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Abstract	Meta-analysis is a statistical technique that is widely used for improving the power to detect associations, by synthesizing data from independent studies, and is extensively used in the genomic analyses of complex traits. Estimates from different studies are combined and the results effectively provide the power of a much larger study. Meta-analysis also has the potential of discovering heterogeneity in the effects among the different studies. This chapter provides an overview of the methods used for meta-analysis of common and rare single variants and also for gene/region-based analyses; common variants are mainly identified via genome-wide association studies (GWAS) and rare variants through various types of sequencing experiments.	
Keywords (separated by '-')	Meta-analysis - Common variants - Rare variants - Aggregation analysis - Single variant analysis - GWAS - NGS	

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Chapter 6

Meta-Analysis of Common and Rare Variants

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Abstract

Meta-analysis is a statistical technique that is widely used for improving the power to detect associations, by synthesizing data from independent studies, and is extensively used in the genomic analyses of complex traits. Estimates from different studies are combined and the results effectively provide the power of a much larger study. Meta-analysis also has the potential of discovering heterogeneity in the effects among the different studies. This chapter provides an overview of the methods used for meta-analysis of common and rare single variants and also for gene/region-based analyses; common variants are mainly identified via genome-wide association studies (GWAS) and rare variants through various types of sequencing experiments.

Key words Meta-analysis, Common variants, Rare variants, Aggregation analysis, Single variant analysis, GWAS, NGS

1 Introduction

Meta-analysis provides a powerful approach to combine data from different resources. It has been widely used in genomics in order to increase the power of single studies to detect associations with a specific trait or disease of interest [1, 2]. Sharing of genotype data between studies is not always possible, even within well-established collaborations, thus the need for alternative approaches for combining the effects from different studies has led to the wide use of meta-analyses in the field. The different meta-analysis techniques use summary statistics, are easy and quick to perform, are powerful, and practically provide the power of a much larger study.

Meta-analysis has been extensively used in order to assess Genome-wide association study (GWAS) data for millions of genotyped or imputed SNPs [2]. GWAS provide a cost-effective method for assessing the effect of common genetic variation across the genome [3, 4] and have been widely informed using imputation to publicly available genotype reference panels such as the HapMap [5], the 1000 Genomes Project Consortium [6], and the

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Haplotype Reference Consortium [7]. Over the last decade thousands of common variants, which are associated with complex diseases or traits, have been identified through imputation and meta-analyses of GWAS [8, 9]. More recently, custom arrays that focus on the replication of rare variants from sequencing experiments have been developed [10]. The identification of rare variants through genotyping arrays and imputation has been more problematic compared to common variants [11]. With the tremendous advances in next generation sequencing (NGS) technologies [12], it is now feasible to conduct large-scale whole-genome, whole-exome, and targeted sequencing experiments. Recently, novel statistical techniques have been developed for the analysis of the rare variants as single entities and also for their collective analysis in gene or regional tests. As in the common variant analysis, meta-analysis techniques will aid the increase of power to detect associations with rare variants. In the following sections, the different aspects of meta-analysis for common and rare variants are discussed.

1.1 Meta-Analysis Pre-Steps and Quality Control

Meta-analysis usually begins with the individual studies sharing summary statistics for each variant, including a regression estimate, standard error, *p*-value, sample size, imputation accuracy, and minor allele frequency (MAF). There are various steps that need to be taken into account before performing the meta-analysis in order to minimize bias. Studies need to harmonize their quality control measures and perform the analysis in the same/comparable way before being able to combine the results. Standardized quality control measures that need to be followed in each of the participating studies, for example in GWAS, include the removal of low call rate individuals and variants, removal of variants with genotype frequencies deviating from those expected under Hardy-Weinberg Equilibrium and variants with poor clusterplots [4]. Parameters for adjustments need to be set upfront so that the analyses are performed in a comparable way. Principal components should be calculated and adjusted for in the analyses [13] and appropriate genomic control can be applied to individual studies [14] in order to minimize bias due to population stratification. If the studies perform imputation they need to use the same reference panel and the same filters afterward (MAF and imputation accuracy) [15]. Different programs produce different quality metrics that need to be accounted for when performing the meta-analysis, for example "info score" from IMPUTE2 [16], "R^2" from MACH [17] and BEAGLE [18]. The individual study effect estimates need to be aligned to the same strand (usually more difficult for the ambiguous SNPs with A/T and C/G genotypes) and variants with large differences from the mean MAF need to be checked in more detail. Short insertion/deletions (INDEL) that are now being successfully imputed, using the 1000 Genomes Project data as reference, can have different annotations across the different

imputation software that need to be carefully matched. For example some software keep the actual alleles from the reference panel whereas others provide the INDELs as D/I. Furthermore, in the 1000 Genomes Project reference dataset one base is subtracted from the genomic location of the INDELs and this results in different genomic locations for the same variants across the different reference resources.

If the data come from sequencing experiments they need to be aligned on the same reference genome and low-quality variants need to be removed before the meta-analysis is performed, to avoid spurious associations due to sequencing errors. Furthermore, special care needs to be taken when combining the results of sequencing experiments that have been produced using different technologies. Different depth/coverage of the regions of interest or genotyping bias due to the differences in sequencing technologies can lead to the wrong conclusions. If the analyses are based on genes or regions with variable thresholds the classifications for SNPs/variants to be included in each gene/region need to be the same (for example minor allele frequency threshold). Before performing meta—analysis, a common statistical analysis plan needs to be adopted to ensure compatibility of the results and to aid a smooth execution [2].

2 Common Variants

Different analytical approaches have been proposed and have been extensively used for common variant meta-analyses [1, 2].

2.1 Fixed Effects

The most widely used technique for meta-analysis of common variants is the fixed-effects meta-analysis [1, 2]. The assumption behind the fixed-effect meta-analysis is that there is a single common underlying genetic effect in the different studies. This has been proven a powerful approach for discovery of common genetic variants and provides practically the same information as a pooled analysis of the raw data [19]. Different weights have been proposed with the most optimal weight being the inverse variance [20]; other weighting methods have also been used, such as the Mantel-Haesnzel method [21] or weights proportional to the sample size. The weighted effect and variance are calculated as:

$$\overline{\beta} = \frac{\sum_i^k w_i \beta_i}{\sum_i^k w_i}$$

$$\bar{v} = \frac{1}{\sum_i^k w_i}$$

where β_i is the effect estimate from each study, w_i is the weight assigned to each study and k is the number of studies. For the fixed-effects inverse variance meta-analysis, $w_i = \frac{1}{v_i}$, where v_i is the variance of each study [22]. The fixed-effect weighted test statistic follows a standard normal distribution:

$$\frac{\overline{\beta}}{\sqrt{\overline{v}}} \sim N(0,1)$$

2.2 Random Effects

When the underlying effects for each of the studies are assumed to be different but drawn from the same distribution with variance τ, random-effects meta-analysis should be used. When there is no presence of heterogeneity across the different studies, the random- and fixed-effects estimates will give approximately the same results. The most common methods for calculating the variance of the effect distribution are the method of moments [23] or likelihood-based methods [24]. Random-effects models have been used mainly for the determination of the generalizability of the results of the meta-analysis rather than for discovery purposes, as they are less powerful [2]. Most commonly researchers report both fixed- and random-effects meta-analysis results. The classic random-effects meta-analysis follows the inverse-variance scheme with the difference that the variance is now the sum of the within-study variance (v_i) plus the between-studies variance (τ^2) [22]:

$$w_i^* = \frac{1}{v_i^*}$$

$$v_i^* = v_i + \tau^2$$

$$\tau^2 = \frac{Q - (k-1)}{\sum_i^k w_i - \frac{\sum_i^k w_i^2}{\sum_i^k w_i}}$$

$$Q = \sum_i^k w_i \beta_i^2 - \frac{\left(\sum_i^k w_i \beta_i\right)^2}{\sum_i^k w_i}$$

where β_i is the effect estimate from each study, w_i is the weight assigned to each study, and k is the number of studies. The weighted effect and variance becomes

$$\overline{\beta}^* = \frac{\sum_i^k w_i^* \beta_i}{\sum_i^k w_i^*}$$

$$\bar{v}^* = \frac{1}{\sum_i^k w_i^*}$$

The test statistic then follows

$$\frac{\bar{\beta}^*}{\sqrt{\bar{v}^*}} \sim N(0,1)$$

Han and Eskin [25] observed that classical random effect models are underperforming even when there is heterogeneity present among the different studies. They proposed an alternative method for performing random effects meta-analysis whereby under the null hypothesis it is assumed that there is no heterogeneity. Han and Eskin [26] proposed the Binary Effects Assumption as another method for random effects meta-analysis. This method is based on two hypotheses; first that the effect is either present or absent in a study and second that if the studies have an effect then the effect is expected to be similar between the studies. A novel random effects model, based on a kernel machine framework, has been proposed by Shi et al. [27] for the meta-analysis of trans-ethnic studies. In the presence of substantial heterogeneity between the results in the different studies, further checking needs to be made to explore the potential reasons behind this heterogeneity [25].

2.3 P Value and Z Score Meta-Analyses

A more simplistic meta-analysis approach is the meta-analysis using the *p*-values of the individual studies [28] and the test statistic takes the form:

$$X_{2k}^2 = -2 \sum_i^k \log(p_i) \sim X_{2k}^2$$

where k is the number of studies and p_i the individual study p-value. Z score statistics-based meta-analysis [29] has also been used, the test statistic can be derived using the p-values together with the sample size information and direction of the effect. The Z-score can be calculated using the following equation:

$$Z = \frac{\sum_i^k Z_i w_i}{\sqrt{\sum_i^k w_i^2}} \sim N(0,1),$$

where

$$Z_i = \Phi^{-1}\left(1 - \frac{p_i}{2}\right) \times \text{direction of effect}$$

where w_i is the square root of the sample size and Φ is the standard normal cumulative distribution function. Although these methods are more straightforward to perform there is a substantial loss of power as no information regarding the direction of the effects in

each study is used (for the *p*-value meta-analysis), a combined effect cannot be calculated and measures of heterogeneity cannot be obtained [2].

2.4 Bayesian Meta-Analysis

Although not as widely used as the other classical methods for meta-analysis, different Bayesian methods have been also adapted for different scenarios, with both fixed and random effects. Results obtained from Bayesian meta-analyses are directly comparable among/across different SNPs without the need for comparing power and adjusting for differences in the frequencies among different variants [30]. Choosing a suitable prior to be used for the meta-analysis is an important issue and certain assumptions need to be made. Results obtained via Bayesian meta-analysis include the Bayes Factor (BF). The conventional cutoff for a test to be significant (BF $>$ 10) is not sufficient for GWAS, not due to issues of multiple testing as in frequentist testing but because the number of truly associated variants we expect to have is small [30]. Bayesian methods have also been developed for the meta-analysis of trans-ethnic GWAS data, where the studies are assigned into ethnic clusters and the effects are assumed to be the same in each ethnic group [31].

3 Rare Variants

The significant reduction in the cost of whole-exome and whole-genome sequencing in recent years has enabled large-scale sequencing experiments to be conducted [7, 32]. Chip-based rare variant experiments [10, 33] have also been performed for the assessment of rare variation.

Single variant tests for rare variants are more challenging to perform compared to common variants. The statistical tests need to be adapted for rare variants as there is usually only a small number of alternative allele counts and the current methods might not be as accurate [11]. Score type statistics have been shown to be more stable for rare variant association testing, especially for binary traits [34]; Wald statistics can be too conservative and likelihood type statistics too liberal.

3.1 Aggregation Tests

Rare variant experiments are underpowered to identify single variants associated with modest effects, even within a large sample size [11]. Different methods have been proposed to increase the power to detect associations by grouping variants into units of interest (regions). A large number of different aggregation tests have been developed to combine the effects of a subset of variants in order to obtain a region-level test statistic [11]. Aggregation testing increases the power to detect associations by combining the

cumulative effects of rare variants and by reducing the number of tests performed [11, 34].

The various aggregation tests can be extended to include any subset of variants of interest and for convenience, for the rest of this chapter, these subsets will be referred to as a "region" of interest. For example, in exome or whole-exome studies the region can be a specific exon of a single gene, all the exons of a single gene or all the exons of all the expressed genes in a genome. In the case of whole-genome studies the region can also be a genomic region (for example a sliding window of a pre-specified genomic length) or a subset of variants belonging to the same category (for example non-synonymous variants or variants that have a specific functional annotation according to functional classification software [35]). Some of the most widely used aggregation tests are discussed in more detail elsewhere in this book (*see* Chapter 5). These include the burden test or collapsing test, where a score is created for each set of variants in a region, for each study sample, and then this is compared to the disease/trait of interest. Collapsing tests can either calculate a binary value (0 rare alleles or at least one rare allele) such as CAST [36], or count the rare variants in each gene [37], or calculate a weighted sum of the rare alleles in the region [38]. Another form of collapsing test is the CMC test where the variants are grouped according to their MAF and then CAST is performed [39]. A different set of approaches are the variable threshold (VT) methods, where the decision of the MAF threshold for variants to be included in the region is obtained so that it gives the most significant result [34, 40]. Other methods developed are the variance-component (VC) tests that can detect associations in regions when variants are allowed to have opposite effects such as the C-alpha and SKAT [41, 42]. A combination of the burden and variance-component tests has also been developed such as the optimal unified SKAT-O, which takes the most significant linear combination of the burden and SKAT tests [43]. The majority of the different methods are applicable for quantitative measures, binary traits, and survival analysis data. Even when aggregating rare variants in regions of interest the power to detect associations is still small [11] and thus appropriate techniques for the meta-analysis of regional tests have been developed in order to increase the power to detect associations. Both fixed- and random-effects meta-analysis methods have been proposed for the regional tests. As estimates based on individual rare-variant regression are not stable the most optimal methods for meta-analysis of regional tests have been based on the meta-analysis of score statistics.

3.2 Meta-Analysis of Regional Tests

3.2.1 P Value and Z Score

Following a similar concept as the single variant meta-analysis the most straightforward method for regional meta-analysis is a p-value [28] or a Z score statistic [29] meta-analysis. A regional p-value or Z score is obtained from each study and then these are meta-analyzed. This is an attractive method when effect estimates cannot

be calculated or shared between the different studies. However, these methods have been shown to suffer from substantial loss of information, especially when no information of sample size and direction of the effects is used, and thus these methods are not widely used [44]. Another simple method has been proposed by Lumley et al. [45] and is performed by the summation of the test statistics for each individual study, though it is not as powerful if variants are shared between studies.

More sophisticated methods that do not result in loss of information and provide results that are as powerful as a pooled analysis of individual level data have also been proposed. In the following sections a general framework for the meta-analysis of rare variant regional association tests will be described. These tests are based on the individual study score test summary statistics and the fact that the regional test statistic can be reconstructed using the individual variant score statistic from each study [44–48]. Fixed effects and random effects meta-analysis methods have been proposed for the most widely used regional tests. Individual studies share the summary score statistic for each variant and an average variance-covariance matrix for the region [44–48]. Others have also proposed meta-analysis methods for aggregation tests that are reconstructed using the effects and standard errors from the usual regression analyses together with the correlation matrix of the individual variants (which can also be obtained from public resources) [49]. This is an attractive method when score statistics cannot be obtained but there is a substantial loss of information since variants that do not produce valid effect estimates are not used [49]. In the next sections score-based meta-analysis tests will be described; these methods have been shown to be as powerful as the analysis of the pooled genotype data [44, 46, 47].

3.2.2 Fixed Effects

The main assumption behind fixed effect meta-analysis for regional tests is that there is a shared common genetic effect across the different k studies. If we assume that there are j variants in the region of interest, we can get the combined score (U_j) and combined variance (V_j) for each variant [44, 50]:

$$U_j = \sum_i^k U_{(j,i)} \text{ and } V_j = \sum_i^k V_{(jj,i)}$$

$$w = \left(w_1, w_2, \ldots, w_j\right)^T$$

where w is a vector of weights. If a variant is not present in a specific study then the corresponding score and variance are set equal to 0. The regional score (U) and variance/covariance matrix (V) are defined as

$$U = \left(U_1, U_2, \ldots, U_j\right)^T$$

$$V = \mathrm{cov}(U)$$

The majority of the different methods use a normalized score statistic (for quantitative traits) for each variant [34, 46, 47] whereas Liu et al. [44] uses non-normalized scores.

Burden Tests

For the burden test, the assumption is that the combined score for the region is the same across the different studies $\beta_1 = \beta_2 = \ldots = \beta_k$. For testing the null hypothesis that $\beta_k = 0$, under the additive mode of inheritance, the meta-analysis test statistic takes the form [46]:

$$Q_{M-Burden} = \frac{U^{*2}}{V^*} \sim X_1^2$$

where $U^* = w^T U$ and $V^* = w^T V w$.

Equivalently form Liu et al. [44]:

$$Q_{M-Burden} = \frac{w^T U}{\sqrt{(w^T V w)}} \sim N(O, 1)$$

Weights are usually based on MAF threshold cutoff (for example MAF < 0.05 or MAF < 0.01) or the Madsen-Browning [38] weights, which up-weight rarer variants.

Variable Threshold (VT) Tests

A VT test can be constructed by calculating the burden test statistic at each MAF threshold (p):

$$Q_{M-VT} = \max_p Q_{M-\mathrm{Burden}}(p)$$

The p-value can then be calculated by comparing the test statistic to a multivariate normal distribution of U [34].

Variance Components (VC) Test, SKAT, and SKAT-O

For the VC tests, the mean of the genetic variants in the region is assumed to be the same across studies. The mean μ of the variants in the region is assumed to follow a multivariate normal distribution with mean 0 and covariance matrix $\tau_w W$. For testing the null hypothesis that the mean of the variant level effects $\mu = 0$, the meta-analysis test statistic takes the form [44, 46]:

$$Q_{M-\mathrm{SKAT}} = U^T W U$$

where W is an jxj diagonal matrix of rare variant-specific weights, usually a function of the MAF, for example if W is a diagonal Beta $(\mathrm{MAF_i}, a_1, a_2)$ this is equal to the SKAT statistic [41] and if W is an identity matrix then it produces the meta-analysis statistic for the C-alpha test [42]. The p-value of the test statistic can then be obtained by comparing the test statistic to a mixture of χ_1^2 distributions, $\sum_i^j \lambda_i \chi_{1,i}^2$ where λ_i is the i^{th} eigenvalue of $\mathrm{V}^{1/2}\mathrm{WV}^{1/2}$ [44, 46, 47].

An optimal unified test, SKAT-O [43], has been proposed; for this test the most optimal linear combination of SKAT and burden test is selected. The meta-analysis formula for SKAT-O takes the form [34, 43]

$$Q_{\text{hom}-\text{SKAT}-\text{O}} = \rho Q_{M-\text{Burden}} + (1-\rho) Q_{M-\text{SKAT}}$$

Intuitively if $\rho = 1$ the test correspond to the meta-analysis of burden test and if $\rho = 0$, to the meta-analysis of the SKAT statistic. ρ is calculated so that it produces the most significant result, the p-value of the test can be obtained using a one-dimensional numerical integration [43].

3.2.3 Random-Effects

Random-effects meta-analysis for regional tests assumes that the genetic effects of the different studies are not the same but are derived from the same distribution [51]. Heterogeneity between and across studies is expected to be a bigger issue for rare variants compared to common variants, as they are population specific [51]. Random-effects models for aggregation tests have been based on the Han and Eskin [25] single variant meta-analysis method that has been shown to be more powerful compared to other classical methods.

Burden Test

Under the random effects model for the burden test, the combined effects of the different studies are drawn from:

$$\beta_i = \mu + \xi_i, \quad i = 1, \ldots, k$$

where μ represents the average combined genetic effect among studies and ξ_i represents the deviation of the effect of study k from the mean μ, and is assumed to follow a multivariate normal distribution with mean 0 and variance σ. The test for $\mu = 0$ and $\sigma = 0$ takes the form [51]:

$$Q_{RM-\text{Burden}} = Q_{M-\text{Burden}} + \frac{\left(\sum_i^k U_i^2 - \sum_i^k V_i\right)^2}{2\sum_i^k V_i^2}$$

For the VT test we test:

$$Q_{RM-VT} = \max_p Q_{RM-\text{Burden}}(p),$$

which is the maximum of the random-effects burden tests obtained with a MAF threshold p [51].

SKAT and SKAT-O

In order to obtain the test statistic for the SKAT meta-analysis we assume that the mean of the genetic effects of the variants in each region, for each study, is drawn from the distribution:

$$\beta_i = \mu + \xi_i, \quad i = 1, \ldots, k$$

where μ is the mean of the effects of the j variants across the k studies and ξ_i is the deviation of the effects of the k^{th} study from the mean effect [34, 51]. Random effects models have been proposed for the SKAT and SKAT-O tests, by Lee et al. [47], the Het-SKAT and Het-SKAT-O and by Tang et al. [51], the RE-SKAT and RE-SKAT-O. RE-SKAT aims to detect mean effects and heterogeneity and HET-SKAT aims to detect heterogeneity in the absence of mean effects [46].

Tang and Lin [46] performed extensive simulation testing to compare the power of the different statistical models. They compared three different genetic structure models: rare variant model (for this model it is assumed that 50% of the variants with MAF $< 0.5\%$ are causal), low-frequency-variant model (where 50% of all variants are assumed to be causal), and opposite effects model (where 50% of the variants are assumed to be causal, 80% of the causal variants are risk, and 20% of the causal variants are protective) [46]. Under these genetic models they evaluated the different meta-analysis methods for the fixed- and random-effects burden, VT, SKAT, and SKAT-O models using normalized score statistics [46]. The tests were performed assuming that the effect of each study is a random variable with mean μ and variance τ. Two different mean effect values, $\mu = 0$ and $\mu = 0.25$, were tested and the variance τ was allowed to vary between 0 and 0.25. For $\mu = 0.25$ and when genetic heterogeneity was small the fixed-effects burden and VT models were more powerful than their equivalent random-effects models and the fixed-effect SKAT and SKAT-O had similar power to their corresponding random-effects models [46]. In the presence of strong heterogeneity the random-effect models were more powerful to the equivalent fixed-effects. The simulations showed that under the rare variant model VT tests were the most powerful whereas for the opposite effects model SKAT and SKAT-O were more powerful than burden and VT models [46]. Under the opposite effects structure model, the random effects models for SKAT and SKAT-O (HET-SKAT and HET-SKAT-O) proposed by Lee et al. [47] were less powerful compared to random effects models proposed by Tang et al. [51] (RE-SKAT and RE-SKAT-O) when the heterogeneity was low, and slightly more powerful when the heterogeneity was large [46]. Under the assumption that $\mu = 0$, the random effects models were more powerful in the presence of strong heterogeneity. For the rare and low-frequency variant models RE-SKAT-O was the most powerful whereas for the opposite effects model HET-SKAT and HET-SKAT-O were more powerful [46]. Tang and Lin [46] further compared the different methods using normalized and non-normalized score statistics and illustrated that the use of non-normalized score-type statistics can result in power loss. It is obvious that no single test is more

powerful under all different genetic models and since the underlying genetic model is not known upfront, it is important that the different methods are explored in each different case.

4 Heterogeneity

There can be numerous reasons underlying between-study heterogeneity in genomic studies, including differences due to different populations being studied and environmental or lifestyle factors. Other potential sources of heterogeneity include genotyping errors, differences in genotyping platforms, variants being imputed or genotyped in the different studies, or differences in the definitions of the phenotype or trait [2]. Different measures of heterogeneity have been proposed and used in the literature for assessing the between-study differences in underlying models for the common variants in GWAS. The Cochran's Q-statistic [20] and the I^2 metric [52] have been widely used in common variant meta-analyses. Q statistic is used for testing the hypothesis of no heterogeneity between studies and I^2 is a measure of the proportion of the total variability that is due to heterogeneity and takes values between 0 and 100%. Usually, a Q statistic p-value of <0.1 is regarded as significant heterogeneity, $I^2 > 50\%$ is considered moderate heterogeneity and $I^2 > 75\%$ is considered as high heterogeneity [52].

Heterogeneity is expected to play a more significant role in the meta-analyses of rare variant studies as rare variants are population specific [53] and can be more sensitive to sequencing technologies errors, quality control measures, and differences in regional annotations [51].

5 Meta-Analysis Software

The majority of the software developed for the common variant meta-analysis will perform both fixed- and random–effects meta-analysis for binary and quantitative traits and also allow for genotyped and imputed variants. For the common variant single variant meta-analysis, METAL [54], META [55], GWAMA [56], PLINK [57] and different R packages [58] like MetABEL [59] have been most widely used. MANTRA [31] and TransMeta [27] have been used for the meta-analysis of results from multiethnic studies and METASOFT for the new random effects model proposed by Han and Eskin [25] and the binary effects assumption [26]. The different software require input of individual study summary statistics including p-values, sample size, estimate of the regression coefficient, standard errors, and imputation quality metrics. GWAMA, META, and METASOFT perform both fixed- and

random effects meta-analysis whereas METAL, PLINK, and MetABEL perform only fixed effects meta-analysis. META and METAL also perform *z*-score-based meta-analysis and METASOFT implements two additional random-effects meta-analysis tests; a test that is optimal for detecting associations in the presence of heterogeneity and a test that is optimal when some studies have an effect and others do not. All software allow for genomic control adjustments and also produce measures of heterogeneity across the different studies (Cochran's Q *p*-value and I^2).

Different software have been developed for the meta-analysis of the regional rare variant test statistics including meta-analysis of score statistics (MASS) [50], RAREMETAL [60], MAGA and different R packages like MetaSKAT [47] and seqMeta [48]. Each package has their own function or complementary software for the calculation of the score statistics and covariance matrices of each individual study to be used subsequently for the meta-analysis. MASS, MetaSKAT, and seqMeta can be used to obtain summary statistics for both quantitative and binary traits whereas RAREMETAL can currently only be used for quantitative traits. seqMETA can also be used for survival analysis data and allows for different selection weights for the Burden and SKAT part of the SKAT-O. RAREMETAL and seqMeta also support the analysis of family data and conditional analyses. Furthermore, Tang and Lin developed a software to convert the summary statistics of the different rare variant meta-analysis software (PreMeta) as they are not always compatible [46]. This allows for the easier exchange of summary level statistics across the different studies without the need for each analyst to perform the analysis using the same software. Tang and Lin [46] have also proposed different transformation methods that are implemented in PreMeta which aim to achieve normality and reduce the type I error; the inverse-normal transformation (INT) and rescaled INT (R-INT). MAGA allows for the reconstruction of the regional test statistic using the single variant results from each study (effect estimates and standard errors) and uses correlation matrices from one of the component studies or publicly available resources [49]. The method implemented in MAGA is attractive when each of the component studies cannot obtain the score statistics and information matrices, but has limitation due to the fact that rare variants for which the effect cannot be estimated are not used in the analysis [49].

6 Discussion

Meta-analysis provides a powerful tool for the combination of the results of different studies in order to identify associations that would have not been found through a single study. This has proven an extremely successful method in GWAS and aided the

identification of thousands of robustly associated common variants with complex traits. Although different meta-analysis techniques have been proposed, the fixed-effects meta-analysis has been most widely used in GWAS. The field of genetics is currently being driven by rare variant studies and a large number of sequencing experiments are currently being performed. Larger power will need to be achieved in order to identify rare variant associations and aggregation methods and meta-analysis will clearly play an important role in this identification. As the underlying genetic effects of rare-variants are not known upfront and currently there is not a universally more powerful rare variant meta-analysis method, a collection of approaches need to be explored. Further validation will need to be performed to regions identified through meta-analyses of aggregation tests.

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