Review of genetic analyses for sex differences in intracranial aneurysms

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Abstract

Intracranial aneurysms (IA) are relatively common among the population with a prevalence of 3 to 5%. They have the potential to rupture, which leads to a subarachnoid haemorrhage (SAH). Subarachnoid haemorrhages have a severe impact on health as they can cause permanent disability and can even be life-threatening. There is a small window for treatment from the moment an IA develops until an IA ruptures, however, current treatment is only used for severe cases where the chance of rupture is high and the IA might not be found before it ruptures. The prevention, detection and treatment of IAs still need further improvement to help prevent serious negative health outcomes from occurring.

Previous research on IA has made developments regarding the genetic background and sex differences. It has been found that multiple factors of IA such as prevalence, location and age of occurrence are influenced by genetics. The same factors also differ between sexes and females are more likely to develop an IA or SAH. Currently, it is not yet known to what extent the known sex differences are influenced by genetic background and this topic is very understudied.

However, many scientific methods and tools are available that could provide important information and valuable insights into genetic sex differences. Therefore, this review aims to provide an overview of the available methods and what each method could provide. The categories of genetic associations, shared biology, and risk prediction are described. The methods and tools described have the potential to improve the prevention, detection and treatment of IAs/SAHs by creating personalised or sex-specific models. Nonetheless, it is important to remember that studies must be designed properly and results must be interpreted carefully. On top of that, methods and tools are being developed and improved rapidly and therefore monitoring these developments could be helpful when deciding which method or tool is most appropriate.

Layman's Summary

An intracranial aneurysm (IA) is a dilation of an artery in the brain that fills with blood. They have a chance to rupture from the pressure and cause blood to spill into the surrounding tissue causing a subarachnoid haemorrhage (SAH). IAs are also relatively common among the general population with an occurrence of 3 to 5%. SAH causes severe health issues in a large portion of patients such as permanent disability and has a high mortality rate. This means that although the amount of IAs that rupture is low, it affects the patient's health severely when it does. However, current treatment still has many limitations and IAs go unnoticed until it ruptures in most cases. That's why it is important to further improve the assessment and treatment of IA.

To better understand IAs, two topics are especially important: heritable factors (genetic background) and differences between males and females. People with first or second-degree family members who already have suffered from an SAH are more likely to develop IAs and the occurrence of IAs in this subgroup is 7%. Factors of IAs such as risk of development, age at which the IA develops and location in the brain at which the IAs occur, are all found to be influenced by genetics. Some of the genetic factors that cause IAs also influence other health issues which are also influenced by genetics, such as Ehlers-Danlos syndrome. Overall, these findings mean that IAs are influenced by genetics. Regarding sex differences, females are more likely to develop an IA or an SAH. Besides that, factors of IAs such

as the location of the IA or the number of IAs that have developed are also found to be different between males and females. Although information is known about the genetic background and sex differences separately, the combination is currently severely understudied. It is as of yet unknown how and to what extent the genetic background influences and causes the sex differences.

However, multiple options to study the genetic background of sex differences are available and since these options could be very useful in better understanding IAs, this review aims to provide an overview of current available methods. Three categories are described in this review. The first category is finding new genetic association studies which look for new genes that cause IAs. The second category is shared biology, meaning to look if there are any similarities in genetic background when comparing two diseases with each other. The third category is risk prediction, meaning looking at a person's DNA and predicting the risk of developing an IA. By doing these different methods for a male group and female group separately, differences between the two groups might be found.

The methods that are described in the paper and summarized in the paragraph above, have the potential to provide insights that are beneficial to better understanding IAs but could also aid the development of the prevention, assessment and treatment of IAs. Thereby lowering the number of patients and severe health issues that IAs can cause. Despite the methods being promising, it is vital to develop genetic studies carefully and keep in mind limitations. It is important to choose the right method and to understand the results well. Besides that, the methods and tools that can be used for genetic studies are being improved very quickly. This means new methods are being developed constantly and staying on top of those developments should be a priority.

Introduction

Intracranial aneurysms

Intracranial aneurysms (IAs) are characterized as a bulge in a blood vessel that most often occurs at the arterial bifurcations.[1] With a population prevalence of 3-5%, IA's are relatively common.[1-4] They can grow unpredictably and carry a risk of rupture, leading to a subarachnoid haemorrhage (SAH).[2,3,5,6] Once ruptured IAs have a severe impact on health as they can be life-threatening and cause severe permanent damage. Previous studies have reported that SAH has a mortality rate of 25% to 50% where, of the survivors, about 50% develop a permanent disability as a result of the SAH.[1,2,5,6] Multiple risk factors have been reported to increase the risk of IA's including having a family history of IA's, high blood pressure, smoking habits and high alcohol consumption. Furthermore, the female sex also has a higher chance of developing an IA compared to the male sex.[1,5,7]

Most IAs rupture around 50 years of age.[1,5] There are multiple methods and treatments available which can be divided into two groups: surgical methods and endovascular treatments (EVT)[8]. The standard treatment is shifting from open surgical methods to EVTs as innovations and advancements are developed.[4,8] Unruptured IAs carry a lot fewer health risks than ruptured IAs which gives a window to treat the IA before it does rupture. Despite the treatment possibilities during this window, only a small number of patients get treatment. Only patients with a high risk for IA rupture are treated. Furthermore, some cases are too complex and cannot be treated due to complications and limitations of the currently available treatments and in some cases, ruptured aneurysms aren't properly identified until after the treatment window.[8,9] Therefore the assessment and treatment of IAs must be improved.

The prevalence of IA, its possible severe health issues as outcomes and the many limitations in treatment show that more research is still needed to better understand how IAs could be prevented, detected and treated.

Genetic background of intracranial aneurysms

One aspect of IAs that has been extensively researched is their genetic background. As described above, one of the risk factors for IA is an existing family history of IA. Previous studies have found that patients with an existing family history are more likely to develop multiple IA's and suffer from an IA rupture at a younger age compared to patients without a family history of IA. Moreover, the location of the rupture was also found to differ when comparing patients with a family history of IA to patients without.[5] The IA prevalence of 3-5% in the general population increases to 7% in the first or second degree of the relatives of patients who have suffered a SAH. These findings indicate that IAs in general and characteristics of IAs are influenced by a genetic predisposition and have led to even more research being conducted showing that genetics indeed has a strong influence on IAs.[2,5] A 21,6% SNP-based heritability was discovered[6,10] as well as strong evidence that this heritability is mostly polygenic, meaning that aneurysms are influenced by several genes as opposed to a single gene.[10]

Better understanding this genetic predisposition could help with identifying the risk of developing an IA, the risk of severe health issues as an outcome and the effectiveness of specific treatment methods. For example, a recent review reported that so far 19 risk loci have been identified that could be used to calculate the risk of developing IAs per patient.[5,10]

Certain genetic correlations have also been revealed. Firstly, a strong genetic correlation has been found between ruptured and unruptured IAs. This suggests that they have very similar genetic backgrounds.[10] Secondly, as mentioned before smoking and high blood pressure are risk factors for IAs which have been found through genetic research. A previous study found that having a predisposition for these two traits is the main genetic risk factor for developing IAs as well as for other types of strokes.[5,10] Lastly, there also seems to be an overlap between certain genetic diseases and the genetic background of IAs since the prevalence of IAs is increased in genetic diseases such as Ehlers-Danlos syndrome. Since the genetic background of IAs is strong, researching this background could potentially reveal more information that could help to create a better understanding of IA, and also improve the prediction, prevention and treatment of IAs.

Research opportunities

Although the genetic background and the sex differences of IAs have been studied independently, an understanding of the interaction between the two, or how the genetic background influences these sex differences is lacking. Previous studies have mostly focused on the influence of hormones since they are one of the aspects that are fundamental to sex differences.[1] One sex-stratified Mendelian randomization study has discovered that female patients with higher predicted levels of SHBG are associated with an increased risk of developing SAH. This association was not found when analysing male patients.[11]

Although there is a lot of research potential and certain studies are in the works, currently, there is a lack of published studies on the genetic causes of sex differences of IAs specifically. Several options are available to study genetic sex differences, and studies of other diseases could be used as examples of which the method could be leveraged to study IAs and SAHs.
For example, besides the sex hormones, sex chromosomes are also of great influence on sex differences since genes which are found on the X and Y chromosomes could also be of influence on a specific trait besides sex. Studying the sex chromosomes could therefore reveal information about the genetic sex differences of IAs. A second example is a study where the authors used data from the UKBiobank to analyse sex differences in genetic architectures for 530 different traits. They found differences in the estimate of heritability between sexes for certain traits, but also genetic correlations between sex and specific traits.[12] This study is one of the many cases that show that genetic sex differences are present in a large number of traits which most likely is also the case for IAs

and SAHs. Studying the genetic sex differences of IAs and SAHs is therefore a promising direction to better understand the genetic background and pathogenesis of these diseases.
The first example is a study on chronic pain. In this study, the authors performed a sex-stratified GWAS using data from the UKBiobank and also investigated genetic correlations and calculated polygenic risk scores specific to the sex. This was done by performing the analysis separately for males and females, which indeed revealed genetic sex differences at multiple levels which could not be found in a study with one combined cohort. Now that these differences have been discovered, more accurate sex-specific risk scores can be calculated which in turn can improve the screening and choice of treatment.[13] A second example is a study where the authors performed sex interaction and sex-stratified genetic analyses to investigate sex-specific predictors for Alzheimer's disease. This study also found that certain associations were stronger in female patients compared to male patients. They found pathways that influence Alzheimer's disease differently when comparing sexes which indicate which genes to prioritize in future research.[14]

Using similar strategies as mentioned in the examples above, useful information about IAs could be revealed which could contribute to the better understanding of IAs in multiple ways. Firstly, a more personalised risk assessment and screening could be created by including the biomarkers or risk loci that differ between the sexes. Secondly, prevention could be more personalised if a sex-stratified study shows that risk factors differ between sexes. Lastly, understanding the underlying disease mechanism better could lead to the development of sex-specific treatments which could hopefully improve outcomes and lessen complications. However, there are many methods and tools available that each have their benefits and limitations and each is useful for a different research question. Therefore, the remaining portion of this literature review aims to provide a thorough overview of possible methods that could be used to analyse the genetic sex differences of intracranial aneurysms.

Methods for analysing sex-differences

The methods that will be discussed in this review can be divided into three categories: 1. Finding new genetic associations, 2. shared biology studies and 3. risk prediction. This review discusses each category and possible examples and tools that could be used in the case of studying genetic sex differences for IAs/SAHs. An overview that summarizes these methods can be found in Table 1.

Finding new genetic associations

Genetic association studies aim to identify which genetic variants are associated with a trait. Three subtypes of studies can be used to find such associations for analysing genetic sex differences: sex-stratified GWAS, chromosome X-wide association study (XWAS) and sex-interaction GWAS. Understanding the genetic associations can improve the understanding of the pathogenesis of IAs/SAHs. Furthermore, the results of a genetic association can be used as input in downstream analysis which will be discussed in the other two categories.

Sex-stratified GWAS

A sex-stratified study means that the same analyses are repeated in a female-only and a male-only cohort. By comparing the two sets of results that these studies produce, genetic sex differences can be discovered. An example of a sex-stratified study is one where a sex-specific GWAS was performed to analyse genetic sex differences in blood insulin levels. Here, the authors found single nucleotide polymorphisms (SNPs) that differed between females and males. 13 SNPs were found to be associated with blood insulin levels in the female group as well as 13 other SNPs in the male group.[15] This could be repeated for IA or SAH and potentially show a difference in the genetic association between the sexes and create a better understanding of its genetic pathways as well.

Chromosome X-wide association study

A chromosome X-wide association study refers to GWAS studies that specifically focus on the X chromosome. These studies can help in the better understanding of sex differences with a genetic basis in a specific trait. This could reveal specific variants of interest on the X chromosome that could be used in further research or downstream analysis. Most GWAS studies have not included the X chromosome even though it could provide useful additional information and previous studies have addressed how to combat limitations.[16,17] A study has successfully created sex-specific gene expression prediction models for Alzheimer's disease showing that studying the X chromosome can be a promising method for other traits where there are known sex differences.[18] Including the X chromosome in a GWAS for IAs/SAHs can improve the understanding of the sex differences in the genetic background and downstream analysis could provide sex-specific models for either prevention, diagnosis or treatment of IA.

Sex-interaction GWAS

Sex-interaction GWAS aim to identify genetic variants that interact with sex and influence the development of IAs/SAHs or other traits and diseases. This could lead to finding sex-specific interactions and identifying genetic variants that have different effects or associations in females and males. These findings could potentially help in the better understanding of IAs/SAHs and their genetic sex differences.

GENESIS[19] (GENetic Estimation and Inference in Structured samples) and REGENIE[20] (whole-genome regression modelling) are two of the tools that can be used for sex-interaction association studies. A big advantage of these tools is that, unlike other available options, they allow for the analysis of SNPs versus sex interactions. Therefore, these are both promising tools to analyse IAs/SAHs while taking into account sex differences but do differ in certain factors. GENESIS takes into account phenotypic heteroskedasticity, which refers to the fact that the variance of a trait can differ between different groups such as different sexes.[19] Disadvantages of GENESIS is that it might be less accurate when the data is family-based. Therefore, if the dataset includes a lot of participants who are closely related to each other, REGENIE might be a more appropriate option as this tool handles samples with relatedness. On top of that, inconsistent results were found in cases where the p-values of the discovered associations were close to the genome-wide levels of significance.[21] On the other hand REGENIE is a lot more memory efficient and is therefore cost-effective as well as it accepts local segments of genotype matrices as input data. Therefore, if resources are limited it might be more appropriate to use compared to GENESIS. The tool runs faster than alternatives but is still able to provide statistical efficiency and even when the minor allele count is low, both the standard errors and effect-size estimates stay reasonable. Another big advantage of REGENIE is that it can handle samples with relatedness as mentioned before.[21] The clearest factor when comparing GENESIS and REGENIE to one another is that REGENIE outputs results that might be too conservative when using a small but highly correlated dataset while GENESIS still performs well in such cases.[21] Both tools are promising options for analysing sex differences in IAs and SAHs but when selecting the tool aspects such as available memory, sample size and correlation of the dataset will help decide which is the more appropriate option in a study. Performing a sex-interaction GWAS can improve further study design as sex-specific effects can be taken into account in downstream analysis. Besides that, it can provide a better understanding of sex-specific genetic influences and provide biological insights into these sex differences.

Shared biology studies

Genetic studies can show whether certain traits share a genetic background and provide better insights into biological relationships.[22] The most commonly used methods for these types of studies are (local) correlation studies and Mendelian randomization.

Genetic correlation studies

Genetic correlation studies aim to assess to what extent there is a genetic overlap between different traits and diseases. It analyses shared genetic factors that contribute to the development of the diseases. This can help in understanding the underlying biology and shared pathways of diseases such as IA/SAH. Two tools can be used to study correlations between diseases which are linkage disequilibrium score regression (LDSC) and genomic restricted maximum likelihood (GREML). They are both methods that are commonly used to estimate the genetic correlation of complex traits and are both based on SNPs across the entire genome.[23] The biggest difference between the two tools is that LDSC uses summary statistics while GREML uses individual genotype data. However, GREML is found to be more accurate. Therefore, if individual genotype data is available, it might be more appropriate to use GREML instead of LDSC.

In more detail, LDSC is a tool that is commonly used to estimate the SNP-based heritability of a trait, and can also be used to estimate the genetic correlation between two traits and to pinpoint specific genetic variants that are influencing multiple traits.[24] This method has multiple advantages compared to GREML. Firstly, it only uses the summary statistics that are a result of a GWAS and does not require the use of individual genotypes. Summary statistics are aggregated association statistics of every variant that was analysed in a GWAS, which uses a lot less storage than individual-level genotype data which is required for certain other tools such as GREML as mentioned before. Besides that, LDSC is not biased by sample overlap and is computationally fast.[24] However there are also limitations to this method. Although LDSC is not biased by sample overlap it can be biased by factors such as misclassification. Besides that, LDSC requires a larger size to be able to reach an equivalent standard error compared to methods that use individual-level genotypes. Lastly, LDSC is more effective when the disease has a polygenic genetic architecture otherwise it could be more effective to only analyse significant SNPs because LDSC assumes a trait is polygenetic.[24] However, as mentioned in the introduction, IA/SAH is deemed a polygenic trait and therefore LDSC is an appropriate tool to use to study this disease.

Although LDSC is most commonly used and has its advantages, when comparing it to GREML, GREML might be favourable in certain studies. A previous study has found that GREML is less biased, more accurate at calculating heritability and also more accurate at analysing correlations. The downside of GREML is that it uses individual genotype data rather than summary statistics like LDSC and therefore requires more memory and time to run.[23] LDSC and GREML could be used to find correlations in a sex-stratified study. For example, relevant SNPs or SNP-based heritability can be analysed for female and male patients separately. The results can then be compared to research how much of the genetic background of IA and SAH is shared and different when comparing female and male results. However, depending on whether individual genotype data is available or not, it might be more favourable to use GREML instead of LDSC or use both tools and compare the results for additional validation of the results.

Local genetic correlation studies

A limitation of genetic correlation studies as described above is that they cannot identify specific genomic regions that contribute to the genetic overlap between traits. Local genetic correlation studies assess genetic correlation between traits at specific regions within the genome. One tool that could be used for local genetic correlation studies is the Local Analysis of (co)Variant Associations (LAVA).[25] LAVA is a tool that can be used to study the local genetic correlation between two phenotypes but also to evaluate local heritability.[25] Advantages of LAVA are that the tested regions can be defined by the user and it accepts any degree of sample overlap.[25] However, there are also limitations. There might be residual association signals from adjacent regions and therefore local genetic correlation may be confounded. Besides that, there need to be enough SNPs overlapping to have enough power to detect true correlations. Lastly, LAVA pinpoints which location is most likely to

cause pleiotropy (single gene influencing multiple phenotypes) however, experimental validation is required to analyse whether the observed pleiotropy is indeed driven by a shared variant.[25]

A previous study used LAVA to study local genetic sex differences in a total of 157 quantitative traits where they found 146 loci across 47 traits that were significantly different between male and female data. This method could be repeated for IA/SAH to analyse whether and how much loci that influence this disease differ per sex.[26] This would help to better understand the sex differences in the genetic background and identify which loci should be focused on in further analysis.
Another example is a study that analysed local genetic correlations between neurodegenerative and neuropsychiatric diseases with LAVA. They found correlations locally that were not found globally which could also be the case for IA/SAH.[27] This showcases that it could be beneficial for IA/SAH to study both local and genetic correlations since they might differ. By performing these types of studies sex-stratified, both the global and local pathways can be compared between sexes to better understand the genetic sex differences.

Mendelian Randomization:

Mendelian Randomization (MR) is a commonly used method to analyse genetic overlap which focuses only on SNPs that are significant genome-wide to analyse causal relationships between risk factors and diseases. The major benefit of MR compared to other methods for genetic correlation is that MR can be used to test for causality. To analyse whether there is a causality, three assumptions must be confirmed. Firstly, the genotype needs to be associated with the trait. Secondly, the genotype is not associated with outcomes other than the trait. Lastly, the genotype is not dependent on other factors that affect the trait.[28] This method is highly effective when analysing traits that have multiple genetic variants that are found to be associated with the trait which explains a significant portion of the trait's heritability.[24] Besides that, the method is not affected by reverse causality and results in a largely unconfounded estimate.[29] However, there are also limitations. MR studies can potentially be biased when genetic variants are pleiotropic and could be confounded by ethnic or racial groups.[29,30] Furthermore, a large sample size is needed to ensure the estimate is precise when statistical power is low.[29,30] Lastly, MR estimates are less accurate for complex traits where the heritability is spread out over a large number of variants.[24]

Multiple studies have used Mendelian randomization to analyse the association between intracranial aneurysms and other traits. The first example is a study that performed two sets of Mendelian randomization and confirmed a causal relationship between gut microbiome and IA risk.[31] A second example shows that biomarkers for tobacco consumption have a causal relationship with IA risk as well. In this study, the authors performed a two-sample Mendelian randomization to estimate what the relationship is between the two traits and results showed that genetically predicted tobacco use is indeed associated with IA. It also showed that regularly smoking is associated with the increased risk of both ruptured and unruptured IAs.[32] and lastly a causal relationship was also found between elevated blood pressure and risk of IA after performing three Mendelian randomization tests.[33]
The above examples could be replicated in a sex-stratified study, which could potentially reveal differences in the causality of certain risk factors of IA/SAH. This would further improve the understanding of differences in the influence of risk factors on IA/SAH between sexes.

Risk prediction:

The genetic background of a disease can be used to predict the risk or the development of certain diseases. This has been done many times in previous studies for all kinds of traits and diseases by calculating polygenic scores. Polygenic scores (PGS), polygenetic risk scores (PRS) and genetic risk scores (GRS) are often used interchangeably within the literature and have subtly different meanings.[34] In this review the general term PGS is used. These risk scores are very useful tools to

group patients into low, average and high risk and are calculated with tools such as bgenix, QCTOOL and PLINK(2).[34-36]

Although PGS are a promising solution to improve the prediction of IA and SAH development, there are also some limitations. Firstly, calculating scores requires individual genotype data, meaning a file for every individual's genome which is hard to obtain mainly due to limited consent.[24] Besides that, the size of the data can be limited due to storage limitations. Biobanks such as the UKBiobank have such datasets available for usage, however, these datasets tend to mostly consist of one ancestry. This is a problem as there might also be differences in the development of a disease when comparing different ancestries. Therefore, training PGS in one ancestry might result in scores that aren't accurate for another ancestry. Furthermore, even though these biobanks contain a large amount of participants (500k in UKBiobank) when studying a disease with relatively low occurrence the number of cases might still be low. [22] Another limitation is the fact that the scores can be biased when there is sample overlap between the dataset that is used to train the risk scores and the dataset that is used to validate the risk scores.[24,34] Although limitations exist, PGS are a common method to predict the risk of developing a disease and could be used in a sex-stratified study for intracranial aneurysms where scores are calculated separately for male and female patients to more accurately predict IA risk for both sexes. One previous study has calculated general genetic risk scores for intracranial aneurysms to use for the prediction of developing subarachnoid haemorrhage. The authors validated the scores in both female and male persons and found that the prediction of SAHs performed better when the scores were trained with female data and worse when trained with male data. Indicating that calculating sex-specific risk scores might be a solution to accurately predict the risk for both female and male patients.[6]

Table 1: Overview of current methods that are promising and beneficial for studying genetic sex differences in intracranial aneurysms and other traits or diseases.

Category	Method	Description	
Finding new genetic associations	Sex-stratified GWAS	A GWAS that is performed separately for females and males	
	XWAS	GWAS specifically focuses on the X chromosome which could reveal specific variants of interest on the X chromosome.	
	Sex-interaction GWAS	Study to identify genetic variants that have a different effect size in female and male persons.	
Category	**Method**	**Description**	**Input**
Shared biology	Genetic correlation studies	A study that analyses whether there is a correlation between genetic associations between two traits or diseases on a genome-wide level. Can be done either in a sex-stratified or male-vs-female setting.	GWAS summary statistic or individual genotype data
	Local genetic correlation studies	Study that analyses whether there is a correlation between genetic variants and a trait or disease on a local level.	GWAS summary statistics
	Mendelian randomization	Test for analysing genetic overlap and causality. For sex difference perform in a sex-stratified setting.	GWAS summary statistics
Risk prediction	PGS	Method that uses genetics to calculate the risk of developing a trait or disease per individual.	Individual genotype data

Discussion

Although intracranial aneurysms when unruptured do not cause severe health outcomes, they always carry the risk of rupture which does result in serious health issues in a large group of patients. Besides that, intracranial aneurysms are relatively common in the general population. Previous studies have mostly focused on the genetic background of IAs and have shown that IAs are heavily influenced by certain genetic variants. Furthermore, previous studies have also focused on how IAs present in female patients compared to male patients and have shown that the outcomes after developing an IA/SAH differ greatly. However, the underlying causes of these sex differences have not yet been thoroughly explored. The sex differences in genetic background could potentially provide insight into the mechanisms underlying these differences.

This paper has described multiple methods that could potentially be used for analysing genetic sex differences. Studies investigating genetic associations, shared biology, and risk predictions could provide useful insights that could be beneficial in multiple ways. Firstly, the described methods will improve the understanding of the pathogenesis of intracranial aneurysms and subarachnoid haemorrhage. Secondly, risk factors might differ between sexes. By performing sex-stratified association and correlation studies, these differences could be better understood. The outcomes of these studies might show that females and males need to pay extra attention to different lifestyle aspects to prevent an IA from occurring. Thirdly, polygenetic scores can be used as risk assessment. Sex-specific risk models have the potential to predict the development or rupture of an IA more accurately, which could be beneficial information when deciding which patients to prioritize for screening or treatment. Lastly, sex-stratified studies might reveal that there is a difference in treatment response between the sexes. With that knowledge, sex-specific models can support the decision for treatment. This way, side effects and complications may be minimized.

As described in this paper, different methods will result in different information, however, each has its limitations. With every method, it is also needed to interpret the results carefully. Misinterpreting results can lead to the finding of false associations and true associations might be overlooked. As the results will hopefully lead to better clinical decisions, each study must be performed according to a well-thought-out design and with carefully validated results, to ensure any outcomes are not misguided. Besides that, methods and tools for genetic studies are being developed and improved rapidly. Therefore, it is important to be aware of new methodologies and tools that could potentially provide useful insights with fewer or different limitations.

To conclude, as medicine in general moves to a more personalised approach, it is important to move IA research along with it. To gain the most information, using a combination of the methods described will provide the most complete insights into the genetic sex difference of IAs/SAH. Each method will need to be performed carefully to minimise limitations, but in general, it is important for further research to include chromosome X and perform the method in a sex-stratified setting. This will give a better understanding of the genetic sex differences of IAs/SAHs that are currently not yet known. By understanding these sex differences, a personalised approach can be provided.

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