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SNP barcodes generated using particle swarm optimization to detect susceptibility to breast cancer

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ABSTRACT

Considerable research has been devoted to investigating variations in disease susceptibility using SNPs associated with the individual co-occurrence of single nucleotide polymorphisms (SNPs) in genetic and phenotypic variability. Without the raw genotype data, these association studies are difficult to conduct and often omit SNP interactions, thus limiting their reliability and potential applicability. In this study, we apply a particle swarm optimization (PSO) algorithm to detect and identify the best protective SNP barcodes (*i.e.*, SNP combinations and genotypes with a maximum difference between cases and controls) associated with chronic dialysis patients. SNP barcodes containing different numbers of SNPs were computed. We evaluated the combined effects of 27 SNPs related to nine published epigenetic modifier-related genes on breast cancer. Eleven different SNP combinations were found to be protective associated with the risk of breast cancer (odds ratio, *OR* < 1.0; *p*-value < 0.05). The results suggest that SNPs 1 and 2 (gene BAT8), 9, 10, 11 and 13 (DNMT3A), 20 and 21 (EHMT1), 24 (HDAC2), 25 (MBD2), and 27 (SETDB1) are statistically very significant and that there may be interactive effects that play a role in the prevalence of breast cancer. A PSO-based on the Chi-Square test process allowed us to quickly identify the significant SNP combinations in a multi-locus association analysis, and then further detect interactive effects on complex genotypes amongst the SNPs. The PSO algorithm is robust and precisely identifies the best protective SNP barcodes. It can identify potential combined epigenetic modifier-related genes together with the SNP barcodes that were deemed protective against breast cancer by *in silico* analysis.

Keywords: Single Nucleotide Polymorphism; Particle Swarm Optimization; SNP-SNP Interaction; Breast Cancer

1. INTRODUCTION

Genome-wide association studies (GWAS) involve a vast amount of single nucleotide polymorphism (SNP) data from several genes which is associated with genotype frequencies between cases and controls and can be used to investigate disease susceptibility. Studies of gene variations associated with hereditary phenotypes are becoming increasingly popular and contribute to the detection of significant effects on disease susceptibility [1-6].

A total of 27 SNPs from nine epigenetic modifier-related genes (BAT8, DNMT1, DNMT3A, DNMT3B, EHMT1, HDAC2, MBD2, MTHFR and SETDB1) were selected to investigate their association with breast cancer [7]. Previous research only considered the analysis of the effect of individual SNPs, but investigating their association with SNPs can provide deep insight into disease susceptibility. Although the individual role of these epigenetic modifier-related genes was addressed in [7], the combined effect of gene (or SNP) interactions in relation to breast cancer was not addressed. This study is similar to many association studies in that only genotype frequencies were published without supplementary genotypic raw data.

Analysis of SNP-SNP interactions is used to investigate polygenic diseases. However, it remains a challenge to collect large-scale combinations of SNP data and analyze the possible SNP-SNP interactions. The simultaneous evaluation of multiple SNPs generates many possible combinations of alleles in SNP-SNP interactions. The possible combinations of SNP interactions between cases and controls is estimated to be $C(N,M)*3^M = N!/[M!(N-M)!]*3^M$, where N is the total number of SNPs or factors, and M is the selected number of SNPs. Machine learning and data mining methods are widely used in GWAS data analysis, but current methods aren't robust enough to simultaneously evaluate the complex interactions for all tested SNPs, though some computational approaches have been developed to examine epistasis in family-based and case-control association studies [8-16].

We hypothesize that interactions between the polymorphisms of epigenetic modifier-related genes may have a synergistic or non-additive effect on the pathogenesis of a disease and can explain differences in disease susceptibility. We propose the PSO method to generate SNP barcodes of genotypes to predict disease susceptibility and evaluate risk factors. The best combination of SNPs with genotypes can be verified by determining its risk factor in terms of odds ratio and confidence intervals. We systematically evaluated the joint effects of 27 SNP combinations of nine related genes involved in breast carcinogenesis. The SNP barcodes generated by the PSO algorithm were statistically evaluated by the odds ratio (*OR*) to predict dialysis susceptibility in breast cancer.

2. METHODS

We introduce a particle swarm optimization method that generates the best SNP barcodes to combine SNPs with their corresponding genotypes. A characteristic of PSO is its fast convergence, allowing for the quick identification of optimal solutions in a wide solution space, meaning that we can look for the optimal protective SNP barcodes.

2.1. Particle Swarm Optimization

PSO is an efficient evolutionary computation learning algorithm developed by Kennedy and Eberhart [17] to describe an automatically evolving system through the simulation of the social behavior of organisms, e.g., the social behavior of birds in a flock or fish in a school. PSO was designed for use in practical applications and simulates social behavior based on information exchange. Within a problem space, each potential result can be regarded as a vector in a swarm, where the vector is referred to as a particle. Each particle uses its own memory and knowledge gained from the swarm as a whole to find an optimal solution. Each particle is evaluated by an objective function to detect good experience, and particles can share the experience amongst the swarm. These experiences can be inform the search direction to lead the swarm toward the optimal solution. This superior strategy effectively mines the optimal regions of complex search spaces. The basic elements of PSO are as follows:

1) Particle: In this study each particle can be regarded as a problem solution.

2) Population: A swarm population consisting of *n* particles.

3) Particle position, x_i: Each candidate solution can be represented by a *D*-dimensional vector; the i^{th} particle can be described as $x_i = (x_{i1}, x_{i2}, \cdots, x_{iD})$, where x_{iD} is the position of the i^{th} particle with respect to the D^{th} dimension. Each dimensional vector in the particle position is defined by the number of selected SNPs and the corresponding genotypes for the associated SNPs.

4) Particle velocity, v_i: The velocity of the i^{th} particle is represented by $v_i = (v_{i1}, v_{i2}, \cdots, v_{iD})$, where v_{iD} is the velocity of the i^{th} particle with respect to the D^{th} dimension. The new locations of particles are chosen by adding v_i to the coordinate of the particle position x_i; PSO operates this process by adjusting v_i. In addition, the velocity of a particle is restricted within $[V_{\min}, V_{\max}]^D$.

5) Inertia weight, *w*: The inertia weight is used to control the impact of a particle's previous velocity on its current velocity. This control parameter affects the tradeoff between the particle's abilities for exploration and exploitation.

6) Individual best value, *pbest*$_i$: *pbest*$_i$ is the position of the i^{th} particle with the highest value of the objective function during a given iteration. It can be regarded as a best current solution for the i^{th} particle.

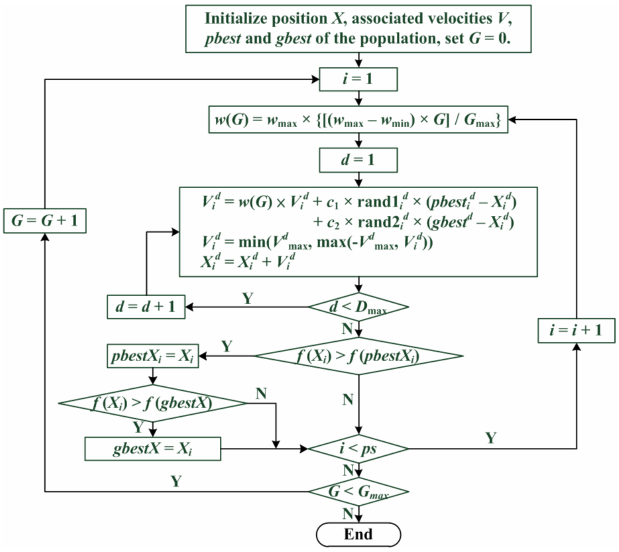
7) Global best value, *gbest*: The best position of all *pbest* particles is called the global best *gbest*. It can be regarded as the best current solution of SNP barcodes in all particles.

8) Termination criteria: The process is stopped after the maximum allowed number of iterations is reached.

The PSO procedure is shown in **Figure 1** and can be divided into the following steps: 1) initialization of particles; 2) particle evaluation with an objective function; 3) selection of the particles' *pbest* and *gbest*; and 4) updating of the particles' velocity and position. These procedures are repeated in successive iterations until the termination conditions are reached.

2.2. Encoding Schemes

In PSO, each particle was designed in a format that enabled us to express a particular amount of SNP and genotype combinations. A particle is defined in a vector that consists of the number of selected SNPs and their corresponding genotypes; SNPs cannot be repeatedly selected. In this paper, we define the SNP barcode to represent a solution with selected SNPs and their corresponding genotypes. The particle encoding can thus be represented by:


Initialize position X, associated velocities V, pbest and gbest of the population, set G = 0.
i = 1
w(G) = w_max × {[(w_max − w_min) × G] / G_max}
d = 1
V_i^d = w(G) × V_i^d + c_1 × rand1_i^d × (pbest_i^d − X_i^d) + c_2 × rand2_i^d × (gbest^d − X_i^d)
V_i^d = min(V^d_max, max(-V^d_max, V_i^d))
X_i^d = X_i^d + V_i^d
d < D_max
Y
d = d + 1
N
f (X_i) > f (pbestX_i)
Y
pbestX_i = X_i
f (X_i) > f (gbestX)
N
Y
gbestX = X_i
N
i < ps
Y
i = i + 1
N
G < G_max
Y
G = G + 1
N
End

i: the *i*th particle from 1 to *ps*; *G*: the *G*th generation from 1 to $G_{\max}$; *d*: the *d*th dimension from 1 to $D_{\max}$; X_i^d : the value of *d*th dimension of *i*th particle; *f*(): objective function; *ps*: population size; $G_{\max}$: maximum generations; $D_{\max}$: maximum dimensions; *w*: intertia werght.

Figure 1. PSO flowchart.

$$X_i = \left(SNP_{i,j}, \text{Genotype}_{i,j}\right),\ i = 1, 2, \cdots, n,\ j = 1, 2, \cdots, m$$

where $SNP_{i,j}$ represents the selected SNP, $\text{Genotype}_{i,j}$ represents the three possible genotypes once $SNP_{i,j}$ is selected, *n* represents the size of the population, and *m* represents the number of SNPs selected.

2.3. Population Initialization

Based on the above encoding schemes, the population of particles is randomly generated over the search space. SNPs are selected based on a randomly generated value between $X_{SNP\text{min}} = 1$ and $X_{SNP\text{max}} = 27$. In addition, the three genotypes are randomly generated between 1 and 3. In each particle, the selected SNPs between SNP_1 and SNP_m are not the same. If a given SNP is found to be repeated in a particle, the SNP is randomly generated between $X_{SNP\text{max}}$ and $X_{SNP\text{min}}$ until it is different. For example, let *X* = (2, 3, 7, 3, 1, 3). In this representation of the particle, SNPs 2, 3, and 7 and genotypes 3, 1, 3 are chosen. In this case, the selected SNPs with their corresponding genotypes are represented as (2, 3), (3, 1), and (7, 3). The initial velocity for a particle is based on a randomly generated value in the interval (0, 1).

2.4. Objective Function

In this study, an objective function is used to compute the SNP barcode for the difference between the cases and controls; the particle fitness is represented as a value computed by this objective function. The maximum difference between cases and controls has the highest fitness value. We use set theory to evaluate the fitness value, with the equation defined as follows:

$$F\left(X_i\right) = \left(\text{controls} \cap X_i\right) - \left(\text{cases} \cap X_i\right) \tag{1}$$

where controls represents the total number of SNP interactions in the control group and cases represents the total number of SNP interactions in the case group. X_i represents the *i*th particle. The total number of intersections of the controls and the *i*th particle is calculated as $n(\text{controls}\cap X_i)$. The total number of intersections of the cases and the *i*th particle is calculated as $n(\text{cases}\cap X_i)$. For example: $X = (SNP_{1,2}, \text{Genotype}_{2,1})$ is used to evaluate the number of matching conditions in the cases and controls. The case group contained 89 cases and the control group had 191 controls. According to **Eq.1**, the value is determined by subtracting 89 from 191, leaving 102.

2.5. Selection of *pbest* and *gbest*

When moving, particles keep a record of their personal best position (*pbest*) and the global best position (*gbest*). If a particle's fitness value in the current iteration is better than the fitness value of *pbest* form the previous iteration, then the position and fitness value of *pbest* are updated with the current position and fitness value. If the fitness value of *pbest* is better than *gbest* in the previous iteration and is the best value in the current iteration, *gbest* is updated by *pbest*. Each particle then adjusts its direction based on *pbest* and *gbest* in the following iteration.

2.6. Updating Particle Velocity and Position

The PSO algorithm updates the particle's velocity and thus moves its position (*i.e.*, possible solution), thus allowing us to search for a better solution. In each generation, the position and velocity of the *i*th particle are updated with the $pbest_i$ and *gbest* of the swarm population. The updating equations can be formulated as:

$$w_{LDW} = \left(w_{\max} - w_{\min}\right) \times \frac{\text{Iteration}_{\max} - \text{Iteration}_i}{\text{Iteration}_{\max}} + w_{\min} \tag{2}$$

$$\begin{aligned} v_{id}^{\text{new}} = w_{LDW} \times v_{id}^{\text{old}} + c \times r_1 \times \left(pbest_{id} - x_{id}^{\text{old}}\right) \\ + c \times r_2 \times \left(gbest_d - x_{id}^{\text{old}}\right) \end{aligned} \tag{3}$$

$$x_{id}^{\text{new}} = x_{id}^{\text{old}} + v_{id}^{\text{new}} \tag{4}$$

where *w* is the inertia weight, $w_{\max}$ is 0.9, $w_{\min}$ is 0.4 and $\text{Iteration}_{\max}$ is the maximum number of allowed iterations. This inertia weight is a positive linear function of time that changes with the generations; r_1 and r_2 are random numbers between (0, 1), and c_1 and c_2 are acceleration constants that control how far a particle moves in a single generation. The velocities v_{id}^{new} and v_{id}^{old} respectively denote the velocities of the new and old particles;

x_{id}^{old} is the current particle position, and x_{id}^{new} is the updated particle position. The velocity implies the degree to which a particle's position should be changed at a particular moment in time, such that equals that of the global best position, *i.e.*, the velocity of the particle flying toward the best position. To obtain a search solution, the particle velocities in each dimension are restricted within $[V_{\min}, V_{\max}]^D$, and the particle positions are restricted within $[X_{\min}, X_{\max}]^D$, thus determining the size of the steps the particle is allowed to take through the solution space.

2.7. Parameter Settings

The population size parameter was set to 50. PSO termination condition is reached at a pre-specified number of iterations (100 in this case). The starting value of the inertia weight *w* is set to 0.9 and the final value is set to 0.4 [18]. The acceleration (learning) factors c_1 and c_2 are 2 [19]. These parameters have been optimized by Kennedy and Eberhart [17].

2.8. Statistical Analysis

We used the odds ratio (*OR*) and *p*-value of Pearson Chi-Square test, which are commonly used criteria to determine performance [20].

$$\text{Odds Ratio} = \frac{TP \times TN}{FP \times FN} \tag{5}$$

TP, *TN*, *FN*, and *FP* respectively represent the number of true positives, true negatives, false negatives and false positives. For statistical analysis with SPSS version 19.0 (SPSS Inc., Chicago, IL), the odds ratio are used to determine the best SNP barcode and quantitatively measure the risk of disease; the *p*-values are used to prove that the SNP barcode is statistically significant for the difference between cases and controls.

3. RESULT

3.1. Data Set

The datasets were obtained from the epigenetic modifiers (49 SNPs for 10 genes) in a breast cancer association study [7] and consisted of the SNPs and clinical statuses for 4373 cases and 4556 controls. Except for the MTHFR gene which, according to [7] contains only one SNP, the other genes chosen for this study (BAT8, DNMT1, DNMT3A, DNMT3B, EHMT1, HDAC2, MBD2, and SETDB1) all had 27 SNPs, with details shown in **Table 1**. The SNPs in the original data [7] consist of different numbers of individuals; therefore, the number of each SNP needs to be normalized to fit the same number. The new data was randomly generated according to the frequency of the original data; however, the output still obeyed the final frequency for each SNP for the whole dataset. All the SNP data from the data source are adjusted to the same sum number, (5000) for all genotype distributions. For example, in the SNP_1 (gene, BAT8; dBSNP ref, rs535586), the sums of the values with three genotypes (*i.e.*, AA, Aa, and aa) in cases is 4373. First, the percentage for each genotype in SNP_1 is calculated as "original data*/sum (%)", *i.e.*, 1930/4373 (44%) for AA, 1936/4373 (44%) for Aa, and 507/4373 (12%) for aa, where the symbol * indicates that the original data was derived from the SNP dataset before normalization. According to this percentage, the modified data for SNP_1 was calculated by multiplying the percentage with the sum of the complete dataset (SNP number adjusted to 5000), *i.e.*, 44% × 5000 (= 2200) for AA, 44% × 5000 (= 2200) for Aa, and 12% × 5000 (= 600) for aa. Therefore, the modified data for SNP_1 has been adjusted to the sum of 5000 (2200 + 2200 + 600 = 5000).

3.2. Evaluation of Breast Cancer Susceptibility in 27 Separate SNPs from Nine Epigenetic Modifier-Related Genes

Table 1 shows the performance (*OR* and 95% CI) for each SNP from nine epigenetic modifier-related genes (BAT8, DNMT1, MTHFR, DNMT3A, DNMT3B, EHMT1, HDAC2, MBD2, and SETDB1). Some SNPs (such as SNPs 1, 11, and 21 listed in **Table 1**) with certain genotypes display a statistically significant *OR* (*p*-value < 0.05) for breast cancer with *OR* values ranging from 1.16 to 0.90. The other SNPs show no statistically significant *OR* for chronic dialysis patients.

3.3. Identification of SNP-SNP Interactions with Maximum Differences between Cases and Controls Using PSO

Table 2 shows the 11 2-SNP barcodes selected by systematic sampling, listed in order of the magnitude of difference between cases and controls from maximal to minimal. Among the combinations, 2-SNP barcode with their corresponding genotypes, namely SNPs (1, 2) with genotype 1-1, [rs535586-AA]-[rs652888-AA], showed the maximal difference (135) between the controls and cases (1479 vs. 1344). Similarly, 3 and 27 combined-SNP barcodes with the best performance (the largest difference between controls and cases) were mined using the GA. The left side of **Table 3** shows only two to nine SNPs for the combinational analysis. For example, in a 3-SNP combination, the barcode consists of SNPs (1, 2, 21) with genotype 1-1-2, *i.e.*, [rs535586-AA]-[rs652888-AA]-[rs6559218-Aa]. In a 4-SNP combination, the barcode consists of SNPs (1, 2, 11, 20) with genotype 1-1-1-2, *i.e.*, [rs535586-AA]-[rs652888-AA]-[rs7581217-AA]-[rs452 6432-Aa]. Therefore, the PSO provides the highest

Table 1. Effect in individual SNPs of 27 epigenetic modifier-related genes on the occurrence of breast cancer.

SNP[a]	Genotypes	Controls/Cases	OR	95% CI	*p*-value	SNP	Genotypes	Controls/Cases	OR	95% CI	*p*-value
1 BAT8	1	2353/2208				15 DNMT3B	1	1788/1849			
rs535586	2	2098/2213	1.12	1.034 - 1.222	0.006	rs2424932	2	2406/2363	0.95	0.871 - 1.036	0.241
	3	549/579	1.12	0.987 - 1.281	0.079		3	806/788	0.95	0.844 - 1.069	0.350
2 BAT8	1	3079/2995				16 DNMT3B	1	1546/1515			
rs652888	2	1676/1748	1.07	0.986 - 1.166	0.103	rs2424928	2	2440/2447	1.02	0.932 - 1.116	0.616
	3	245/257	1.08	0.899 - 1.293	0.417		3	1014/1038	1.04	0.930 - 1.163	0.444
3 DNMT1	1	1251/1280				17 DNMT3B	1	1871/1878			
rs2290684	2	2534/2565	0.99	0.899 - 1.088	0.825	rs992472	2	2351/2354	1.00	0.918 - 1.090	0.955
	3	1215/1155	0.93	0.831 - 1.039	0.198		3	778/768	0.98	0.870 - 1.103	0.783
4 DNMT3A	1	1412/1425				18 DNMT3B	1	1539/1536			
rs7587636	2	2486/2506	1.00	0.911 - 1.095	0.980	rs2424913	2	2460/2469	1.01	0.923 - 1.105	0.903
	3	1102/1069	0.96	0.859 - 1.075	0.488		3	1001/995	1.00	0.893 - 1.119	0.944
5 DNMT3A	1	1395/1355				19 DNMT3B	1	1733/1799			
rs6749992	2	2442/2477	1.04	0.951 - 1.146	0.363	rs1333469	2	2405/2340	0.94	0.862 - 1.026	0.145
	3	1163/1168	1.03	0.926 - 1.155	0.553		3	862/861	0.96	0.856 - 1.077	0.512
6 DNMT3A	1	1568/1579				20 EHMT1	1	1544/1560			
rs749131	2	2466/2481	1.00	0.914 - 1.092	0.984	rs4526432	2	2460/2436	0.98	0.896 - 1.072	0.661
	3	966/940	0.97	0.862 - 1.082	0.555		3	996/1004	1.00	0.894 - 1.119	0.968
7 DNMT3A	1	1747/1744				21 EHMT1	1	2035/2056			
rs7560488	2	2398/2406	1.01	0.921 - 1.097	0.910	rs6559218	2	2385/2267	0.94	0.864 - 1.022	0.155
	3	855/850	1.00	0.887 - 1.118	0.944		3	580/677	1.16	1.022 - 1.317	0.026
8 DNMT3A	1	2726/2765				22 EHMT1	1	1968/2003			
rs734693	2	1925/1885	0.97	0.888 - 1.048	0.404	rs7852475	2	2340/2314	0.97	0.891 - 1.056	0.505
	3	349/350	0.99	0.845 - 1.158	0.888		3	692/683	0.97	0.858 - 1.097	0.624
9 DNMT3A	1	2745/2784				23 HDAC2	1	2051/2067			
rs2276599	2	1901/1874	0.97	0.895 - 1.056	0.501	rs352063	2	2293/2324	1.01	0.929 - 1.099	0.895
	3	354/342	0.95	0.814 - 1.116	0.546		3	656/609	0.92	0.811 - 1.044	0.202
10 DNMT3A	1	1628/1706				24 HDAC2	1	2695/2664			
rs2289195	2	2477/2425	0.93	0.855 - 1.020	0.130	rs3778216	2	1971/1974	1.01	0.930 - 1.097	0.755
	3	895/869	0.93	0.826 - 1.040	0.195		3	334/362	1.10	0.939 - 1.288	0.254
11 DNMT3A	1	2152/2010				25 MBD2	1	2147/2146			
rs7581217	2	2213/2362	1.14	1.051 - 1.243	0.002	rs1259936	2	2239/2267	1.01	0.929 - 1.098	0.762
	3	635/628	1.06	0.934 - 1.201	0.374		3	614/587	0.96	0.845 - 1.091	0.496
12 DNMT3A	1	1703/1698				26 MTHFR	1	2237/2286			
rs2304429	2	2428/2387	0.99	0.903 - 1.076	0.753	rs1801133	2	2217/2105	0.93	0.856 - 1.011	0.084
	3	869/915	1.06	0.942 - 1.184	0.351		3	546/609	1.09	0.958 - 1.241	0.185
13 DNMT3A	1	1656/1644				27 SETDB1	1	2111/2163			
rs6722613	2	2429/2513	1.04	0.954 - 1.138	0.359	rs4970986	2	2313/2250	0.95	0.874 - 1.033	0.222
	3	915/843	0.93	0.827 - 1.042	0.206		3	576/587	0.99	0.870 - 1.127	0.935
14 DNMT3B	1	1262/1322									
rs6058897	2	2480/2494	0.96	0.873 - 1.056	0.400						
	3	1258/1184	0.90	0.804 - 1.003	0.058						

[a]Data collected from literature [7].

Table 2. Representative difference between cases and controls for two SNP combinations amongst 27 SNPs, listed from maximal to minimal.

SNPs	Genotype	Cases		Controls		Difference of percentage
		No.	%	No.	%	
SNP (1, 2)	1 - 1	1344	26.88	1479	29.58	2.70
SNP (7, 14)	2 - 3	601	12.02	632	12.64	0.62
SNP (10, 19)	3 - 2	424	8.48	441	8.82	0.34
SNP (11, 13)	3 - 3	109	2.18	118	2.36	0.18
SNP (15, 27)	2 - 3	284	5.68	288	5.76	0.08
SNP (17, 19)	1 - 3	342	6.84	342	6.84	0.00
SNP (5, 19)	2 - 3	411	8.22	406	8.12	–0.10
SNP (16, 27)	2 - 3	294	5.88	284	5.68	–0.20
SNP (9, 16)	1 - 2	1360	27.20	1342	26.84	–0.36
SNP (19, 25)	1 - 1	782	15.64	752	15.04	–0.60
SNP (1, 11)	2 - 2	1010	20.20	896	17.92	–2.28

Table 3. Estimated effects of SNP barcode on the occurrence of breast cancer.

Combined SNP	SNP genotypes	Cases		Controls		Difference (%)	OR	95% CI	*p*-value
		No.	%	No.	%				
SNPs (1, 2)	1-1	1344	26.88	1479	29.58	2.70	0.875	0.802 - 0.955	0.003
		3656		3521					
SNPs (1, 2, 21)	1-1-2	623	12.46	718	14.36	1.9	0.849	0.756 - 0.953	0.005
		4377		4282					
SNPs (1, 2, 11, 20)	1-1-1-2	280	5.60	337	6.74	1.14	0.821	0.697 - 0.967	0.018
		4720		4663					
SNPs (1, 2, 10, 21, 24)	1-1-2-2-1	155	3.10	193	3.86	0.76	0.797	0.643 - 0.988	0.039
		4845		4807					
SNPs (1, 2, 10, 21, 24, 27)	1-1-2-2-1-1	57	1.14	84	1.68	0.54	0.675	0.481 - 0.947	0.023
		4943		4916					
SNPs (1, 2, 9, 10, 21, 24, 27)	1-1-1-2-2-1-1	32	0.64	51	1.02	0.38	0.625	0.401 - 0.974	0.038
		4968		4949					
SNPs (1, 2, 9, 10, 21, 24, 25, 27)	1-1-1-2-2-1-2-1	14	0.28	27	0.54	0.26	0.517	0.271 - 0.987	0.046
		4986		4973					
SNPs (1, 2, 9, 10, 13, 21, 24, 25, 27)	1-1-1-2-2-2-1-2-1	7	0.14	18	0.36	0.22	0.388	0.162 - 0.930	0.034
		4993		4982					

difference in terms of SNP barcodes between the cases and controls for fixed numbers of SNPs.

3.4. Prediction Scores of the Best PSO-Generated SNP Barcodes in Breast Cancer

Table 3 lists the best *n*-SNP barcodes (*n* = two to nine) calculated by the PSO algorithm. The right side of the table shows the estimated effect (odds ratio, 95% CI, and *p*-value) of certain SNP barcodes with respect to breast cancer susceptibility. SNP combinations (two to nine SNPs) with group differences between cases and controls are shown in "Difference" field. The difference between cases and controls are reduced from 2.70% to 0.22% between two to nine SNP barcodes. The *OR* of the best SNP barcodes is in the range of 0.875 to 0.388, and the 95% CI of *OR* is in the range of 0.162 to 0.988. The SNP barcodes involving two to nine SNPs show significantly decreasing *OR* values (*p*-value < 0.050 to 0.001). Since the SNP barcodes listed in **Table 3** show that the control numbers are greater than the case numbers, the SNP barcodes are regarded as protective SNP barcodes against breast cancer.

4. DISCUSSION

Accumulating evidence on SNP-SNP interaction supports polygenic models for breast cancers [21-23] and suggests that, in terms of disease analysis, breast cancers are associated with combinations of SNPs rather than individual SNPs. Possible protective effects are also important for the prediction of cancer morbidity by SNPs. However, computational methods used to identify the complex interactions are still difficult to implement for high order SNP-SNP interactions. In this study, the PSO method was used to overcome this problem. We analyzed the contributions of 27 SNPs from nine breast cancer related genes to generate protective SNP barcodes in a case-control study of 5000 cases and 5000 controls with genotype data simulation. The genotype information is simulated at random with the genotype frequency as the only dependent variable. In general, a larger number of simulated datasets may provide more stable results for certain types of SNP barcodes associated with disease. However, the purpose of this study was to develop a methodology for detecting cancer-associated SNP barcodes using case-control studies where only the genotype frequencies are known.

As **Table 1** shows, SNPs 1, 11, and 21 show significant differences with respect to the risk of breast cancer based on the odds ratio (*p*-value < 0.05) and share a common effect between individual and combined SNPs for the occurrence of breast cancer. However, SNPs 2, 9, 10, 13, 20, 24, 25, and 27 are not found to be significant as individual SNPs in relation to the occurrence of breast cancer. These results reveal that the SNPs involved in SNP-SNP interactions may be detectable using association studies. The analysis of the results in **Table 3**, which depicts the maximum difference information calculated by the PSO algorithm, can be used to predict the relative strength of the impact of an SNP on breast cancer protection. For example, the difference between controls and cases for SNP barcode [SNPs (1-2-21)-genotype (1-1-2)] is higher than that of [SNPs (1-2-11-20)-genotype (1-1-1-2)], suggesting that SNPs 1 and 2 are more relevant for breast cancer protection than SNPs 11, 20, and 21. Hence, an order of impact on breast cancer for the SNPs listed in **Table 3** can be arranged as SNPs 1/2 > SNP 21 > SNP 11/20 > SNP 10/24 > SNP 27 > SNP 9 > SNP 25 > SNP 13. The PSO-generated SNP barcodes involve two to nine SNPs and show significantly decreasing *OR* values, ranging from 0.875 to 0.388 in **Table 3** (*p*-value, 0.003 to 0.034). In contrast, some individual SNPs with breast cancer protection display *OR* values ranging from 0.99 to 0.90 (**Table 1**). **Figure 2** shows the relationship amongst *OR*, cases and controls. The value of *OR* is reduced from the low order to high order SNP-SNP interaction. Also, the difference between cases and controls decreases quickly, indicating that a very significant SNP barcode can be found in the high order SNP-SNP interaction. However, this rapidly decrease in difference increases the difficulty in identifying optimal SNP barcodes. PSO successfully overcome this difficulty, and the results show the SNP barcode is statistically significant.

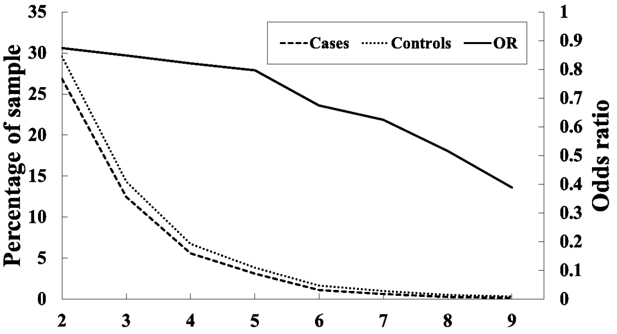


Figure 2. Frequency analysis among cases and controls, and odds ratio in 2- and 9-order SNP-SNP interactions.

We analyze the PSO in term of the computational complexity and parameters. Computational complexity is a key issue in detecting SNP-SNP interactions, and is estimated by the objective function computation for the PSO algorithm. Given I iterations and P solutions (particles) in the population, then the objective function computation has a computational complexity of O(IP). Optimal PSO parameters were investigated by Kennedy and Eberhart [17]. Population size and the number of iterations can be adjusted according the dataset. The suggested population size ranges from 50 to 100 and the suggested number of iterations ranges from 100 to 1000. The acceleration constants c_1 and c_2 control how far a particle moves in a single generation, *i.e.*, they respectively control the exploitation and exploration ability in each search. To balance exploitation and exploration, it is suggested that c_1 and c_2 are set equal to 2. PSO can overcome the limitations imposed on computational time for complex SNP interactions for GWAS because PSO has the following advantages: 1) PSO allows robust analysis of high-order SNP combinations for GWAS studies and generates the best SNP barcodes; 2) PSO is an evolutionary algorithm without exhaustive search; 3) PSO only needs two parameters for computation without complex settings; and 4) PSO's computational complexity is unaffected by the data set size.

5. CONCLUSION

Analysis of association studies is made difficult by the huge number of SNPs involved, especially when multiple SNPs are investigated simultaneously. Our proposed PSO algorithm was shown to successfully identify 27 SNP cross-interactions, and provides representative SNP-SNP interactions for breast cancer. The PSO algorithm can help identify the best fitness of cases and controls. Results involving two to nine SNPs show the *OR* of the best SNP barcodes is in the range of 0.875 to 0.388, and the 95% CI of *OR* is in the range of 0.162 to 0.988. All SNP barcodes show significantly decreasing *OR* values (*p*-value < 0.050 to 0.001). These results demonstrate that PSO, coupled with odds ratio analysis, can successfully account for complex SNP interactions and provides the best SNP barcode profile for predicting breast cancer cases. This suggests that the method is suitable for the systematic exploration of genome-wide SNP interactions.

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