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Statistical Bias and Inflated Variance in the Genehunter Nonparametric Linkage Test Statistic

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Abstract

Evidence of linkage is expressed as a decreasing trend of the squared trait difference of two siblings with increasing identical by descent scores. In contrast to successes in the application of a parametric approach of Haseman-Elston regression, notably low powers are demonstrated in the nonparametric linkage analysis methods for complex traits and diseases with sib-pairs data. We report that the Genehunter nonparametric linkage statistic is biased and furthermore the variance formula that they used is an inflated one, and this is one reason for a low performance. Thus, we propose bias-corrected nonparametric linkage statistics. Simulation studies comparing our proposed nonparametric test statistics versus the existing test statistics suggest that the bias-corrected new nonparametric test statistics are more powerful and attains efficiencies close to that of Haseman-Elston regression.

Keywords: Haseman-Elston method, nonparametric linkage analysis, Genehunter, sib-pairs.

1. Introduction

Previously there have been spectacular successes in the application of parametric linkage analysis to monogenic traits and diseases of cystic fibrosis and neurofibromatosis, but unsuccessful outcomes have been noted in the application of nonparametric linkage(NPL) analyses to complex traits and diseases, such as hypertension, diabetes mellitus and psychiatric disorders. This lack of success led some researchers to re-examine the NPL approach, because they believe that if the problems of NPL approach are identifiable, it can potentially be corrected. Schork and Greenwood (2004a) pinpointed a source of bias during data compilation; that is, keeping the sib or relative pairs data whose alleles shared identical by decent(IBD) are uncertain and thus assigning the expected IBD values to these cases will produce bias. In response to Schork and Greenwood (2004a) paper, there have been much debates subsequently (Mukhopadhyay *et al.*, 2004; Visscher and Wray, 2004; Sieberts *et al.*, 2004; Abecasis *et al.*, 2004; Schork and Greenwood, 2004b). On the other hand, a large-scale simulation study conducted by Davis and Weeks (1997), on the analysis methods and software packages so far developed, is helpful to recognize in a concrete way the degree of problems of the NPL statistic. Davis and Weeks (1997) have found that, while the Haseman-Elston (1972) method is powerful for all situations and also with different data sets of sib-pairs, the Genehunter NPL statistic of Kruglyak and Lander (1995) have surprisingly low powers, being placed in the lowest quartile in power rankings.

In this report, we give a conclusive proof of the statistical bias of the Genehunter NPL statistic of Kruglyak and Lander (1995) under the assumption that the information of alleles shared IBD in sib-pairs is known with certainty, and we demonstrate further that their variance estimate is not the minimal one. Thus, we are led to propose more precise variance estimate than that Kruglyak and Lander (1995) have suggested. In certain cases, biased test statistics are preferred because, despite of

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possessing an undesirable property of being biased, the test statistic may demonstrate better overall performance compared to the unbiased test statistic with a smaller variance. However, this is the not case with the Genehunter NPL statistic which has both statistical bias and inflated variance, and that certainly affected negatively on the performance, even if the sources of bias mentioned by Schork and Greenwood (2004a) and other researchers are absent.

2. Methods

Suppose that we have a sample of n independent sib-pairs and we assume random mating, linkage equilibrium, and no epistasis. We will restrict to a single point situation, which is enough for the purpose of comparing the powers of several linkage test statistics. The Haseman-Elston regression, the commonly used linkage analysis method in sib pair studies is based on the value of squared trait difference $y_i = (x_{i1} - x_{i2})^2$ as a dependent variable, where x_{i1} and x_{i2} are the trait values of each sib within sib-pair i ($i = 1, \ldots, n$), and the number of alleles shared IBD at a marker locus as an independent variable. On the other hand, the NPL statistic is based on ranks of all values of the squared trait difference y_i's. In the notation of Kruglyak and Lander (1995), the rank of y_i is denoted by rank(i). The Genehunter NPL statistic is defined as $X_{NPL} = \sum_{i=1}^{n} \text{rank}(i) \times f(v_i)$, where $f(v_i)$ is a simple function (chosen to have expected value 0) of v_i, the number of alleles shared IBD by two siblings at the locus of interest. Thus, under the assumption that there is complete genetic information, each sib-pair shares 0, 1, or 2 alleles IBD. In the absence of dominance effects, for example, Kruglyak and Lander (1995) suggested $f(0) = -1$, $f(1) = 0$ and $f(2) = 1$. As a result of this particular function chosen, this intuitive nonparametric NPL statistic resembles to testing a linear relationship in the Haseman-Elston regression. In other words, the NPL statistic is basically $R_2 - R_0$, the difference of rank sums, where R_2 and R_0 is the observed rank sum of sib-pairs belonging to the IBD group of score 2 and 0, respectively, in the joint ordering of all n values of the squared trait differences of sib-pairs.

2.1. Expectation and variance of the Genehunter NPL statistic

Kruglyak and Lander (1995) stated that, in the absence of linkage, the statistic X_{NPL} has expectation 0 and variance $V_{NPL} = n(n + 1)(2n + 1)/12$. Thus they proposed $Z_{NPL} = X_{NPL}/\sqrt{V_{NPL}}$ as a test statistic, which is asymptotically distributed as a standard normal; significance is determined by a one-sided testing of $Z_{NPL} < 0$ that matches with a negative slope in the Haseman-Elston regression, in which a decreasing squared trait difference of two siblings with increasing IBD scores is considered as evidence of linkage. The authors stated that X_{NPL} is equivalent up to rescaling to the two-sample Wilcoxon rank sum statistic.

2.2. Expectation and variance of the new NPL statistic

We now derive the expectation and variance in a new approach. The rank sum R_2 and R_0 in the NPL statistic is obtained from the joint ordering of n squared trait differences of sib-pairs, even if only difference of two rank sums appears in the statistic. Therefore, we have to rely on the formulas of expectations, variances and covariances of the rank sums for more than two groups. For the case of three IBD groups in the sib-pair linkage study, when n_0, n_1 and n_2 are the observed number of sib pairs in each IBD group of score 0, 1 and 2, respectively, with $n = n_0 + n_1 + n_2$, Hettmansperger (1984, p.180) provided the formulas: $E(R_i) = n_i(n + 1)/2$, $\text{var}(R_i) = n_i(n - n_i(n + 1))/12$ and $\text{cov}(R_i, R_j) = -n_i n_j(n + 1)/12$, for $i = 0, 1, 2$. Thus, the difference of rank sums has the following expectation and

variance:

$$E(R_2 - R_0) = \frac{(n_2 - n_0)(n+1)}{2}, \tag{2.1}$$

$$\text{var}(R_2 - R_0) = \frac{(n+1)\left\{n(n_2 + n_0) - (n_2 - n_0)^2\right\}}{12}. \tag{2.2}$$

The new NPL test is then based on

$$Z_{New} = \frac{R_2 - R_0 - (n_2 - n_0)(n+1)/2}{\sqrt{(n+1)\left\{n(n_2 + n_0) - (n_2 - n_0)^2\right\}/12}}, \tag{2.3}$$

which may be referred to a table of the standard normal distribution if n is large. Under random sampling, the proportion of sib-pairs sharing 0, 1 and 2 alleles IBD is assumed to be approximately equal to 1/4, 1/2 and 1/4 and this relationship is used in many situations, particularly in sample size calculations in the planning stage of a linkage study. However, we have to remind ourselves that this is just an approximation and, in the application of linkage analysis to a sample of observed data, this condition is seldom satisfied. The two IBD groups can be of quite different sizes. When the numbers of sib-pairs in the two IBD groups differ, the expectation has non-zero value and its magnitude is non-ignorable if the total number of sib-pairs is large. We have come across biomedical researchers who believe that the condition of no linkage (more precisely under no genetic effect) naturally imply that the two IBD groups of score 0 and 2 have always equal numbers of sib-pairs, which is not true. Different interpretations of the null hypothesis in the linkage analysis, that is, hypothesis of no linkage are presented in Hädicke *et al.* (2008), and neither of these hypotheses implies that the sample sizes of the two IBD groups are equal. They are assumed to be equal, out of computational convenience, in the demonstration of an example or simulations.

The magnitude of variance is also important in determining test efficiencies. Notice that the variance V_{NPL} given by Kruglyak and Lander (1995) is a function only of the total number n of sib-pairs in a sample, while the variance of the new NPL statistic is a function of n_0 and n_2, in addition to n. Therefore, when the two variances are compared under the sample sizes of $n_0 = n_2 = m$, $n_1 = 2m$, the variance of the new NPL statistic is $\text{var}(R_2 - R_0) = n(n+1)(2m)/12$, while that of X_{NPL} is $V_{NPL} = n(n+1)(8m+1)/12$, and thus V_{NPL} is roughly four times larger than the variance estimate used in the new NPL statistic. Furthermore, V_{NPL} would be much larger than four times if $n_0 \neq n_2$.

Notice that the variance of X_{NPL}, which is $V_{NPL} = n(n+1)(2n+1)/12$, is in fact the expected variance under the assumption of no linkage, not the one which is estimated each time differently for a sample of observed data. In other words, based on the fact that Kruglyak and Lander (1995) have considered that the expected value of X_{NPL} is 0, and that the two IBD groups of score 0 and 2 approximately involve only a half of all sib-pairs, the expected variance of X_{NPL} would be identical to

$$\text{var}(X_{NPL}) = E\left(X_{NPL}^2\right) = \frac{1}{2}\sum_{i=1}^{n} i^2 = \frac{n(n+1)(2n+1)}{12}. \tag{2.4}$$

We understood in this way how the variance of X_{NPL} is derived. In summary, non-ignorable magnitude in the variance reduction and bias correction in the new NPL statistic are expected to lead to possible improvements in power over the Genehunter NPL statistic. We will examine with a simulation study in which situations this new NPL statistic is powerful and also robust compared to the Genehuneter NPL statistic.

2.3. Extension of the NPL statistic

Several other rank-based test statistics can be derived in the process of proposing the new NPL statistic. One of the closest variant of new NPL statistic is to construct a test statistic based on the observed rank averages rather than rank sums in each IBD groups. When the discrepancy between n_0 and n_2 is large, rank averages may differ from rank sums. The rank average is defined as $\bar{R}_i = R_i/n_i$, where R_i is the rank sum of the IBD group of score $i\,(i = 0, 1, 2)$ with a sample size n_i. The expectations, variances and covariances of the rank averages for more than two groups, are easily derived using the formulas found in Hettmansperger (1984): $E(\bar{R}_i) = (n + 1)/2$, $\mathrm{var}(\bar{R}_i) = (n - n_i)(n + 1)/12n_i$ and $\mathrm{cov}(\bar{R}_i, \bar{R}_j) = -(n + 1)/12$. Under the null hypothesis of no linkage, the expectation of a difference of two rank averages, $\bar{R}_2 - \bar{R}_0$, is 0, and the variance is equal to

$$\mathrm{var}\left(\bar{R}_2 - \bar{R}_0\right) = \frac{n(n+1)}{12}\left(\frac{1}{n_0} + \frac{1}{n_2}\right). \tag{2.5}$$

Thus, the ratio $Z_{Ave} = (\bar{R}_2 - \bar{R}_0)/\sqrt{\mathrm{var}(\bar{R}_2 - \bar{R}_0)}$ is asymptotically standard normally distributed and linkage testing is done similarly as above.

Other nonparametric statistic is worth mentioning due to its close nature of a decreasing trend alternative. Terpstra (1952) and Jonckheere (1954) independently proposed a test for the ordered alternative which is based on two-sample Wilcoson statistics. Jonckheere-Terpstra trend statistic has the attractive feature that it is based on separate ranking of different pairs of two groups each, *i.e.*, comparing the two groups of IBD 0 and 1, IBD 1 and 2, and IBD 0 and 2, instead of ranking the observations in all three groups. A detailed description of the Jonckheere-Terpstra trend statistic in linkage testing can be found in Kim *et al.* (2006).

3. Simulation Studies

A simulation study was undertaken to compare the performance of the Genehunter NPL statistic Z_{NPL} with the test statistics mentioned above, in which the new NPL statistic Z_{New}, the rank average-based statistic Z_{Ave}, the Jonckheere-Terpstra trend statistic, and finally the Haseman-Elston regression on raw data are included. Following Haseman and Elston (1972), consider a quantitative trait locus with two alleles B and b with population frequency p and $q = 1 - p$, respectively. For m^{th} individual in a pair, it is assumed that a trait value x_m follow a linear model $x_m = \mu + g_m + e_m$, where $m = 1$ or 2 with an arbitrary ordering; μ is the overall mean, g_m is the genetic effect of the trait locus and e_m is the residual effect. And, g_m and e_m are stochastically independent and $e = e_1 - e_2$ has mean 0 and variance σ_e^2. The difference in residuals for different sib-pairs, that is, e's are mutually independent, but e_1 and e_2 of a sib-pair are correlated. Define $g_m = a$, if genotype of the m^{th} member of a sib-pair at quantitative trait locus is BB, d if it is Bb, and $-a$ if it is bb. A genetic model is recessive if $d = -a$, and additive if $d = 0$, and dominant if $d = a$ with $-a \le d \le a$. The genetic variance σ_g^2 due to the trait locus is given by $\sigma_g^2 = \sigma_a^2 + \sigma_d^2$ with $\sigma_a^2 = 2pq\{a - d(p - q)\}^2$ and $\sigma_d^2 = (2pqd)^2$ are the additive and dominance variance, respectively. The broad sense trait locus heritability h^2 is defined as $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$.

We adopted the simulation plans of Kim *et al.* (2006); 2,000 replicates with 200 and 500 sib-pairs per replicate were generated under additive, dominant and recessive genetic models for empirical powers and 5,000 replicates were generated to obtain empirical type I error rates. The simulations with zero heritability $h^2 = 0$ or equivalently $\sigma_g^2 = 0$, implying no differences among trait distributions of three IBD groups, correspond to the null hypothesis. The simulations steps were: first, we

Table 1: Empirical type I error rates with 5,000 replicates for a completely informative marker at the trait locus at nominal $\alpha = 0.05$ level under normal, contaminated normal and lognormal residual distributions. The residual correlation is 0.5 and allele frequency p at the diallelic trait locus varied with the genetic model. JT corresponds to the type I error rates with the Jonckheere-Terpstra trend statistic and HE to the type I error rates with Haseman-Elston regression on raw trait values.

Residual distribution	p	$n = 200$					$n = 500$				
		Z_{NPL}	Z_{New}	Z_{Ave}	JT	HE	Z_{NPL}	Z_{New}	Z_{Ave}	JT	HE
Normal	0.1	0.050	0.051	0.052	0.052	0.044	0.045	0.055	0.055	0.054	0.060
	0.3	0.051	0.048	0.050	0.047	0.046	0.047	0.049	0.049	0.048	0.048
	0.5	0.046	0.052	0.053	0.051	0.057	0.048	0.047	0.047	0.047	0.058
Contaminated normal	0.1	0.048	0.055	0.056	0.056	0.057	0.050	0.054	0.055	0.055	0.051
	0.3	0.048	0.047	0.048	0.048	0.051	0.045	0.043	0.042	0.042	0.055
	0.5	0.048	0.051	0.053	0.051	0.058	0.053	0.049	0.050	0.050	0.052
Lognormal	0.1	0.050	0.051	0.051	0.050	0.049	0.051	0.042	0.043	0.042	0.054
	0.3	0.048	0.048	0.047	0.047	0.046	0.058	0.051	0.051	0.052	0.047
	0.5	0.048	0.050	0.049	0.050	0.044	0.055	0.053	0.052	0.052	0.040

generated the trait IBD scores of sib-pairs by use of a trinomial random number generator with cell probabilities of 1/4, 1/2 and 1/4, respectively. Secondly, we generated the trait genotypes of the sib-pairs from a multinomial distribution, with cell probabilities given by the conditional probabilities of the generated trait IBD scores, given the trait genotypic pair (given in Table 1 of Haseman and Elston (1972)). Thirdly, the trait residual values for sib 1 and sib 2, each had the same marginal distribution with a specified residual sibling correlation of 0.5, were simulated from the following distributions: symmetric trait distributions of normal and contaminated normal and asymmetric trait distribution of lognormal (Fernàndez *et al.*, 2002). The contaminated normal and lognormal distributions are examples for which there exists intrinsic non-normality in the trait distributions. The contaminated normal distribution of trait residuals is generated by $e \sim (1-c)N(0,\ \sigma_e^2) + cN(\delta,\ (f\sigma_e)^2)$, where $0 \leq c \leq 1$. When $c \neq 0$, each error term is a random observation from either a $N(0,\ \sigma^2)$ distribution with probability $1-c$ or a $N(\delta,\ (f\sigma_e)^2)$ distribution with probability c. The values of c were chosen to be 0.025 in our simulations, and σ_e^2 was set to equal 1. The probability model with $\delta = 0$, $c > 0$ and $f = 5$ is a popular non-normal distribution in the literature. In our simulated data, the contaminated normal distribution with $c = 0.025$ has a skewness of approximately 0.3 and kurtosis of approximately 15. Under various genetic models, the genetic effect a is determined for a given heritability h^2. Heritability h^2 takes the values of 0, and 0.1, 0.2, 0.5.

The observed type I errors, given in Table 1, agree reasonably well with nominal value of $\alpha = 0.05$, and thus all tests are comparable in the tail region for symmetric and asymmetric trait residual distributions. Non-normality of the distribution of trait values can influence the power of various linkage tests. When the residuals of individual trait values are normally distributed, the observed empirical powers of the HE regression are substantially higher compared to other nonparametric linkage tests based on ranks, regardless of the inheritance models (Table 2, 3 and 4). However, for non-normal trait residual distributions of contaminated normal and lognormal that are considered in our simulations, the rank-based nonparametric linkage tests are more efficient. Since the rank average-based statistic Z_{Ave} and the Jonckheere-Terpstra trend statistic demonstrate almost equivalent power levels to the new NPL statistic, the power results of these two tests are not presented in the tables. Genehuner NPL statistic demonstrates low powers than the new NPL statistic throughout all situations of genetic models, heritability, population disease allele frequency, and sample sizes of sib-pairs (Table 2, 3 and 4).

The power increases for all tests with the heritability and with increasing sample size from 200

Table 2: Empirical power to detect a quantitative trait locus under additive models with 2,000 replicates for a completely informative marker at the trait locus at nominal $\alpha = 0.05$ level. Normal, contaminated normal and lognormal residual distributions, with the residual correlation is 0.5, were generated and allele frequency p at the diallelic trait locus varied with the genetic model. HE corresponds to the power with Haseman-Elston regression on raw trait values.

Residual distribution	p	h^2	$n = 200$			$n = 500$		
			Z_{NPL}	Z_{New}	Z_{Ave}	Z_{NPL}	Z_{New}	Z_{Ave}
Normal	0.1	0.1	0.091	0.132	0.172	0.121	0.203	0.277
		0.3	0.194	0.438	0.623	0.309	0.762	0.945
		0.5	0.306	0.722	0.934	0.556	0.975	1.000
	0.3	0.1	0.097	0.143	0.179	0.109	0.231	0.316
		0.3	0.233	0.522	0.689	0.370	0.865	0.957
		0.5	0.437	0.911	0.976	0.756	1.000	1.000
	0.5	0.1	0.077	0.133	0.167	0.109	0.223	0.294
		0.3	0.233	0.563	0.699	0.387	0.874	0.958
		0.5	0.448	0.931	0.984	0.780	1.000	1.000
Contaminated normal	0.1	0.1	0.073	0.119	0.077	0.093	0.190	0.086
		0.3	0.159	0.401	0.204	0.291	0.690	0.259
		0.5	0.284	0.684	0.415	0.507	0.955	0.628
	0.3	0.1	0.084	0.130	0.086	0.110	0.207	0.088
		0.3	0.195	0.493	0.195	0.349	0.814	0.250
		0.5	0.392	0.862	0.451	0.723	0.997	0.639
	0.5	0.1	0.094	0.127	0.087	0.108	0.204	0.089
		0.3	0.178	0.495	0.192	0.363	0.808	0.251
		0.5	0.419	0.902	0.444	0.727	0.999	0.647
Lognormal	0.1	0.1	0.070	0.104	0.077	0.099	0.152	0.109
		0.3	0.127	0.245	0.121	0.186	0.448	0.152
		0.5	0.151	0.380	0.102	0.291	0.703	0.127
	0.3	0.1	0.075	0.101	0.072	0.091	0.147	0.084
		0.3	0.145	0.304	0.115	0.234	0.587	0.150
		0.5	0.228	0.587	0.148	0.443	0.902	0.188
	0.5	0.1	0.065	0.102	0.067	0.090	0.144	0.074
		0.3	0.136	0.316	0.111	0.234	0.582	0.138
		0.5	0.233	0.589	0.148	0.444	0.922	0.202

to 500. The rate of gaining power as sample size increases is slightly faster for HE regression under normally distributed residuals, but on the contrary this rate is faster for the new NPL statistic under non-normal trait distributions. From the results of simulations, shown in Table 2, 3 and 4, we conclude that the HE regression outperform for normally distributed residuals, but they are not as powerful as nonparametric trend tests for non-normal trait distributions, especially for high heritability and for lognormal distributed residuals. For a small addition, 2.5%, of highly dispersed normal to the standard normal distribution of trait residuals, the higher efficiency of the HE regression, holds only for a rare allele with $p = 0.1$ or 0.3 under the recessive model, or conversely for $p = 0.7$ (or 0.9 whose results are not shown) under the dominant model; otherwise, the observed power of the new NPL statistic is higher.

4. Discussion

One of the commonly used Genehunter NPL statistic for sib-pair linkage analysis is re-examined in this report. We wish to make some points clear: First of all, the Genehunter NPL statistic is not a Wilcoxon statistic, since Wilcoxon statistic is defined for a two-group situation and is expressed by a rank sum of a group, when the ranks are obtained from the joint ordering of observations of

Table 3: Empirical power to detect a quantitative trait locus under dominance models with 2,000 replicates for a completely informative marker at the trait locus at nominal $\alpha = 0.05$ level. Normal, contaminated normal and lognormal residual distributions, with the residual correlation is 0.5, were generated and allele frequency p at the diallelic trait locus varied with the genetic model. HE corresponds to the power with Haseman-Elston regression on raw trait values.

Residual distribution	p	h^2	$n = 200$			$n = 500$		
			Z_{NPL}	Z_{New}	Z_{Ave}	Z_{NPL}	Z_{New}	Z_{Ave}
Normal	0.1	0.1	0.093	0.148	0.175	0.105	0.205	0.302
		0.3	0.195	0.454	0.644	0.335	0.787	0.947
		0.5	0.301	0.740	0.941	0.561	0.979	1.000
	0.3	0.1	0.079	0.137	0.159	0.124	0.227	0.300
		0.3	0.216	0.569	0.649	0.417	0.882	0.959
		0.5	0.460	0.939	0.971	0.787	1.000	1.000
Contaminated normal	0.1	0.1	0.091	0.122	0.075	0.120	0.201	0.094
		0.3	0.164	0.416	0.204	0.305	0.733	0.260
		0.5	0.292	0.694	0.441	0.520	0.961	0.628
	0.3	0.1	0.076	0.118	0.074	0.107	0.219	0.086
		0.3	0.204	0.502	0.196	0.367	0.849	0.261
		0.5	0.427	0.897	0.447	0.718	1.000	0.651
Lognormal	0.1	0.1	0.077	0.103	0.081	0.091	0.142	0.090
		0.3	0.126	0.256	0.119	0.206	0.507	0.173
		0.5	0.173	0.384	0.168	0.298	0.725	0.234
	0.3	0.1	0.079	0.101	0.066	0.106	0.156	0.075
		0.3	0.159	0.349	0.093	0.245	0.613	0.125
		0.5	0.254	0.655	0.141	0.449	0.930	0.178

Table 4: Empirical power to detect a quantitative trait locus under recessive models with 2,000 replicates for a completely informative marker at the trait locus at nominal $\alpha = 0.05$ level. Normal, contaminated normal and lognormal residual distributions, with the residual correlation is 0.5, were generated and allele frequency p at the diallelic trait locus varied with the genetic model. HE corresponds to the power with Haseman-Elston regression on raw trait values.

Residual distribution	p	h^2	$n = 200$			$n = 500$		
			Z_{NPL}	Z_{New}	Z_{Ave}	Z_{NPL}	Z_{New}	Z_{Ave}
Normal	0.5	0.1	0.091	0.138	0.162	0.106	0.211	0.284
		0.3	0.194	0.512	0.624	0.361	0.843	0.939
		0.5	0.372	0.842	0.940	0.668	0.998	1.000
	0.7	0.1	0.078	0.144	0.172	0.108	0.221	0.286
		0.3	0.230	0.559	0.665	0.403	0.890	0.954
		0.5	0.463	0.935	0.971	0.791	1.000	1.000
Contaminated normal	0.5	0.1	0.079	0.123	0.076	0.113	0.209	0.088
		0.3	0.180	0.449	0.203	0.335	0.779	0.243
		0.5	0.320	0.786	0.422	0.611	0.987	0.614
	0.7	0.1	0.075	0.136	0.078	0.105	0.184	0.079
		0.3	0.201	0.513	0.205	0.384	0.843	0.242
		0.5	0.418	0.906	0.433	0.732	0.999	0.639
Lognormal	0.5	0.1	0.070	0.105	0.071	0.096	0.144	0.081
		0.3	0.142	0.301	0.127	0.204	0.540	0.160
		0.5	0.203	0.492	0.159	0.344	0.827	0.218
	0.7	0.1	0.076	0.116	0.061	0.090	0.157	0.082
		0.3	0.161	0.325	0.103	0.254	0.605	0.127
		0.5	0.255	0.641	0.160	0.483	0.941	0.185

two groups. But, the ranks of the NPL statistic are virtually derived from the observations of three IBD groups of score 0, 1 and 2, even though only the ranks of two IBD groups of score 0 and 2

appear in X_{NPL}. Secondly, it is evident that the new NPL statistic, obtained with correction of the bias in the Genehunter NPL statistic and use of a more appropriate variance estimate, enhanced the test performance. Thirdly, another support for the appropriateness of the use of observation-dependent expectation and more precise variance in the new NPL statistic is that it demonstrates almost equivalent powers to the well-known Jonckheere-Terpstra trend statistic. We have compared the power of the Haseman-Elston regression on raw data while other tests are all based on ranks. In order to match with the nonparametric settings we have also examined the Haseman-Elston regression on ranked data, whose results are not presented in the table, and, interestingly, our simulation revealed that the Haseman-Elston regression with ranked data resulted almost equivalent powers to the new NPL statistic for non-normal trait distributions.

The problems of bias and inflated variance discussed in this report for the Genehuner NPL statistic also apply to a multipoint testing situation. Therefore, we believe that many software programs that handle nonparametric multipoint statistical procedures for sib-pair linkage analysis may need further evaluation in the future.

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