The Relationship between Seven Common Polymorphisms from Five DNA Repair Genes and the Risk for Breast Cancer in Northern Chinese Women

Peijian Ding¹,²,³,⁴, Yang Yang⁴, Luyang Cheng⁴, Xuejun Zhang⁴, Limin Cheng⁴, Caizhen Li⁵, Jianhui Cai¹,²,³,⁴

¹ Department of Surgery, Hebei Medical University, Shijiazhuang, Hebei, China, ² Department of Surgery, Hebei General Hospital, Shijiazhuang, Hebei, China, ³ Department of Oncology & Immunotherapy, Hebei General Hospital, Shijiazhuang, Hebei, China, ⁴ Department of Surgery, The Affiliated Hospital of Chengde Medical College, Chengde, Hebei, China, ⁵ Hebei Eracon Bio-tech Co. Ltd, Shijiazhuang, Hebei, China

Abstract

Background: Converging evidence supports the central role of DNA damage in progression to breast cancer. We therefore in this study aimed to assess the potential interactions of seven common polymorphisms from five DNA repair genes (XRCC1, XRCC2, XRCC3, XPA and APEX1) in association with breast cancer among Han Chinese women.

Methodology/Principal Findings: This was a case-control study involving 606 patients diagnosed with sporadic breast cancer and 633 age- and ethnicity-matched cancer-free controls. The polymerase chain reaction - ligase detection reaction method was used to determine genotypes. All seven polymorphisms were in accordance with Hardy-Weinberg equilibrium in controls. Differences in the genotypes and alleles of XRCC1 gene rs25487 and XPA gene rs1800975 were statistically significant between patients and controls, even after the Bonferroni correction (P<0.05/7). Accordingly, the risk for breast cancer was remarkably increased for rs25487 (OR = 1.28; 95% CI: 1.07–1.51; P = 0.006), but decreased for rs1800975 (OR = 0.77; 95% CI: 0.67–0.90; P = 0.001) under an additive model at a Bonferroni corrected alpha of 0.05/7. Allele combination analysis showed higher frequencies of the most common combination C-G-G-C-G-G (alleles in order of rs1799782, rs25487, rs3218536, rs861539, rs1800975, rs1760944 and rs1130409) in controls than in patients (P_sim = 0.002). In further interaction analysis, two-locus model including rs1800975 and rs25487 was deemed as the overall best model with the maximal testing accuracy of 0.654 and the cross-validation consistency of 10 out of 10 (P = 0.001).

Conclusion: Our findings provide clear evidence that XRCC1 gene rs25487 and XPA gene rs1800975 might exert both independent and interactive effects on the development of breast cancer among northern Chinese women.

Introduction

Breast cancer is the most common invasive cancer in women, and like other forms of cancer it results from multiple hereditary and environmental modulators, possibly in an interactive manner. Many risk factors such as ionizing radiation and alcohol consumption have been established to account for approximately 30% of breast cancer patients [1]. Family studies found that the risk for those with first-degree relatives of affected individuals is more than two times higher than the risk of general population [2,3], confirming a strong genetic component underlying the etiology of breast cancer [4]. Pasche et al have written an excellent review on the genetic underpinnings of breast cancer [5]; however, to determine many genes and which genetic determinants are actually involved in the pathogenesis of breast cancer remains an interpretive challenge.

Evidence is converging supporting the central role of DNA damage in progression to breast cancer. In fact, exposure to ionizing radiation, which can cause double-strand DNA breaks, increased the risk of developing breast cancer [6,7]. In-vitro studies also observed that radiation-induced damage can remarkably reduce the repair proficiency of DNA double-stranded breaks in breast cancer patients [8]. As such, it is reasonable to hypothesize that deficiency in DNA repair proteins induced by genetic mutations can initiate or aggravate the development of breast cancer. However, there is a general impression that most published studies assessing the relationship between DNA repair genes and breast cancer risk have often focused on a single gene or a single polymorphism, but overlooked the potential gene-to-gene interactions, a ubiquitous phenomenon in human genetics. Accordingly, we conducted the present study to assess the genetic interactions of seven common polymorphisms from five DNA repair genes and breast cancer risk in northern Chinese women.
repair genes in association with breast cancer among Han Chinese women.

Materials and Methods

Study participants
In total, 1239 study participants were enrolled on a hospital-based design from Chengde city, Hebei province, China. Approval of this study was obtained from the Ethics Committee of Chengde Medical College, and each participant read and signed the informed consent before entering this study, which was carried out according to the principles of the Declaration of Helsinki.

All breast cancer patients who had no prior history of any cancers and reported no family history of breast cancer were for their first time diagnosed as invasive ductal carcinoma based on pathological confirmation, and then they received surgical intervention plus adjuvant chemotherapy at the Affiliated Hospital of Chengde Medical College. Clinical information of breast cancer was obtained via a full clinical examination by specialists. All controls were women who underwent breast cancer screening and were clinically confirmed to be free of breast cancer at the same hospital, and they had a negative history of all forms of cancer in their first-degree relatives. All study participants were genetically unrelated women of Han Chinese descent who were consecutively recruited from the Affiliated Hospital of Chengde Medical College between September 2009 and March 2013.

All study participants were classified into two study groups: the breast cancer group and the cancer-free control group. Overall, 606 patients 54.36 (standard deviation: 12.33) years of mean age were diagnosed with sporadic breast cancer, and the rest 633 participants who had no manifest of cancers formed the age- and ethnicity-matched control group with mean age of 55.15 (standard deviation: 9.38) years.

At enrollment, baseline data on age, family history of cancers, age at menarche and menopausal status were recorded. Moreover, additional data on tumor size (from T1 to T4), tumor grade (from G1 to G3), and lymph node (positive or negative) were exclusively presented for breast cancer patients.

Selection of polymorphisms
The five DNA repair genes under study were X-ray repair complementing defective repair in Chinese hamster cells 1 gene (XRCC1: rs1799782 and rs25487), XRCC2 gene (rs3218536), XRCC3 gene (rs861539), xeroderma pigmentosum, complementation group A gene (XPA: rs1800975) and APEX nuclease (multifunctional DNA repair enzyme) 1 gene (APEX1: rs1760944 and rs1130409). The selection of these functional polymorphisms was based on their wide evaluation in association with various forms of cancer [9–15].

Genotyping
EDTA blood samples were obtained from all study participants at the time of enrollment. Genomic DNA was isolated from peripheral blood leukocytes by using TIANamp Blood DNA Kit (Tiangen Biotec Co., Beijing, China), and then was stored at −40°C until required for batch genotyping. The polymerase chain reaction-ligase detection reaction (PCR-LDR) method [16] was adopted to determine the genotypes of seven examined polymorphisms in this study.

To discriminate specific bases of each polymorphism, we synthesized two specific probes and one common probe, and labeled the common probe 6-carboxy-fluorescein (FAM) at the 3′ end and phosphorylated at the 5′ end. The multiplex ligation reaction was conducted in a volume of 10 μl containing 2 μl of PCR product, 1 μl of 10×Taq DNA ligase buffer, 1 μM of each discriminating probe, and 5 U of Taq DNA ligase. After ligation,

Table 1. The baseline characteristics of all study participants.

| Characteristics                        | Patients (n = 606) | Controls (n = 633) |
|--------------------------------------|-------------------|-------------------|
| Age (years)                          | 54.36±12.33       | 55.15±9.38        |
| Family history of other cancers      |                   |                   |
| Menarche age (years)                 |                   |                   |
| ≤12                                  | 14.60±1.63        | NA                |
| 13–14                                | 22.44%            | NA                |
| ≥15                                  | 40.43%            | NA                |
| Menopausal status                    |                   |                   |
| Premenopause                         | 50.17%            | NA                |
| Postmenopause                        | 49.83%            | NA                |
| Tumor size (T1–T4)                   |                   |                   |
| T1                                   | 49.80%            | NA                |
| T2                                   | 42.54%            | NA                |
| T3                                   | 3.83%             | NA                |
| T4                                   | 3.83%             | NA                |
| Tumor grade (G1–G3)                  |                   |                   |
| G1                                   | 4.87%             | NA                |
| G2                                   | 50.81%            | NA                |
| G3                                   | 44.32%            | NA                |
| Lymph node (+)                       | 42.13%            | NA                |

Data were expressed as mean ± standard deviation unless otherwise indicated. doi:10.1371/journal.pone.0092083.t001
reaction, 1 μl of LDR reaction product was mixed with 1 μl of ROX passive reference and 1 μl of loading buffer before being denatured at 95°C for 3 min and chilled rapidly on ice. The fluorescent products of the LDR were differentiated using an ABI 3730XL sequencer (Applied Biosystems, California, USA).

To test the accuracy of the PCR-LDR method, 48 DNA samples were randomly selected and run in duplicates with 100% concordance.

Statistical analysis

Continuous and categorical variables were compared between breast cancer patients and controls by the unpaired t-test and the χ² test, respectively. A Pearson goodness-of-fit test was conducted to assess the Hardy-Weinberg equilibrium. Binary Logistic regression models were used to evaluate the additive (major homozygotes versus heterozygotes versus minor homozygotes), dominant (major homozygotes versus heterozygotes plus minor homozygotes), and recessive (major homozygotes plus heterozygotes versus minor homozygotes) models of inheritance after controlling for age at enrollment, and risk estimates were expressed as odds ratio (OR) and 95% confidence interval (95% CI). The statistical analyses described above were completed with the SAS software for Windows (version 8.1) (SAS Institute, Cary, North Carolina, USA). Statistical power was estimated by PS (Power and Sample Size Calculations) software (version 3.0.7, Nashville, TN, USA).

Analysis of allele combinations was adopted to examine the joint effect of seven polymorphisms on breast cancer risk, and their frequencies were estimated by the haplo.em program implemented in Haplo.stats software (version 1.4.0, Rochester, MU, USA). The haplo.em program computes the maximum likelihood estimates of allele combination probabilities using the progressive insertion algorithm which progressively inserts batches of loci into the allele combinations of growing lengths. To avoid false-positive results, only allele combination with frequency of over 3% in all study participants was considered in this analysis. P values were calculated based on 1000 simulations.

To explore the potential interactions of multiple polymorphisms of DNA repair genes, a promising data-mining open-source approach multifactor dimensionality reduction (MDR) was employed (version 3.0, available at the website http://www.epistasis.org [17,18]). This approach aims to identify the overall best combination of all quantities (from one locus to seven loci). The accuracy of each best model was evaluated by a Bayes classifier in the context of 10-fold cross-validation. A single best model has the maximal testing accuracy and cross-validation consistency simultaneously. The cross-validation consistency is a measure of the number of times of 10 divisions of the dataset that the best model is extracted. Permutation testing corrects for multiple testing by repeating the entire analyses on 1000 datasets that are consistent with the null hypothesis.

Results

Baseline characteristics

Details of the study population are shown in Table 1. Age at enrollment did not differ significantly between breast cancer patients and controls (P = 0.205). The percentage of family history

| Gene: polymorphism | W/M | Status | WW | WM | MM | M (%) | Three genetic models (OR; 95% CI; P*) |
|--------------------|-----|--------|----|----|----|-------|--------------------------------------|
| XRCC1: rs1799782   | C/T | Patients | 279 | 263 | 64 | 32.26 | Additive 0.97; 0.82–1.15; 0.749 |
| XRCC1: rs25487     | G/A | Patients | 318 | 209 | 79 | 30.28 | Additive 0.97; 0.82–1.15; 0.749 |
| XRCC2: rs3218536   | G/A | Patients | 166 | 280 | 160 | 49.50 | Additive 1.11; 0.95–1.29; 0.196 |
| XRCC3: rs861539    | C/T | Patients | 510 | 91  | 5  | 8.33  | Additive 1.38; 1.02–1.88; 0.038 |
| XPA: rs1800975     | A/G | Patients | 201 | 268 | 137 | 44.72 | Additive 0.77; 0.67–0.9; 0.001 |
| APEX1: rs1760944   | G/T | Patients | 177 | 293 | 136 | 46.62 | Additive 1.01; 0.87–1.19; 0.866 |
| APEX1: rs1130409   | G/T | Patients | 389 | 168 | 49  | 21.95 | Additive 1.15; 0.95–1.38; 0.144 |

Abbreviations: W/M, wild allele/mutant allele; OR, odds ratio; 95% CI, 95% confidence interval. P for χ² test was calculated based on the 3×2 contingency tables for genotype comparisons and on the 2×2 contingency tables for allele comparisons. *Controlling for age at enrollment.
of cancers was 10.56% in breast cancer patients. The average age at menarche was 14.60 (standard deviation: 1.63) years. Breast cancer patients with menarche age of 12 years or less, 13–14 years and 15 years or more accounted for 22.44%, 37.13% and 40.34%, respectively. Nearly half of breast cancer patients had postmenopausal status at enrollment (49.83%), 49.80% and 42.54% of patients had tumor size of T1 and T2, and 50.18% and 44.32% patients had tumor grade of G2 and G3, respectively. The percentage of positive lymph node was 42.13% in breast cancer patients.

### Single-locus analysis

Table 2 shows the genotype and allele comparisons of seven polymorphisms under study between patients and controls and their risk prediction for breast cancer under three genetic models of inheritance. No deviation from Hardy-Weinberg equilibrium was noted in controls for all polymorphisms. The association of this polymorphism with breast cancer was slightly substantiated (P for χ² test: 0.036 for genotype and 0.010 for allele), while no significance was reached after applying the stringent Bonferroni correction (Bonferroni significance threshold P = 0.05/7).

### Interaction analysis

A data-mining analytical approach MDR was adopted to explore the potential interactions of multiple polymorphisms of five DNA repair genes, and the results are summarized in Table 4. Each overall best model of all quantities is weighed by testing accuracy and cross-validation consistency. Overall, the two-locus model including rs1800975 and rs25487 emerged as the best MDR model. This model had the maximal testing accuracy of 0.654 and the maximal cross-validation consistency of 10 out of 10, which was significant at 0.001, indicating that a model this good or better was observed one out of 1000 permutations and thus unlikely hinged on the null hypothesis of null association.

### Discussion

In this study, we sought to explore the potential interactions of seven common polymorphisms of five DNA repair genes in association with breast cancer among 1239 Han Chinese women. The key finding was that two polymorphisms, XRCC1 gene rs25487 and XPA gene rs1800975, might exert both independent and interactive effects on the development of breast cancer. This study, to the authors’ knowledge, is the first report assessing the association of multiple DNA repair genes and polymorphisms, both individually and interactively, with breast cancer risk in Han Chinese women.

In view of the ubiquity of epistasis in determining susceptibility to common human diseases [19], to examine the interactions of...
multiple genes in common pathologic pathways should be a priority. In such context, MDR has been developed as a promising data-mining approach for overcoming some limitations of traditional parametric statistics such as logistic regression for the detection and characterization of high-order gene-gene and gene-environmental interactions [17,18]. This approach is nonparametric and model-free in design, and has been successfully applied to detect and characterize high-order gene-gene and gene-environment interactions in studies with relatively small samples [20,21]. For the present study, application of MDR to breast cancer case-control data set identified a statistically significant two-locus best model from five DNA repair genes. It is not surprising to note that the two polymorphisms in overall best model were strikingly significant in our single-locus analysis, reinforcing the robustness of MDR approach. Moreover, the interactive role of these two polymorphisms was particularly evident in protection against the development of breast cancer, as our allele combination analysis indicated that the estimated frequencies of combinations were consistently higher in controls than patients for those carrying rs25487-G and rs1800975-G alleles, especially for the most common allele combination. Although empirical and theoretical studies have suggested that MDR is a useful method for identifying epistasis, the power of MDR in the presence of noise that is common to many epidemiological studies is unknowable. Furthermore, we cannot exclude the possible existence of residual confounding from the incompletely measured or unmeasured physiologic covariates. Considering the magnitude of risk estimates and the mutual validation of different analytical methods, it seems unlikely that our findings could be explained by confounding.

Although epidemiological studies on DNA repair genes and breast cancer risk have been undertaken extensively across different populations, the results are inconsistent and inconclusive. For example, Roberts et al in Caucasians observed an increased risk of XRCC1 gene rs25487 for breast cancer in postmenopausal women [22], which was consistent with the results of the present study, as well as a recent meta-analysis by Wu et al on 44 independent case-control studies [23]. However, Al Mutairi et al in Saudi patients failed to confirm this association, and instead they found that another polymorphism rs1799782 in XRCC1 gene was associated with the significant risk of breast cancer [24]. Besides the environmental and cultural divergences, it cannot be totally ruled out that the evolutionary history of linkage disequilibrium patterns will vary significantly across different ethnic populations. Generally, a locus is in close linkage with another nearly causal locus in one ethnic group but not in another [25]. As a consequence, there is a need to construct a database of breast cancer-susceptibility genes or polymorphisms in each racial/ethnic group. Also it is of clinical importance to incorporate joint and synergistic analytical strategies for the potential disease-susceptibility genetic effects by scanning DNA repair genes to facilitate the identification of individuals at high risk of developing breast cancer in future clinical screening.

Several limitations of the present study merit consideration. First, this study was conducted on a retrospective case-control design, which has inherent drawbacks and precludes causal inferences [26]. Second, this study of 1239 participants might not be powered enough to address small risk effects. Third, due to our design flaw, some baseline data on age at menarche and menopausal status were not available for controls, as well as other reproducible risk factors for breast cancer, which prevented further adjustment in risk estimates and may have overestimated the true effect size. However, this lack of information is unlikely to affect the validity of our findings, because this study involved homogenous breast cancer patients and well-matched controls. Fourth, only seven common polymorphisms from five DNA repair genes were evaluated in this study, and it is highly encouraged to incorporate other polymorphisms, especially the low-penetrance polymorphisms of DNA repair genes. Fifth, although MDR is a method to improve the identification of polymorphism combinations associated with disease risk, it is not without drawbacks, such as computational intensiveness, indistinct interpretation, lack of sensitivity, and heterogeneity-free assumption [27,28]. Last but not the least, because our study sample was entirely of Han Chinese ancestry, we avoided confounding by ethnicity but at the same time, we reduced the generalizability of our findings to other ethnic populations.

In conclusion, our findings provide clear evidence that XRCC1 gene rs25487 and XPA gene rs1800975 might exert both independent and interactive effects on the development of breast cancer. As breast cancer is a multifactorial complex disorder, large well-designed longitudinal studies attempting to account for high-order gene-gene and gene-environment interactions, as well as in-vitro and in-vivo studies seeking to provide biological or clinical implications of DNA repair genes in susceptibility to breast cancer, are required in future investigation.

Supporting Information

Table S1 Genotype distributions and allele frequencies of seven polymorphisms under study between breast cancer patients without a family history of other cancers and controls, as well as their risk prediction for breast cancer under three genetic models of inheritance. (DOC)

Author Contributions
Conceived and designed the experiments: JC. Performed the experiments: PD YY. Analyzed the data: PD YY JC. Contributed reagents/materials/analysis tools: Layang Cheng XZ Lumin Cheng CL. Wrote the paper: PD JC.

References
1. Madigan MP, Ziegler RG, Benichou J, Byrne C, Hoover RN (1995) Proportion of breast cancer cases in the United States explained by well-established risk factors. J Natl Cancer Inst 87: 1601–1605.
2. Nelson HD, Zakher B, Cantrall A, Fu R, Griffith J, et al. (2012) Risk factors for breast cancer for women aged 40 to 49 years: a systematic review and meta-analysis. Ann Intern Med 156: 635–648.
3. [Collaborative Group on Hormonal Factors in Breast Cancer] (2001) Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies of breast cancer in postmenopausal women of European ancestry. Lancet 358: 1389–1399.
4. Gage M, Wattendorf D, Henry LR (2012) Translational advances regarding hereditary breast cancer syndromes. J Surg Oncol 105: 444–451.
5. Pasche B (2008) Recent advances in breast cancer genetics. Cancer Treat Res 141: 1–10.

6. Holmberg E, Holm LE, Lundell M, Mattsson A, Wallgren A, et al. (2001) Excess breast cancer risk and the role of parity, age at first childbirth and exposure to radiation in infancy. Br J Cancer 85: 362–366.
7. Webb PM, Hopper JL, Newman B, Chen X, Kelemen L, et al. (2005) Double-strand break repair gene polymorphisms and risk of breast or ovarian cancer. Cancer Epidemiol Biomarkers Prev 14: 319–323.
8. Parshad R, Price FM, Bohr VA, Cowans KH, Zujewski JA, et al. (1996) Deficient DNA repair capacity, a predisposing factor in breast cancer. Br J Cancer 74: 1–5.
9. Przybylowksa-Sygut K, Stanczyk M, Kusinska R, Kordek R, Majsterk I (2013) Association of the Arg194Trp and the Arg399Gln polymorphisms of the XRCC1 gene with risk occurrence and the response to adjuvant therapy among Polish women with breast cancer. Clin Breast Cancer 13: 61–68.
10. Lin WY, Camp NJ, Cannon-Albright LA, Allen-Brady K, Balasubramanian S, et al. (2011) A role for XRCC2 gene polymorphisms in breast cancer risk and survival. J Med Genet 48: 477–484.

11. Loizidou MA, Michael T, Neuhausen SL, Newbold RF, Marcou Y, et al. (2008) Genetic polymorphisms in the DNA repair genes XRCC1, XRCC2 and XRCC3 and risk of breast cancer in Cyprus. Breast Cancer Res Treat 112: 575–579.

12. Sangrajrang S, Schnezer P, Burkholder I, Boffetta P, Brennan P, et al. (2007) The XRCC3 Thr241Met polymorphism and breast cancer risk: a case-control study in a Thai population. Biomarkers 12: 323–332.

13. Han W, Kim KY, Yang SJ, Noh DY, Kang D, et al. (2012) SNP-SNP interactions between DNA repair genes were associated with breast cancer risk in a Korean population. Cancer 118: 594–602.

14. Sangrajrang S, Schnezer P, Burkholder I, Waas P, Boffetta P, et al. (2008) Polymorphisms in three base excision repair genes and breast cancer risk in Thai women. Breast Cancer Res Treat 111: 279–288.

15. Zhou B, Shan H, Su Y, Xue K, Shao X, et al. (2011) The association of APE1 -656T > G and 1349 T > G polymorphisms and cancer risk: a meta-analysis based on 37 case-control studies. BMC Cancer 11: 521.

16. Niu W, Zhang Y, Ji K, Gu M, Gao P, et al. (2010) Confirmation of top polymorphisms in hypertension genome wide association study among Han Chinese. Clin Chim Acta 411: 1491–1495.

17. Pattin KA, White BC, Barney N, Gui J, Nelson HH, et al. (2009) A computationally efficient hypothesis testing method for epistasis analysis using multifactor dimensionality reduction. Genet Epidemiol 33: 87–94.

18. Hahn LW, Ritchie MD, Moore JH (2003) Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. Bioinformatics 19: 376–382.

19. Moore JH (2003) The ubiquitous nature of epistasis in determining susceptibility to common human diseases. Hum Hered 56: 73–82.

20. Qi Y, Niu W, Zhu T, Zhou W, Qiu C (2008) Synergistic effect of the genetic polymorphisms of the renin-angiotensin-aldosterone system on high-altitude pulmonary edema: a study from Qinghai-Tibet altitude. Eur J Epidemiol 23: 143–152.

21. Niu W, Qi Y, Hou S, Zhai X, Zhou W, et al. (2009) Haploype-based association of the renin-angiotensin-aldosterone system genes polymorphisms with essential hypertension among Han Chinese: the Fangshan study. J Hypertens 27: 1384–1391.

22. Roberts MR, Shields PG, Ambrosone CB, Nie J, Marian C, et al. (2011) Single-nucleotide polymorphisms in DNA repair genes and association with breast cancer risk in the web study. Carcinogenesis 32: 1223–1230.

23. Wu K, Su D, Lin K, Lao J, Au WW (2011) XRCC1 Arg399Gln gene polymorphism and breast cancer risk: a meta-analysis based on case-control studies. Asian Pac J Cancer Prev 12: 2237–2243.

24. Al Mutairi FM, Alanazi M, Shalaby M, Al Abdalkarim HA, Pathan AA, et al. (2013) Association of XRCC1 gene polymorphisms with breast cancer susceptibility in Saudi patients. Asian Pac J Cancer Prev 14: 3809–3813.

25. Niu W, Qi Y, Wu Z, Lin Y, Zhu D, et al. (2012) A meta-analysis of receptor for advanced glycation end products gene: four well-evaluated polymorphisms with diabetes mellitus. Mol Cell Endocrinol 358: 9–17.

26. Gu M, Dong X, Zhang X, Wang X, Qi Y, et al. (2012) Strong association between two polymorphisms on 15q25.1 and lung cancer risk: a meta-analysis. PLoS One 7: e37970.

27. Moore JH, Ritchie MD (2004) STUDENTJAMA. The challenges of whole-genome approaches to common diseases. JAMA 291: 1642–1643.

28. Gui J, Andrew AS, Andrews P, Nelson HM, Kelsey KT, et al. (2010) A simple and computationally efficient sampling approach to covariate adjustment for multifactor dimensionality reduction analysis of epistasis. Hum Hered 70: 219–225.