COMPARISON OF DIFFERENT TESTS FOR DEVIATION FROM HARDY-WEINBERG EQUILIBRIUM OF AMPFLP POPULATION DATA

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INTRODUCTION

Since its introduction in 1985, VNTR polymorphism analysis has rapidly become a routine technique for forensic biology and population genetics. But so far there are still some controversies surrounding VNTR polymorphisms, one of them being whether they violate the assumptions of Hardy-Weinberg equilibrium (HWE). Concerning biostatistical tests the only significant difference between VNTR loci analysed by AMP-FLP and traditional serological markers is that there are more alleles at a particular VNTR locus, so that not all genotypes can be observed in a relatively small sample. Therefore, some indirect methods have been suggested to test HWE for these VNTR loci (1-4), but the efficiency of them was not evaluated. Our purpose here is to compare the efficiency of different statistical tests and to suggest a more sensitive new modification.

MATERIALS AND METHODS

EDTA-blood samples were collected from 121 unrelated Chinese individuals in Chengdu, Sichuan province, PR China. The amplification of VWA and COL2A1 loci and the electrophoretical analysis of PCR products were carried out as previously described (5,6).
The different tests for HWE include 1) observed versus expected number of heterozygotes (1-3), 2) observed versus expected number of distinct heterzygotes (1,2), 3) binning alleles (4). In addition, we suggest a modified statistical approach to test HWE (test 4).

$$\chi^2 = \sum_{j=1}^{h} \frac{(Oj-Ej)^2}{Ej} + \sum_{i=1}^{g} \frac{(Oi-Ei)^2}{Ei} = \sum_{j=1}^{h} Ej + \sum_{i=1}^{g} \frac{(Oi-Ei)^2}{Ei} \qquad (1)$$

$$(j=1,2,\ldots,h;\ i=1,2,\ldots,g;\ k=g+h)$$

where Oi and Ei are the number of the i th observed and expected genotype, respectively, while Oj and Ej are the number of the j th observed and expected genotype, respectively, which is not virtually observed in a population sample. k is the number of genotypes at a this locus. If m is the number of alleles, the degree of freedom (df) of the approximate chi-square distribution to test HWE can be obtained by

$$df = (k-h+1) - m = (g+1) - m \qquad (2)$$

ΣEj can be obtained by $\Sigma Ej = N - \Sigma Ei$ (3)

where N is the number of individuals in a sample. If a significant deviation from HWE was observed using equation (1) with ΣEj greater than the critical value with one degree of freedom, a relatively small sample size may be one of causes of deviation from HWE and further tests need to be carried out. Because the sampling error of the allele frequency is much larger in relatively rare alleles with a lower frequency than in relatively common alleles

with a higher frequency, it is suggested to pool relatively rare alleles into one group. Afterwards a further Hardy-Weinberg test with equation (1) or a normal chi-square test can be carried out. If now the deviation from HWE is not significant any more, it can be concluded that for this VNTR locus on a particular pooling allele level the population sample is in HWE, and that the deviation from HWE observed for the first test is due to relatively small sample size.

RESULTS AND DISCUSSION

The results of the different tests for HWE are shown in Tables 1 and 2. In order to compare the efficiency of the different statistical tests, subsamples within these Chinese were sampled in random and tested for HWE (n=25 and n=50, tables 1,2). If the sample size was very small (n=25), no test revealed that there was a significant deviation from HWE in both VWA and COL2A1 loci. This is easily understood because with a smaller sample size the probability of a type II error (ß) is very large for any statistical test. The power of test (1-ß) becomes so low that any conclusion, that the sample is in HWE, is not reliable. With increasing sample size (n=50 and n=120), by test 4 a significant deviation from HWE was observed at the COL2A1 locus. Since HWE is an equilibrium of the distribution of alleles in the population among the various genotypes, a significant deviation from HWE should be observed, if many of the genotypes are not present in the sample. But tests 1, 2 and 3 failed to reveal this deviation from HWE at the COL2A1 locus (P>0.05), because they do not contrast the observed with the expected frequencies of all possible genotypes in a sample. Therefore, they are less sensitive approaches for testing HWE.

Table 1. Tests for HWE of genotype frequencies at the COL2A1 locus

	n = 25		n = 50		n = 120	
Test	Observed	Expected	Observed	Expected	Observed	Expected
1. Number of heterozygotes	20	17.37	36	36.45	90	84.32
homozygotes	5	7.63	14	13.55	30	35.68
	P>0.05		P>0.05		P>0.05	
2. Number of distinct heterozygote genotypes	6	5.52	14	12.51	19	16.71
homozygote genotypes	3	2.53	3	2.85	4	3.34
	P>0.05		P>0.05		P>0.05	
3. Binning alleles 3-allele model	x^2 =3.37 df=3 P>0.05		x^2 = 2.01 df=3 P>0.05		x^2 =1.93 df=3 P>0.05	
4. Modified x^2 1). ΣEj	ΣEj=1.16 x^2 =6.11 df=5 P>0.05		ΣEj=6.01 x^2 =67.44 df=7 P<0.05		ΣEj=7.78 x^2 =76.85 df=10 P<0.05	
2). Pooling rare alleles	-		ΣEj=2.15 x^2 =10.82 df=9 P>0.05		ΣEj=0 x^2 =7.95 df=10 P>0.05	

Table 2. Tests for HWE of genotype frequencies at the VWA locus

Test	n = 25		n = 50		n = 121	
	Observed	Expected	Observed	Expected	Observed	Expected
1. Number of						
heterozygotes	20	19.47	38	39.92	97	95.69
homozygotes	5	5.53	12	10.08	24	25.31
	P>0.05		P>0.05		P>0.05	
2. Number of distinct						
heterozygote genotypes	10	8.58	13	11.99	16	15.20
homozygote genotypes	2	2.75	5	3.92	5	4.67
	P>0.05		P>0.05		P>0.05	
3. Binning alleles						
3-allele model	x^2 =1.02		x^2 =4.16		x^2 =1.33	
	df=3		df=3		df=3	
	P>0.05		P>0.05		P>0.05	
4. Modified x^2						
	ΣEj=2.56		ΣEj=1.75		ΣEj=3.50	
	x^2 =11.44		x^2 =10.41		x^2 =16.18	
	df=7		df=12		df=14	
	P>0.05		P>0.05		P>0.05	

Compared with the above three tests, test 4 possesses some different features. Firstly, it is a contrasting of every observed genotype frequency with its respective expectation, and hence it can detect deviations of each specific genotype frequency from its HWE prediction. Secondly, it is noted that in a relatively smaller sample there is always a deficit between the total of observed and expected genotypes. This deficit is essentially equal to the totality of chi-square between the not observed genotypes and their respective expectations ($\Sigma((Oj-Ej)^2/Ej) = \Sigma Ej = N-\Sigma Ei = x^2$, df = 1). This feature is very useful for testing HWE with the chi-square test, because a relatively smaller sample size can be allowed and it is not necessary to observe all genotypes. Thirdly, it is ΣEj that offers a reference standard to judge whether or not the observed deviation from HWE is due to the absence of a large fraction of genotypes in a sample. Since ΣEj is defined with one degree of freedom, if Σj is smaller than the critical value with one degree of freedom, it will be assumed that the absence of some genotypes in a sample has only little influence on a test for HWE. For example, at the VWA locus, equation (1) can be directly applied to test HWE, if the sample size is large (n=121). In contrary, if ΣEj is larger than the critical value with one degree of freedom, the absence of a large fraction of genotypes will be considered as the main cause of deviation from HWE, and hence pooling relatively rare alleles to cancel this effect may be necessary for assessing the goodness of fit of the distribution of genotypes from their HWE in a relatively small sample.

It should be emphasized that the approach of pooling rare alleles (test 4) is different from that of binning alleles (test 3). With the approach of binning alleles, alleles with similar electrophoretic mobility or molecular weight are classified as one group and all alleles in a sample are divided into a few classes according to their electrophoretic mobility or molecular weight. Unfortunately, this classification is not significant for testing HWE, because there is no relationship between the electrophoretic mobility or molecular weight of a allele and its frequency in a sample, and for testing HWE only the allele frequency is important. Furthermore, binning alleles will change the characteristics of the distribution of the allele frequencies in a sample, and because of binning most of the independent alleles are not subjected to the test for HWE. Since new alleles and genotypes produced by binning differ from alleles and genotypes observed in a sample, with this approach the sample is not virtually examined for

HWE. In fact, Brenner and Morris, who first suggested the "allele binning" strategy for DNA data analysis, have indicated that using this approach Hardy-Weinberg tests are fraught with artifactual pitfalls, and they would view with skepticism claims that a population is or is not in HWE (7). In contrary, the approach of pooling rare alleles can not only decrease ΣEj caused by the absence of a large fraction of genotypes, but also maintains the characteristics of the distribution of allele frequencies in a sample. With this approach, HWE is defined on a feasible pooling allele level for a relatively small sample. For example, at COL2A1 locus all alleles with a frequency of less than 0.01 were pooled into one group, and on this level the Chinese sample is in HWE ($n=120$, $P>0.05$). This means that each allele with a frequency of more than 0.01 has been subjected to the test for HWE as an independent allele, and its frequency in the sample is the unbiased estimate of the population. Since rare alleles have been examined for HWE as one group, the frequency of this group is the unbiased estimate of all rare alleles in the population, and the frequency of each rare allele observed in the sample is the upper limit of its estimate in the population. Therefore, these data can be directly applied to calculate the relative propabilities dealing with individual identification and paternity analysis.

The analyses here indicate that, even though the genetic variation at VWA and COL2A1 loci is extensive, the chi-square test can be applied to assess the goodness of fit of the distribution of genotypes from their HWE. This method can also be employed to test HWE for other similar AMPFLP systems. Although some of the indirect approaches can also detect any gross divergence of genotype frequencies from their Hardy-Weinberg expectations, they are less sensitive for testing HWE. Some of the modified methods like binning alleles should be avoided because with these approaches the sample is not virtually examined for HWE.

REFERENCES

1 Chakraborty R, Fornage M, Gueguen R, Boerwinkle E: Population genetics of hypervariable loci: analysis of PCR based VNTR polymorphism within a population. In: Burke T, Dolf G, Jeffreys AJ, Wolff R: DNA Fingerprinting: approaches and applications. Birkhäuser Verlag: Basel, 1991:127-143.

2 Edwards A, Hammond H, Jin L, Caskey CT, Chakraborty R: Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 1992;12: 241-253.

3 Deka R, Chakraborty R, DeCroo S, Rothhammer F, Barton SA, Ferrell RE: Characteristics of polymorphism at a VNTR locus 3' to the apolipoprotein B gene in five human populations. Am J Hum Genet 1992;51:1325-1333.

4 Alonso A, Martin P, Albarran C, Sancho M: Amplified fragment length polymorphism analysis of the VNTR locus D1S80 in central Spain. Int J Leg Med 1993;105:311-314.

5 Hou Y, Prinz M, Schmitt C, Staak M: Population genetics of the VNTR-polymorphism COL2A1: Comparison of Chinese and German data. In: Proceedings of 13th International Meeting. International Association of Forensic Sciences: Düsseldorf, 1993; in press.

6 Hou Y, Gill P, Schmitt C, Staak M, Prinz M: Population genetics of three STR polymorphisms in a Chinese population. In: Advances in Forensic Haemogenetics 5. Springer Verlag: Berlin Heidelberg, 1993; in press.

7 Brenner C, Morris JW: Paternity index calculations in single locus hypervariable DNA probes: validation and other studies. In: Proceedings for International Symposium of Human Identification. Promega Corporation: Madison, 1990:21-53.