REVIEW

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Hardy-Weinberg Equilibrium in Meta-Analysis Studies and Large-Scale Genomic Sequencing Era

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Abstract

The Hardy-Weinberg Equilibrium (HWE) is a fundamental principle employed in the analysis of genetic data, encompassing studies of meta-analysis and genomic sequencing. It has been demonstrated that HWE possesses the property of transitivity, wherein a multi-allelic polymorphism in equilibrium will persist in its equilibrium state even when alleles are deleted or combined. Nonetheless, the practice of filtering loci that do not adhere to HWE has been observed to impact the inference of population genetics within RADseq datasets. In response to this concern, the Robust Unified Test for HWE (RUTH) has been devised to consider population structure and genotype uncertainty, thereby offering a more precise evaluation of the quality of genotype data. Furthermore, deviations from HWE, such as extreme heterozygote excess, can be effectively utilized to identify genotyping errors or to pinpoint the presence of rare recessive disease-causing variants. In summary, it is evident that HWE holds immense significance in the field of genetic analysis, and its application in meta-analysis studies and genomic sequencing can yield invaluable insights into the intricacies of population structure and the genetics of diseases.

Keywords: Hardy-Weinberg Equilibrium- Meta-Analysis- Genomic Sequencing- Extreme Heterozygote Excess

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Introduction

The concept of the Hardy-Weinberg Equilibrium (HWE) holds significant importance in the realm of population genetics and the study of evolution [1]. It posits that the frequencies of genotypes within a population remain constant over successive generations, unless external factors disrupt this equilibrium [2]. Rather than solely focusing on the genetic makeup of two parental species, HWE suggests examining the entire population in order to comprehend the process of evolution [3]. By analyzing the variation in allele frequency within the initial generation, one can make predictions about the corresponding variation in future generations [4]. Deviations from HWE may indicate errors in genotyping or the occurrence of natural selection [5,6]. While HWE equations are commonly employed to forecast allele frequencies, their application becomes more complex as population models become more intricate. Computational intelligence techniques, such as the Mamdani Fuzzy Inference System and Back Propagation Theory, have been utilized to automate HWE analysis and prediction [7]. Fuzzy logic inference systems, such as the Mamdani method, are employed to replicate real-life decision-making processes by utilizing fuzzy set theory, fuzzy rules, and fuzzy reasoning. These systems have found application in various domains, including transportation and cybernetics [7,8]. On the other hand, back propagation

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theory serves as a learning algorithm within artificial neural networks, adjusting the weights and biases of the network based on the discrepancy between predicted and actual outputs [9–11]. These techniques amalgamate the fields of engineering and ecology, furnishing researchers with efficient tools to study the diversity of population genetics, infer evolutionary history, and comprehend the ecological relationship between organisms and their environment.

HWE in Meta-Analysis Studies

In the context of meta-analytical research, the evaluation of the association between genetic polymorphisms and the risk of disease is carried out through the utilization of HWE [5,12]. HWE can be violated even without genotyping errors, making it challenging to use HWE testing for data quality assessment [5,13]. In this particular context, the significance of HWE assumes considerable importance, as genetic markers that contravene HWE may be identified as errors in genotyping or subjected to scrutiny for discernible evolutionary patterns [14]. However, the existence of population structure can contribute to the failure of genetic markers in passing HWE tests, thereby posing a predicament, given that a substantial majority of natural populations exhibit varying degrees of structure [15–17]. Furthermore, deviations from HWE can arise due to various factors including consanguinity, population differentiation, or technical issues encountered during the process of genotyping [18–20]. The identification of HWE within control populations indicates the existence of an authentic connection between a genetic locus and a particular trait. Conversely, the presence of Hardy-Weinberg disequilibrium (HWD) affecting both cases and controls can be accounted for by factors such as consanguinity, stratification of the population, or technical complications encountered during the genotyping process [12,21,22]. Within the scope of meta-analytical investigations, the adherence to HWE is of utmost importance as it ensures the reliability and validity of the pooled findings [6,23,24]. Therefore, it is advised that future research endeavors uphold the recommendation of conducting well-designed studies that do not deviate from HWE in control groups [25,26].

The HWE possesses both advantages and disadvantages in the field of meta-analysis studies. On the positive side, HWE tests are widely employed to assess the quality of genotypes and identify errors in genotyping. Furthermore, they can be utilized to estimate the number of individuals who carry homozygous and heterozygous variants, based on the frequency of alleles [5,6,27]. Nevertheless, it is important to note that HWE tests can be sensitive to deviations from equilibrium, potentially resulting in biased estimations of heritability and association test outcomes [28]. Particularly in diverse populations, HWE can be violated even in the absence of genotyping errors, posing a challenge in the evaluation of genotype data quality [15,29]. The disregard of population structure and genotype uncertainty in HWE tests may lead to an inflation of false positive rates [30–32]. Moreover, the drawbacks associated with the utilization of the HWE in the context of meta-analysis encompass the incapability to deduce random mating within a population solely based on the observation of the HWE. Moreover, deviations from the HWE may indicate the occurrence of natural selection, thereby posing challenges in differentiating between genotyping errors and advantageous variations [33,34]. Additionally, it should be noted that the commonly employed linear mixed-effect model in genetic association studies can be highly sensitive to deviations from the HWE, consequently leading to biased estimations of heritability and association test results [28,35]. Thus, it is crucial to consider population structure and potential biases when undertaking HWE meta-analysis, despite its usefulness in evaluating genotype quality and estimating variant carriers [36,37].

HWE in the Large-Scale Genomic Sequencing Era

The HWE is frequently utilized as a means of quality control in genetic investigations, particularly in the context of extensive genomic sequencing studies [3,38]. HWE refers to a state wherein the frequencies of genotypes in a given population remain constant across generations in the absence of evolutionary influences [39]. Departures from HWE can imply errors in genotyping or suggest evolutionary phenomena [40]. Nevertheless, the testing of HWE in diverse populations can be complicated by factors such as population structure and uncertainty surrounding genotypes [4]. Numerous studies have examined the impact of HWE filtering on the inference of population genetics, especially in methods like RADseq that involve reduced representation sequencing (RRS) [41,42]. Research has demonstrated that the elimination of loci that exhibit deviations from HWE can significantly influence the inference of population structure [42,43]. Furthermore, traditional HWE tests may be affected by population structure and genotype uncertainty, resulting in elevated rates of false positives [5,44]. To tackle these challenges, novel methods have been developed to account for population structure and genotype uncertainty during HWE testing [45]. Abramovs et al. have devised a filtering strategy to identify variants displaying extreme excess of heterozygotes (HetExc) and have identified HetExc variants that are enriched in genes associated with autosomal recessive diseases [6]. Kwong et al. have presented the Robust Unified Test for HWE (RUTH), which takes into consideration population structure and genotype uncertainty, and have demonstrated its efficacy in assessing the quality of genotype data [5]. Meisner and Albrechtsen have proposed a method that incorporates population structure in HWE testing, allowing for the identification of other factors that may contribute to deviations from HWE [41]. These methods offer valuable tools for the analysis of HWE in the era of large-scale genomic sequencing [46].

The RUTH is a methodology utilized to assess the equilibrium of the HWE, accounting for both population structure and uncertainty within genotypes [5,47]. This approach tackles the limitations observed in traditional HWE tests, which are susceptible to the influence of population heterogeneity and uncertain genotypes, thereby producing an elevated number of false positive results. RUTH is an accessible and scalable software tool that can

effectively carry out HWE tests across a vast number of markers and individuals [5,48]. It consistently exhibits excellent performance in terms of false positive rates and statistical power, establishing itself as one of the most reliable methods for HWE testing [49].

HetExc can arise from a myriad of factors, encompassing a substantial effective population size , natural selection favoring a surplus of heterozygotes, as well as clonality within populations [33,50,51]. The occurrence of HetExc has been witnessed in various plant species, including Dioon caputoi and Cestrum miradorense [52]. These particular species manifest heightened levels of genetic diversity, as evidenced by elevated genetic diversity values (He) and an overabundance of heterozygotes (FIT and FIS) [51,53]. Another investigation conducted by Cabrera-Toledo et al. discovered an excess of heterozygotes in all four populations of Dioon caputoi, which implies a significant degree of genetic diversity [54–56]. Likewise, Reyes-Zepeda et al. observed a surplus of heterozygotes in all life stages of the shrub Cestrum miradorense, indicating that natural selection and gene flow contribute to this phenomenon [52]. Stoeckel et al. carried out a study on wild cherry populations and found pronounced heterozygote excess, which they attributed to clonality and asexual reproduction [57,58]. Furthermore, Stevens et al. identified a departure from the equilibrium of Hardy-Weinberg due to an excess of heterozygotes in populations of Elymus trachycaulus, potentially due to the polyploid nature of the species [59,60]. In marine species, instances of extreme reproductive events can lead to a scenario where a small number of parents dominate the population, resulting in a decrease in genetic diversity. Nevertheless, complete absence of heterozygosity is not attained, indicating that populations may experience decline without overt loss of genetic variation [61,62]. The distribution of heterozygosity across generations can also exhibit peculiarities, with the positions of these singular points subtly deviating under circumstances of high reproductive variance [63,64]. The overabundance of heterozygotes is believed to be the consequence of natural selection favoring heterozygotes, as well as a combination of reproductive systems and mechanisms of gene flow that foster genetic diversity [63]. The method of heterozygote excess has been proposed as a means to estimate effective population size (Ne) in small populations, including dioecious and self-incompatible species [65,66]. The HetExc observed in the urochordate Ciona savignyi is ascribed to its substantial effective population size and the presence of compelling evidence for robust purifying selection [67,68]. However, the efficacy of this method may be limited to populations with a small number of reproducing individuals [69,70]. This particular approach utilizes the heterozygote surplus detected at neutral markers to evaluate the effective size. It can be utilized with small dioecious populations as well as self-incompatible monoecious species [65,71].

HetExc can exert significant impacts on the population dynamics of species. It has the ability to result in elevated genetic diversity and reduced population differentiation [72–74]. In the investigation conducted by Abramovs et al., they devised a filtering strategy to detect variants with HetExc and successfully identified 161 such variants in 149 genes, predominantly observed in African/African American populations. They put forth the notion that an excess of heterozygotes might indicate natural selection, particularly for advantageous recessive disease-causing variants [6]. In a separate discourse, Milkman discussed the Wahlund effect, which can produce an abundance of heterozygotes when populations with varying allele frequencies intermingle [75,76]. The phenomenon known as the Wahlund effect pertains to the deviation from HWE that arises as a consequence of the division and amalgamation of individuals from distinct genetic populations [42,77]. This effect can lead to an overabundance of homozygotes and a shortage of heterozygotes within a group of individuals under examination [64,78,79]. The Wahlund effect exhibits susceptibility to various factors, including null alleles and sampling variation among limited local breeding clusters [80,81]. Additionally, Balloux suggested employing heterozygote excess as a means to estimate the effective population size in small populations, including self-incompatible monoecious species [50]. On the other hand, there is evidence of genotype-specific selection at a BRCA2 polymorphism, with an excess of heterozygotes in women but a shortage in newborn boys [82]. These investigations underscore the potential significance of heterozygote excess in comprehending genetic diversity, natural selection, and population dynamics in the human species [83,84].

In summary, HWE is frequently utilized for the purpose of quality control in genetic investigations, encompassing case-control studies, meta-analysis studies and large-scale genomic sequencing. Departures from HWE may signify errors in genotyping or evolutionary factors such as natural selection. Several investigations have devised approaches to identify variants that deviate from HWE and applied them to extensive population databases. These investigations have detected numerous variants that HetExc, particularly in African/African American populations. Although the majority of these variants are not linked to known diseases, they are concentrated in genes associated with autosomal recessive diseases. Moreover, variants known to cause recessive diseases and exhibit evidence of heterozygote advantage have been identified. Techniques that consider population structure in HWE testing have been proposed to enhance accuracy. These techniques take into account factors like population heterogeneity and genotype uncertainty, which can influence the outcomes of HWE tests.

Author Contribution Statement

Hossein Neamatzadeh: Data analysis, writing - Seyed Alireza Dastgheib: Conceptualization, methodology - Mahta Mazaheri: Investigation, visualization - Amirhossein Omidi , Ali Masoudi: Project administration, funding acquisition - Amirmasoud Shiri: Software, validation - Abolhasan Alijanpour: Formal analysis, resources - Ahmad Golshan-Tafti: Supervision, review - Maryam Aghasipour: Methodology, writing - Maryam Yeganegi: Data curation, editing - Mohammad Bahrami:

Visualization, project management - Kazem Aghili: Validation, conceptualization - Sahel Khajehnoori: Writing, data analysis - Alireza Mosavi Jarrahi: Editing, supervision.

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Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Availability of data and material

The datasets generated during and/or analyzed during this study are the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

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