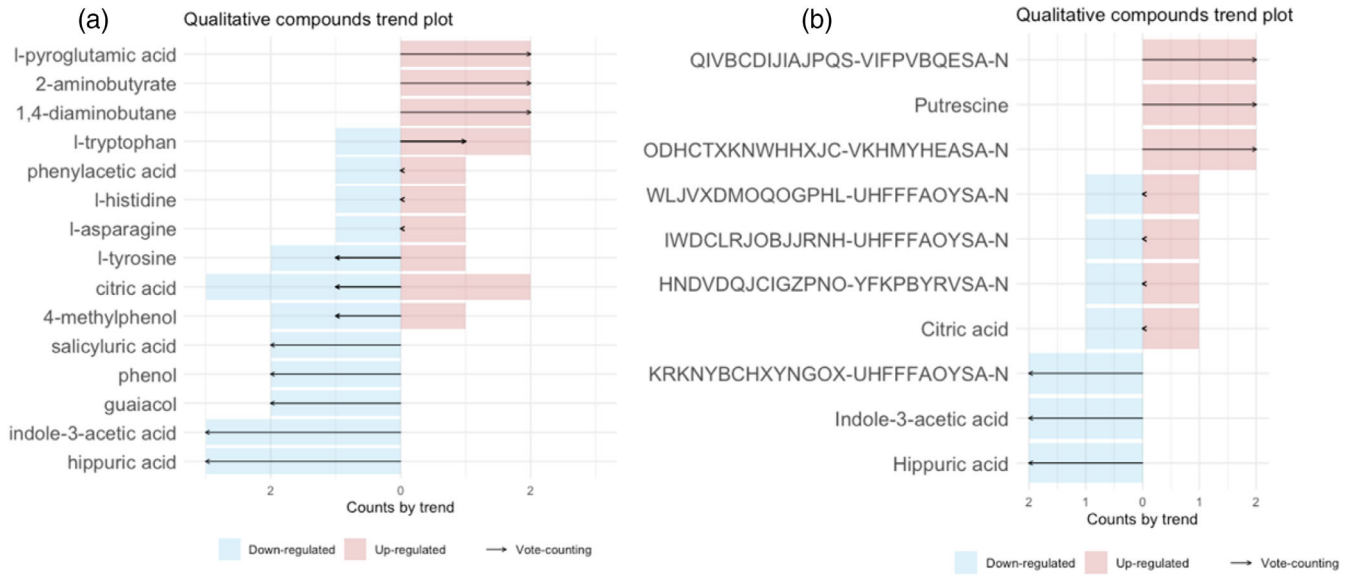

FIGURE 8 Comparative of explore plot when using compounds IDs option (a) or not (b).

7 | CONCLUSION

The Easy-Amanida Shiny web app for meta-analysis successfully combines the aggregate statistical meta-analysis solution Amanida, and the data harmonization with Webchem. We implemented it as a Shiny app to facilitate their use to all the community. Easy-Amanida is optimized for metabolomics studies, it uses the aggregate results (*p*-value and fold-change), to perform a meta-analysis, including several visualizations of the results.

Here, we have illustrated all the functionalities of the Easy-Amanida app, including an example of the possible interpretation of the results. Although the app is designed for metabolomics it can be used with data from other omics.

AUTHOR CONTRIBUTIONS

Raquel Cumeras: Conceptualization; supervision; writing – review and editing; project administration; funding acquisition; methodology. **Maria Llambrich:** Writing – original draft; software; conceptualization; methodology; validation. **Pau Satorra:** Software; writing – review and editing; methodology. **Eudald Correig:** Writing – review and editing; methodology. **Josep Gumà:** Writing – review and editing; conceptualization; funding acquisition. **Jesús Brezmes:** Writing – review and editing; funding acquisition; conceptualization. **Cristian Tebé:** Writing – review and editing; software.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All R scripts, datasets, and source code for Easy-Amanida are available in the Github: https://github.com/mariallr/easy-amanida.

ORCID

Maria Llambrich https://orcid.org/0000-0001-8418-0982
Pau Satorra https://orcid.org/0000-0002-8144-4089
Eudald Correig https://orcid.org/0000-0001-8556-0469
Josep Gumà https://orcid.org/0000-0001-7541-9832
Jesús Brezmes https://orcid.org/0000-0002-7704-8550
Cristian Tebé https://orcid.org/0000-0003-2320-1385
Raquel Cumeras https://orcid.org/0000-0003-4663-4247

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