Bias in the reporting of sex and age in biomedical research on mouse models

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Abstract Lack of accurate method reporting is one of the primary causes of irreproducibility in biomedical research. In animal-based biomedical research, both sex and age affect the disease phenotypes; modifying their susceptibility, presentation and response to treatment. Here we look at these two variables by using text-mining across available full text articles that report investigations where mice were the focus of the study. We found that, although there is an improvement during the last two decades, the lack of reporting of these variables is still a concern; only about 50% of the papers published in 2014 stated these variables. In addition, we observed a sex-bias variability according to the field of study. We hope that this text-mining strategy can be taken as a starting point for future more focused assessment of literature, both in preclinical and clinical studies, and thus impact on the reproducibility of findings and on future study validity.

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Introduction

Studies using animal models are important tools in experimental biomedical sciences for understanding the physiopathological and therapeutic basis of human diseases. In doing this, the results of preclinical studies carried out in animal models provide not only a rationale for justifying clinical evaluation, but also a source of interpretations of unsuccessful translations during clinical development (Kimmelman and Anderson, 2012). Nevertheless, historically, the translation of scientific findings from animal models to humans is far from straightforward. Statistically, more than 80% of potential therapeutics fail in human clinical trials after being successful in animal
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models (Perrin, 2014). This uncomfortable truth is the fundamental reason why animal research is a cause of concern and, therefore, it needs to improve and become more reliable and reproducible (van der Worp et al., 2010). In fact, this has led to doubts as to whether experimental models should be considered as a source of knowledge for clinical evaluation (Perel et al., 2007).

The failure to translate from experimental models to human beings stems from various factors, where the reproducibility of the findings plays an important role (Collins and Tabak, 2014; Freedman et al., 2015). In this way, there is a growing concern over the lack of reproducibility in biomedical studies; a large proportion (75-90%) of the preclinical research findings published in top-ranked journals cannot be replicated (Begley and Ellis, 2012; Prinz et al., 2011). The observed lack of reproducibility may be a result, among other things, of the lack of transparency in reporting biomedical research (Landis et al., 2012; Moher et al., 2008; van der Worp and Macleod, 2011). In the United States, for instance, it has been estimated that about US $28 billion per year is spent on preclinical research that is not reproducible; where the reporting is one of the most common reasons (Freedman et al., 2015).

The Uniform Guidelines of the International Committee of Medical Journal Editors state that authors should include technical information in sufficient detail to allow the experiment to be repeated by other workers (International Committee of Medical Journal Editors, 2013). This is vitally important in animal experimentation, where a detailed description of any animal model is not only in agreement with the principles of the 3Rs (Replacement, Reduction and Refinement) (Burden et al., 2015), but also plays a fundamental role in the interpretation of the data and reproducibility of the findings derived from the animal model used to generate such data. In this context, the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines were developed to improve consistency in reporting animal research (Kilkenny et al., 2010). However, there is still a lag in the implementation of these guidelines (Baker et al., 2014).

In experiments using animals, for instance, the sex and age of the mice should be reported because they influence the outcomes (Diedrich et al., 2007; Wizemann and Pardue, 2001). Both sex and age of organisms are among the variables that affect morphological, physiological, immunological and behavioral parameters and, hence, they are important in reporting both basic science and clinical research. These variables are inextricably linked: it has been proposed that under natural conditions sexual selection has profound effects on the lifespan of organisms (Bale and Epperson, 2015; Maklakov and Lummaa, 2013). Considering some taxa exceptions, the general conclusion is that in many animals (including humans), males have shorter lifespans than females (Clutton-Brock and Isvaran, 2007). Furthermore, from an evolutionary standpoint, these sex differences in lifespan depends to a great extent on sexually dimorphic life-history strategies (Maklakov and Lummaa, 2013), e.g. mating systems, and on genetic architecture; including both the sex chromosomes (Nguyen and Disteche, 2006) and the mitochondrial DNA (Gemmell et al., 2004).

Regarding preclinical and clinical studies, sex and age play key roles in disease phenotypes; modifying their susceptibility, presentation and response to treatment (Arnold, 2010). Some pathologies exhibit a clear sexual dimorphism (Ober et al., 2008). Using stroke as an example, it is known that its incidence is higher in men than women during their lifespan (Mozaffarian et al., 2015). However, recent evidence suggests that after the age of 60 years and thus post-menopause, women have more severe strokes than men (Dehlendorff et al., 2015). In the case of animal models, sex- and age-dependent differences in protein expression profiles were observed in the heart proteome of female and male C57BL/6 mice of two distinct age groups (14 and 100 weeks) (Diedrich et al., 2007). This evidence implies that sex differences must be studied across...
the entire lifespan in order to bring new insights into the pathogenesis of the diseases and identify targets for new drugs for both sexes and different times of life. Guidelines, such as ARRIVE (Kilkenny et al., 2010), have been developed because of the 3Rs (Burden et al., 2015) to highlight the importance of such biological factors in animal experiments, and these have been endorsed by journals with the aim of improving the reporting of bioscience research.

In this study, we have used large scale text mining to evaluate the reporting of information about mouse sex and age as “bibliomarkers” of method reporting quality in a set of over 15 thousand full-text articles. In the last decade, there has been a significant amount of research in the identification of targeted biomedical information in the scientific literature via text-mining (TM) (Cohen and Hersh, 2005; Fleuren and Alkema, 2015). In particular, efforts have been made to recognize protein and gene names in text (Settles, 2005) or other biomedical entities of interest such as electronic health records (Meystre et al., 2008). In comparison with other TM applications that are focusing on the recognition of complex biomedical entities and their shared relationships, our approach addresses a significantly more diverse literature space and questions the reporting of what should be standard information in biomedical research regarding laboratory animals as models for human diseases. Based on syntactic rules and simple dictionary matching, we extracted key characteristics in mouse-based models such as sex and age in order to comprehend the standards of information reporting to assess the possibility of reproducing mouse experiments. Previous work has shown that fundamental criteria of experimental methods are repeatedly omitted in laboratory models (Bramhall et al., 2015; Florez-Vargas et al., 2014). In light of this, our investigation looked at sex and age as two important factors across available full text articles that report investigations where mice were the focus of the study. We investigate questions of whether sex and age of mice is reported, the use of each sex in different types of research area, and the field of analysis for each area.

Results

System evaluation and data

We evaluated the TM system on a set of 50 full-text articles randomly selected from our corpus of study (Supplementary file 1) by comparing its performance with the manual annotations of the same papers performed by two biomedical experts. The F-scores that resulted from this evaluation were around 92% for both sex and age (Table 1), which indicates good quality of the results (Ananiadou et al., 2006).

Table 1. Evaluation of the performance of the text mining system

| Characteristics | True-positives | True-negatives | False-positives | False-negatives | Precision (%) | Recall (%) | F-score (%) |
|-----------------|----------------|----------------|-----------------|-----------------|---------------|------------|------------|
| Sex             | 29             | 16             | 3               | 2               | 90.6          | 93.5       | 92.0       |
| Age             | 31             | 14             | 1               | 4               | 96.8          | 88.5       | 92.4       |

A total of 50 articles were used as the data set to evaluate the performance of the text mining system (Supplementary file 2D). The precision (P), calculated as TP/(TP+FP), determines the accuracy of the system in recognizing desirable terms. The recall (R), calculated as TP/(TP+FN), produces the coverage of the system. F-score is the harmonic mean of precision and recall and it is calculated as 2*P*R/(P+R).
A total of 15,311 full-text articles from the PubMed Central Open Access subset as of February 2015 were processed in this study. These articles correspond to 7.15% and 27.85% of mouse experimentation articles retrieved by the same query in PubMed and PubMed Central, respectively. This corpus of documents were published between 1994 and 2014, of which 50.1% were published after 2011 (n= 7671) (Figure 1) Seventy journals out of the 628 analyzed covered 30 or more articles of the corpus (Figure 1-figure supplement 1), which corresponds to 81.05% of papers retrieved. *PLOS ONE* contained the highest number of articles (n= 5574, 36.41%), followed by *The Journal of Experimental Medicine* (n= 931, 6.08%), and *The Journal of Cell Biology* (n= 363, 2.37%).

**Reporting of sex and age**

The general and historical reporting of sex and age as experimental variables in mouse models is presented in Figure 1. Overall, from 1994 to 2014, about a fifth of papers did not report either the sex or the age of the mouse used in the study (Figure 1a and 1b). Figure 1c shows that the
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The frequency of articles reporting sex and/or age in mouse models has increased steadily during the last two decades, whereas missing information about these two experimental variables showed an important drop from 100% (no papers reported the sex and age of the mice in 1994 and 1995) to about 15% following a slope of approximately -0.045. Nevertheless, since 2012, the percentage of articles reporting both factors had reached only about 50% of the papers published in those years.

When the sex of the mouse model is stated in the article, experiments performed with female mice were more frequently reported than experiments performed with male mice (31.84% vs. 23.38%, Binomial test \( p < 0.001; 95\% \) IC: 56.60 – 58.71) (Figure 1d). Our results showed that, historically, female mice have been reported more often than male mice, reaching a plateau of about 33% since the last decade (2004 – 2014) (Figure 1e). In addition, the use of both sexes in mice experiments stratified by sex showed the lowest improvement over time (Figure 1e); with a maximum of about 10% of the articles since 2006. Reporting of mouse age improved steadily from 1999 to 2006 (Figure 1f), at which point age is reported more than 50% of the time; since 2010 age reporting has plateaued, with between 65 and 70% of articles each year mentioning the age of mice.

In order to identify whether there are general features common on reporting sex and age as experimental variables to any biomedical field, we assessed six main preclinical research topics as defined by their impact on human health (WHO, 2014), including: cardiovascular diseases; cancer; diabetes mellitus; lung diseases; infectious diseases; and neurological disorders. A two-way ANOVA without replication was performed to assess the difference in reporting sex and age for each field. Our results showed statistically significant differences, \( i.e. p < 0.05 \), indicating that the reporting of these experimental factors varies across biomedical fields (Figure 2). In identifying the sex and age of the mouse, for instance, studies on diabetes showed the highest frequency (68%), whereas studies on cancer showed the lowest frequency (48%) (Figure 2a). Studies on cancer reported the worst results regarding missing information about sex (33%) or age (37%) of...
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Overall, the best results in reporting sex and age were achieved by the studies on neurological disorders (Figure 2a, 2b and 2c). For a more detailed analysis of sex-based reporting, the six groups of diseases were divided into four subgroups according to the characterization of the disease models via genetics, immunology, physiopathology and therapy. Our results suggest that there is a preference for studying the immunology of these diseases by using female mouse models, whereas there is a tendency to use male mouse models for studying their genetic basis (Figure 3a and 3b). Both in physiopathology and in therapy subgroups, male mice were more frequently studied in models of cardiovascular diseases, diabetes and neurological disorders, and female mice in models of cancer, lung diseases and infectious diseases (Figure 3c and 3d).

In order to further test whether the observations about the reporting of sex in the experimental mouse models were conserved even in specific cases, we focused the analysis on one particular disease per group as follows: myocardial ischemia (cardiovascular disease); diabetes mellitus type 2 (diabetes); chronic obstructive pulmonary disease (lung disease); Alzheimer’s (neurological disorder). Three diseases were included in the case of infectious diseases that are among the most frequently reported causes of death world-wide (WHO, 2014), i.e. tuberculosis, HIV and malaria. Melanoma was included for the cancer group since it is a highly aggressive and notoriously
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chemoresistant form of cancer; making it a widely used tumor model (Herlyn and Fukunaga-Kalabis, 2010). Overall, our results suggest that in most cases there is a similar pattern of reporting as that found for the biomedical fields assessed to which these diseases belong (Figure 4).

Bibliometric parameters were used to determine if they were associated with the quality of method reporting. We used as journal metrics both the journal impact factor from the Institute for Scientific Information (ISI) Web of Knowledge’s Journal Citation Report (2014), and h-index from the SCImago Journal and Country Rank (2014). No correlation was observed between the reporting of sex or age as experimental variables and the journal impact factor and h-index of the 70 journals that covered 30 or more articles of the corpus (Figure 5).

Discussion

By applying TM as a survey analysis technique on full texts of all available articles in the PubMed Central Open Access subset as of February 2015 we evaluated over 15 thousand papers that used the mouse as an animal model for the study of human biology. Therefore, this analysis constitutes the largest analysis of the quality of mouse experiment reporting, providing the strongest evidence about sex and age bias through biomedical research to date. Nevertheless, this analysis does not represent the entire biomedical literature; not all journals are found in the PubMed Central Open Access subset and some of the journals that deposit their complete contents into PubMed Central include some of their articles in the Open Access subset. This is undoubtedly a limitation of our study. For this survey, we have selected the mouse as a model because of all animal models the mouse is probably the most comprehensive and well-characterized model in life sciences. Researchers rely on mouse models to mimic human disease conditions for several reasons. One of the main reasons is that mouse and human genomes are genetically similar − about 90% of human genes have direct orthologues with mice (Yue et al., 2014). Moreover, as animal models, mice are convenient due to their small size, short lifespan (up to two years), and quick generation time; three weeks for gestation and from 6 to 8 weeks to reach sexual maturity.

Figure 4. Distribution of reporting of the sex in mouse model of diseases. The graph shows the reporting in particular diseases. All these diseases that are among the most frequently reported causes of death world-wide or commonly used models. The distribution is presented in stacked bar charts that illustrate the percentage of the reporting and non-reporting for the sex; stating the number of articles corresponding to each percentage inside the stacks. This analysis was performed in a set of 791 articles; see Figure 1–source data 1.
Therefore, they can be easily housed and maintained, can be genetically manipulated to define gene function in a whole body system and a large number of mice can be studied in a relatively short period of time. This, for instance, allows scientists to study cell/cell interactions in the tissue environment and thus cause and effect relationships in a controlled situation.

Despite the implications for interpretation and reproducibility of experimental findings, the sex and age of the experimental subjects are often not recorded in scientific reports (Kilkenny et al., 2009). In agreement with previous reports, the evidence presented in this study showed that the lack of reporting of key methodological parameters in mouse experiments is still a cause of concern; only about half of the papers published in 2014 stated both sex and age of the mice as experimental variables (Figure 1c). The reason why these variables are not described is unclear, since this simple information is always available to researchers. We do not believe it is a space issue, because in about 40 characters of text it is possible to describe them, including mice number

Figure 5. Scatter plots showing the relationship between the reporting and the bibliometric indices. Journal impact factor in which the papers were published (a) and h-index of journals (b). Spearman’s rank correlation coefficient r square is shown alongside the regression lines. The scatter plots show that there is no correlation between the reporting and impact factor \( r = 0.002, p = 0.984 \) data from the Journal Citation Report (year 2014) and journal h-index \( r = -0.215, p = 0.073 \) data from the SCImago Journal and Country Rank (year 2014). Analysis conducted on the 70 journals that published 30 or more articles of the 15,311 studies returned by searching the PubMed Central Open Access subset as of February 2015.

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Table 2. Summary of the data set used in this study.

| Sets of articles | Number of articles | Task | File |
|-----------------|--------------------|------|------|
| Data 1          | 15,311             | Corpus for assessing reporting of the sex and age of the mice | Supplementary file 1* |
| Data 2          | 40                 | Creating the text-mining rules | Supplementary file 2A |
| Data 3          | 40                 | Manual inspection for finding the location of the mention of the sex and age of the mice | Supplementary file 2B |
| Data 4          | 70                 | Enhancing the performance of the text-mining rules | Supplementary file 2C |
| Data 5          | 50                 | Evaluating the text-mining system | Supplementary file 2D |

*Supplementary file 1 also contains data sets of the six groups of diseases analyzed (cardiovascular diseases; cancer; diabetes mellitus; lung diseases; infectious diseases; and neurological disorders), as well as of the different approaches to assessing the disease models (i.e. genetics, immunology, physiopathology and therapy), and the disease example for each of the six disease groups.
and mouse strain, e.g. ten C57BL/6 female mice (6-8-weeks old). Whilst an improvement in the reporting of mouse sex and age has been observed over time, this is not solely attributable to the introduction of journal guidelines, as improvements were present prior to ARRIVE publication in 2010 [Kilkenny et al., 2010]. In fact, a follow-up study in 2012 showed that while sex and age reporting had improved post-ARRIVE, journals that enforced the ARRIVE guidelines as a condition of publication still failed to publish sex and age in all cases [Baker et al., 2014]. The observed improvements may therefore be a result of a growing recognition of the importance of sex and age as experimental factors that may affect study outcomes, resulting in a movement towards better reported experiments despite, not because of, the introduction of stricter journal guidelines.

An analysis of the scientific literature leads to the general conclusion that the males in both human and other animals are studied much more than their female counterparts. This conclusion is based mainly on the results of two studies that manually surveyed a set of biomedical articles [Beery and Zucker, 2011; Taylor et al., 2011]. However, our results showed otherwise in mouse-based models: 31.84% and 23.38% of all papers assessed were on studies performed on female and male mice, respectively (Figure 1d). This could be explained by some practical advantages of using female rather than male mice: they are cheaper; less aggressive to each other and to experimenters; and they are smaller, requiring less weight-administered drug. In addition, the apparent contradiction between this observation and the previous reports might be related to the sample size and study design; our sample size was the largest to date and we surveyed a much broader range of disciplines. In addition, we focused the survey on mouse models, whereas many more species were included in the other reports [Beery and Zucker, 2011; Taylor et al., 2011], e.g. cat, dog, monkey etc. Nevertheless, although in both studies about 50% (Taylor et al., 2011) and 80% (Beery and Zucker, 2011) of documents relied on rodent models, i.e. mouse and rat, information regarding sex bias by species was not assessed; making comparison with our results difficult. Knowing the sex bias for each particular experimental model is fundamental in the era of decision-making towards reproducible science, which will optimize the design of future studies to fulfil the gap of information regarding sex differences in the model under study.

In preclinical studies, furthermore, we noted an important sex- and age-bias in mouse-based disease models (Figure 2b and 2c). Among the main preclinical research topics assessed, we observed the strongest male-bias in cardiovascular disease models (2.25:1) and the strongest female-bias in infectious disease models (3.54:1) (Figure 2b). This situation still persists: between 2012 and 2014, about 70% and 77% of research articles assessed on these two disease models are still biased towards male and female mice, respectively. These pathologies and many others, exhibit important sexual dimorphisms, which are not only inherent to genetic differences, but also to hormonal influence [Case et al., 2013; Gilks et al., 2014]. For example, in the study of hypertension, one of the major risk factors for cardiovascular disease, a greater increase in blood pressure was reported in gonad-intact XY males than XX females using the four core genotype in the MF1 mouse model. However, the mean arterial pressure was greater in gonadectomized XX mice compared with XY mice regardless of whether the mice were born with testes or with ovaries [Ji et al., 2010]. On the other hand, in the case of infectious diseases, females have a more robust immune system than males – both the innate and adaptive immune responses, which makes them less susceptible to developing many infections (mainly Th1-type infections), although it increases the risk of developing autoimmune diseases due to their trend to develop a stronger pro-inflammatory response [Pennell et al., 2012]. Interestingly, we also observed that the sex-bias could change in a particular disease mouse model according to the biomedical study. Diabetes disease mouse models exemplified this situation. From a global point of view, this disease was found to be male-biased (1.57:1) (Figure 2b). However, in studies related with the immunology of
In order to balance sex of animals and cells in preclinical studies, the National Institutes of Health (NIH) have proposed a multi-dimensional initiative, which includes, among other things, extramural training on experimental design and data analysis by sex (Clayton and Collins, 2014). Regarding this initiative, new ideas have been proposed to achieve, and sustain, the sex balance in biomedical research (McCullough et al., 2014). In this context, our study provides an implementation of TM to assess reporting of experimental factors. By knowing where there is imbalance for a particular variable, it is possible to address it in a cost-effective manner. This not only directly contributes to the comparability of experimental work, but also to the reproducibility of findings. To address this problem some journals are already introducing editorial measures and methods checklists in order to improve the quality of scientific reporting (Nature, 2013). Nevertheless, whilst journal checklists may make reference to species strain, sex and age of animals, most of these checklists focus on statistical analysis to ensure repeatability, which could lead to a biased analysis if it is not made based on biological factors that modify the outcomes. In addition, by checking with the laboratory that conducted the experiment in question it is possible to fix some reproducibility problems; implying a need to adopt more-uniform standards within particular fields. Toward the same direction, we hope that our TM strategy can be taken as a starting point for future more focused assessment of literature; targeting a wider array of characteristics in preclinical and clinical studies. Its potential implementation would enable a straightforward pathway when it comes to reporting key information involved in preclinical and clinical research – e.g. by entering it into the publication cycle as a pre-screening test for submitted manuscripts, which will have an important positive impact on several fronts of the biomedical domain, including the reproducibility of experimental findings and the accuracy of meta-analysis.

Methods

Search strategy and data

A literature search was carried out in Medline via PubMed in order to identify research articles that deal with mouse experimentation. The database was searched in March 2015 for articles that were published between 1st January, 1994 and 31st December, 2014 using the terms as they appear in Figure 1–source data 1. To ensure maximum specificity in the search, searching was limited to articles where the MeSH (Medical Subject Headings) "Mouse" term indicated the major focus of the article; moreover the keywords "Mouse" or "Mice" had to be stated in the title. This also prevented articles that made only passing references to mouse work from entering the dataset and ensured a high quality corpus for analysis. The search was restricted to English language papers and to research articles (excluding review articles). In addition, to obtain full text articles, we restricted the PubMed search to include only those in PubMed Central by adding the special term "pubmed pmc[sb]" in the query. The PubMed Identifiers (PMID) were then converted to the respective PubMed Central (PMC) reference numbers which were acquired by querying the PubMed Central Open Access subset as of February 2015, which contains over one million full-text articles to date.

In order to assess particular areas in which there is strong scientific interest world-wide, we analyzed experiments performed in mouse models for six groups of diseases from the top 10 causes of death according to the W.H.O. in high, low and middle income countries (WHO, 2014).
The six disease groups were as follows: cardiovascular diseases; cancer; diabetes mellitus; lung diseases; infectious diseases; and neurological disorders. Some causes of death did not apply for our study, e.g. road injury. HIV/AIDS, tuberculosis and other infections, for instance, were included in the infectious diseases group. A group for cancer was created in a similar way. An example disease for each of the six disease groups was also included. In addition, as there are different approaches to assessing disease models according to the research field, e.g. immunology, genetics etc., each of these areas were divided into a series of subgroups by using the Subheading MeSH terms “genetics”, “immunology”, “physiopathology” and “therapy” (Figure 1–source data 1). These four approaches were chosen because of their importance for understanding the molecular and physiological basis of diseases, as well as for developing novel therapeutic agents for their treatment. These subjects were used to find if these disease models are being assessed consistently by sex and age.

In 2001 the US Institute of Medicine report (Wizemann and Pardue, 2001) concluded that sex matters in diseases and response to therapy; we therefore decided to explore any changes before and after the report by selecting articles between 1994 and 2014. This time span allows us to assess the impact of this report on the reporting of this experimental factor. In order to avoid misinterpretation due to low number of papers prior to 2001, the analysis for groups and subgroups was applied to articles published after 1st January 2001.

**Sex and Age identification: data sets**

The TM approach involved the design and implementation of generic rule-based patterns, which identify age and sex mentions in text. The rules were based on lexical patterns engineered from a sample of 40 full-text articles manually selected from PubMed through a thematic query of interest as follows: “Mice”[Mesh] AND (mouse[ti] OR mice[ti]) AND “animals”[MeSH Terms:noexp] AND Journal Article[ptyp] AND English[lang]. The first 40 papers that mentioned the sex and/or age of the mice were selected (Supplementary file 2A).

The age rules were based on lexical patterns mentioning age clues, e.g. “aged 3 to 8 weeks old”. Similarly, the sex rules were designed around word matching aiming to identify male, female or both sexes in mice, e.g. “mice of either sex were used”.

The rules were created and applied via GATE (Cunningham et al., 2013) for Windows version 8.1; an open source free software enabling the design and implementation of information extraction systems in unstructured text with the crafted rules following its notation (https://gate.ac.uk/). The number of crafted rules was 12 for sex and 18 for age. Figure 1–source data 2 presents examples of rules for both the sex and age whereas Supplementary file 3 displays all the utilized rules for the two characteristics.

The generated TM results were then integrated at the document level. In cases where several different candidate mentions for a single characteristic, i.e. sex or age, are recognized in a given document, we ‘unified’ them to get document level annotations using the following approach: if multiple mentions of different lengths occur, the longest is selected (usually the most informative) aiming to have one mention for both the sex and age per document, and where mentions are of the same length, the first one is chosen.

Since our method focuses on the recognition of age and sex at the mention level per document, we hypothesize that it is highly unlikely for researchers to report key information about animal models that they did not use. In order to further support this hypothesis, 40 full-text articles were randomly selected from our corpus and through manual inspection, we concluded that indeed, if
there are mentions in text (particularly in the Method section) of specific age and sex (together) these are attributed to the mice used in the animal experiments and no further mentions were reported (Supplementary file 2B). The randomness was modelled by using the “=RANDBETWEEN()” function in Microsoft Office Excel for Windows version 2013 as follows: according to the TM results, each paper of the corpus of articles with a positive mention of the sex and/or age of the mice was assign a random number from 1 to 40. The first 40 papers identified with the random number 1 were selected.

Finally, to further enhance the performance of the rules, we applied this strategy to a development set of 70 full-text documents (Supplementary file 3C). These articles were randomly selected from our corpus by using the “=RANDBETWEEN()” function in Microsoft Office Excel for Windows version 2013; assigning to each paper a random number from 1 to 5. After sorting by the “Year” column, the first five papers identified with the random number 1 were selected by each year group. The mentions of age and sex in both corpus were manually identified and reviewed by the first author, who has a background in the field of biomedical research. A summary of the data sets used in this study is presented in Table 2.

**System evaluation**

The TM system’s performance was evaluated at the document level by considering whether the returned mentions were correctly the sex and age of the mice studied. In order to create an evaluation dataset, 50 full-text articles were randomly selected from our corpus of study (Supplementary file 3D) and were manually double-annotated for both the age and the sex by the first and fourth authors due to their biomedical expertise. There was no disagreement between the manual annotations performed by two biomedical experts. The randomness was modelled by using the “=RANDBETWEEN()” function in Microsoft Office Excel for Windows version 2013 as follows: a random number from 1 to 50 was assigned to each paper. The first 50 papers identified with the random number 1 were selected.

Precision (P), Recall (R) and F-score were calculated for both the age and the sex using the standard metrics (Ananiadou et al., 2006; Hotho et al., 2005), which rely on the number of true- and false-positive (TP and FP), and true- and false-negative (TN and FN) cases. The precision (P), calculated as TP/(TP+FP), determines the accuracy of the system in recognizing desirable terms. The recall (R), calculated as TP/(TP+FN), produces the coverage of the system. Often, there is an inverse relationship between precision and recall; when an increase occurs in precision, a simultaneous decrease is observed in recall and vice versa. Therefore, the F-score was also used for evaluating the performance of information extraction systems due to its harmonic mean of precision and recall and it is calculated as 2*P*R/(P+R). Table 1 shows the results of the evaluation set at the document level.

Despite the overall positive performance of our TM system, there were some results that lead to false-positive and false-negative results due to the relatively complex expressions. False-negative results regarding age mentions occurred because the rules are based on syntactical patterns that require a numeric range between specific time units, i.e., days, weeks and months. For example, in the sentence “Nineteen animals, including males and females, of ages from postnatal day (P) 7 to several months were deeply anesthetized by isoflurane and decapitated” (Arbogast et al., 2013), age is not mentioned as a range concept of days (or weeks or months) but as “postnatal days to several months” without indicating the exact number of months. Cases like this suggest that an extension of the current rule set could lead to an improvement towards the system’s performance. False-negative results regarding sex mentions occurred because the rules for the sex recognition
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is rather straightforward with a simple dictionary matching (minimal), which, as a consequence, does not enable the identification of the sex through inference, e.g. when sex-specific proxy elements are mentioned, such as pregnancy. For example, in the sentence “Primary mouse mammary epithelial (PMME) cells were isolated from 15-d timed-pregnant CD-1 mice” (Lin et al., 1995) are expected to be missed since the sex of the mice used in this experiment is female and is being inferred by the word “pregnant.”

On the other hand, the application of a dictionary approach generated interestingly few false-positives in the sex recognition. This is because the system identified words like “male” or “female” early in text, whereas in the actual experiment the scientists did not report any specific sex for the selected model. For example, in the sentence “The colony of animals carrying the Pak1ip1mray allele is maintained by crossing male carriers with FVB/NJ females. All embryos presented in the phenotypic analysis of this study were produced from carriers crossed for at least four generations onto an FVB/NJ background” (Ross et al., 2013), the sex of the embryos was not established even though the findings relied on them. Other cases were: “Epithelial cells were derived from tracheas of 3-weeks old Gprc5a mice” and “by peritoneal into 8–12 weeks old C56Bl/6 mice”. Cases like these suggest that the implementation of a more sophisticated system that could target common syntactical patterns observed in text (similar to those for the characteristic of age) will contribute to an improvement of the precision and performance of the system. This could explain why sex had the lower precision (90.6%) of the two analyzed factors (Table 1). On the contrary, there was only one false positive (referring to the embryonic stage of the mice) although the real age could not be recognized directly due to not being explicitly expressed; “Genomic DNA and pooled total RNAs were isolated from CRL2196 cells and from various tissues, ages and lineages of mice as indicated, using standard methods and Trizol (Invitrogen), respectively” (Li et al., 2014). The more refined rules led to an increased precision of 96.8% (Table 1).

Although our TM protocol does produce reliable results, the returned results are merely an indication of how TM can be used to improve issues such as the under-reporting of key information in mouse based studies. There is room to improve the applied TM strategy. Crafting more flexible rules for the capture of age and including more specific ones for the recognition of sex could improve the generated results and reveal a clearer picture of the reporting of these variables in the biomedical field. While the variety of the observed common lexical patterns was not wide in the training and development sets (Supplementary files 2A and 2C), a larger set could reveal other patterns that could help increase the recall. Nevertheless, the F-measure of 92% (Table 1) gives enough confidence in using this automated method to assess the incidence of reporting sex and age in biomedical articles.

**Statistical analysis**

The frequencies of reporting of sex and age by articles were determined in Microsoft Office Excel 2013 for Windows. Differences in reporting of sex and age of mice in multiple models of diseases, as well as the use of each sex by the topic of research for each disease were assessed by two-way ANOVA without replication. An index of the reporting for each journal was calculated by dividing the number of articles that report the sex and/or age of the mouse by the number of articles that do not report any of these biological variables. Spearman’s rank correlations were calculated between the reporting index and impact factor from the Journal Citation Report, and h-index journal from the SCImago Journal and Country Rank. All statistical analysis was performed by using the GraphPad Prism software for Windows version 6.05, La Jolla CA, (www.graphpad.com). Graphical representation of the data was performed using Microsoft Office Excel for Windows version 2013.
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Additional information

**Figure 1–figure supplement 1.** Reporting of sex or age in mouse-model experiments by journal.

**Figure 1–source data 1.** PubMed search terms used for each disease group and their approaches.

**Figure 1–source data 2.** Example rules for identification of sex and age.

**Supplementary file 1.** Corpus for assessing reporting of the sex and age of the mice.

**Supplementary file 2A.** Set of articles for creating the text-mining rules.

**Supplementary file 2B.** Set of articles for finding the location of the mention of the sex and age of the mice.

**Supplementary file 2C.** Set of articles for enhancing the performance of the text-mining rules.

**Supplementary file 2D.** Set of articles for evaluating the text-mining system.

**Supplementary file 3.** Rules used to identify the sex and age of experimental mouse models.

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