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Original Article

Chemotherapy-induced neutropenia during adjuvant treatment for cervical cancer patients: development and validation of a prediction model

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Abstract: An artificial neuron network (ANN) model combining both the genetic risk factors and clinical factorsmay be effective in prediction of chemotherapy-induced adverse events. Purpose: To identify genetic factors and clinical factors associated with bone marrow suppression in cervical cancer patient, and to build a model for chemotherapy-induced neutropenia prediction. Methods: We performed a genome wide association study on a cohort to identify genetic determinants. Samples were genotyped using the Axiom CHB 1.0. The primary analyses focused on the scan of 657178 single-nucleotide polymorphisms (SNPs). Artificial neural network were used to integrating clinical factors and genetic factors to predict the occurrence of neutropenia. Results: 32 variants associated with neutropenia in the patients after chemotherapy were found ($P<1 \times 10^{-4}$). During internal validation and external validation, artificial neural network performed well in predicting neutropenia with considerable accuracy, which is 88.9% and 81.7% respectively. ROC analysis had acceptable areas under the curve of 0.897 for the internal validation sample and 0.782 for the external validation sample. Conclusion: Neutropenia may be associated with both genetic factors and clinical factors. Our study found that the artificial neural networks model based on the multiple risk factors jointly, can effectively predict the occurring of neutropenia, which provides some guidance before the starting of chemotherapy.

Keywords: Cervical cancer, genome-wide association study, artificial neuron network, platinum, single-nucleotide polymorphism

Introduction

Cervical cancer is the leading cause of cancer death among females in less developed country according to data in 2012 [1]. And it was also the third common female cancer worldwide, accounting for about 9% (529, 800) of total new cancer patients among women in 2008 [2]. Chemotherapy with platium plus other agents like taxanes or CPT-11 may be effective, but is poorly tolerated by some patients. Bone marrow suppression is one of the most common adverse effects of chemotherapy, leading to neutropenia, with risk of occurring of secondary sepsis. Identifying patients at greatest risk for these complications would be often clinically useful for selecting patients for chemotherapy. This is also useful for planning the frequency of monitor and clinical treatment with colony-stimulating factor. Patients treated with CPT-11 are at highest risk, and bone marrow suppression is also prevalent in patients with other platium-based treatment regimens. It is urgent to identify more accurate factors, including biomarkers and clinical factors.

For complex disorders, both genetics, environment and chance affect the pathogenetic processes [3]. A number of researchers suggest that genetic variants may be associated with chemotherapy-induced cytopenia [4-10]. The relevance of some variants to chemotherapy-induced neutropenia has been realized gradually. Secondly, polymorphism in the region of the UGT1A1 gene has recently been identified to be strongly associated with neutropenia [11-17]. The polymorphism is believed to regulate

neurotoxicity to the anti-tumor agents. Other genetic variants were also reported by a series of studies [11, 13, 15]. Genetic biomarkers from human's genome for predicting risk of chemotherapy induced mylosuppression may be particularly useful if treated as a pre-treatment test.

In this study, we have performed a genome-wide association study (GWAS) for determinants of chemotherapy-related myelosuppression in a large, well characterized cohort of cervical cancer patients treated with platium plus taxanes or platium plus CPT-11. We have focused primarily on chemotherapy-induced neutropenia.

Screening of patients at high risk, may enable preventative medical intervention in an economical way. Nowadays such a predictive model can be made available by using computer-based systems.

Artificial neural network (ANN) models, which are based on a series of multilayered interconnected equations, use non-linear statistical method to discover previously unknown relations between input variables and an output variable [18]. Researchers have revealed that ANN models are accurate and reliable in prediction of diverse clinical settings, including diagnosis and prognosis [19-23].

Our study aims to develop and validate a model through ANN method, which predicts WHO grade II-IV bone marrow depression in a group of cervical cancer patients presenting with neutropenia during chemotherapy.

Methods

Eligibility

Eligible patients were diagnosed with cervical cancer by pathological experts according to cervical biopsy by clinicians according to the International Federation of Gynecology and Obstetrics (FIGO). The exclusion criteria included preexistingsensory or motor neuropathy greater than WHO grade 1, a history of myocardial infarction and cardiac insufficiency ≥grade 3 (New York Heart Association scale). Patients previously treated for cervical cancer (i.e., surgery, chemotherapy orradiotherapy) and a past or current history of other neoplasmwere excluded. Patients with active infectious disease or other medically complicating condition were excluded. Women who were pregnant or lactating were also excluded from this study. This study was approved by each participating center's Institutional Ethical Committee and was conducted according to the principles of the Declaration of Helsinki. Written informed consents were obtained from all subjects (ClinicalTrials.gov Identifier: NCT01628757).

Samples collection and DNA extraction

A case-control analysiswas performed, and all cervical cancer patients were unrelated ethnic Han Chinese. Ethylene diaminetetraacetic acid disodium salt (EDTA-2Na)-anticoagulated venous blood samples were collected from all participants.

Genomic DNA was extracted from peripheral blood by standard procedures using Flexi Gene DNA kits (Qiagen) and the QuickGene DNA whole-blood kit (Fujifilm). The extracted DNA was diluted to working concentrations of 50 ng/μL for genome-wide genotyping.

GWAS genotyping and quality control

Genome-wide genotyping was performed using the Axiom Genome-Wide CHB1.0 Array (Affymetrix). Quality-control filtering of the GWAS data required a dish quality control (DQC) value >0.82 for further data analysis. DQC is a metric developed by Affymetrix that takes both interchannel and intrachannel signal separation and spread into account and is the recommended quality-control metric for Axiom arrays. For sample filtering, arrays with generated genotypes for <98% of the loci were excluded (11 samples). PLINK's identity by descent analysis was used to detect hidden relatedness. When pairs of individuals had PIHAT >0.25, the member of the pair with the lower call rate was excluded from the analysis (no sample was found); 57 cases and 218 controls were retained for further analysis. For SNP filtering (after sample filtering), SNPs with call rates <98% in the samples were removed. SNPs with MAF <5% or SNPs that deviated significantly ($P \leq 1 \times 10^{-5}$) from Hardy-Weinberg equilibrium in controls were also excluded. A total of 627,203 SNPs passed the quality criteria and were used in subsequent analyses. SNP with $P \leq 1 \times 10^{-4}$ was selected for follow-up study to construct the ANN model.

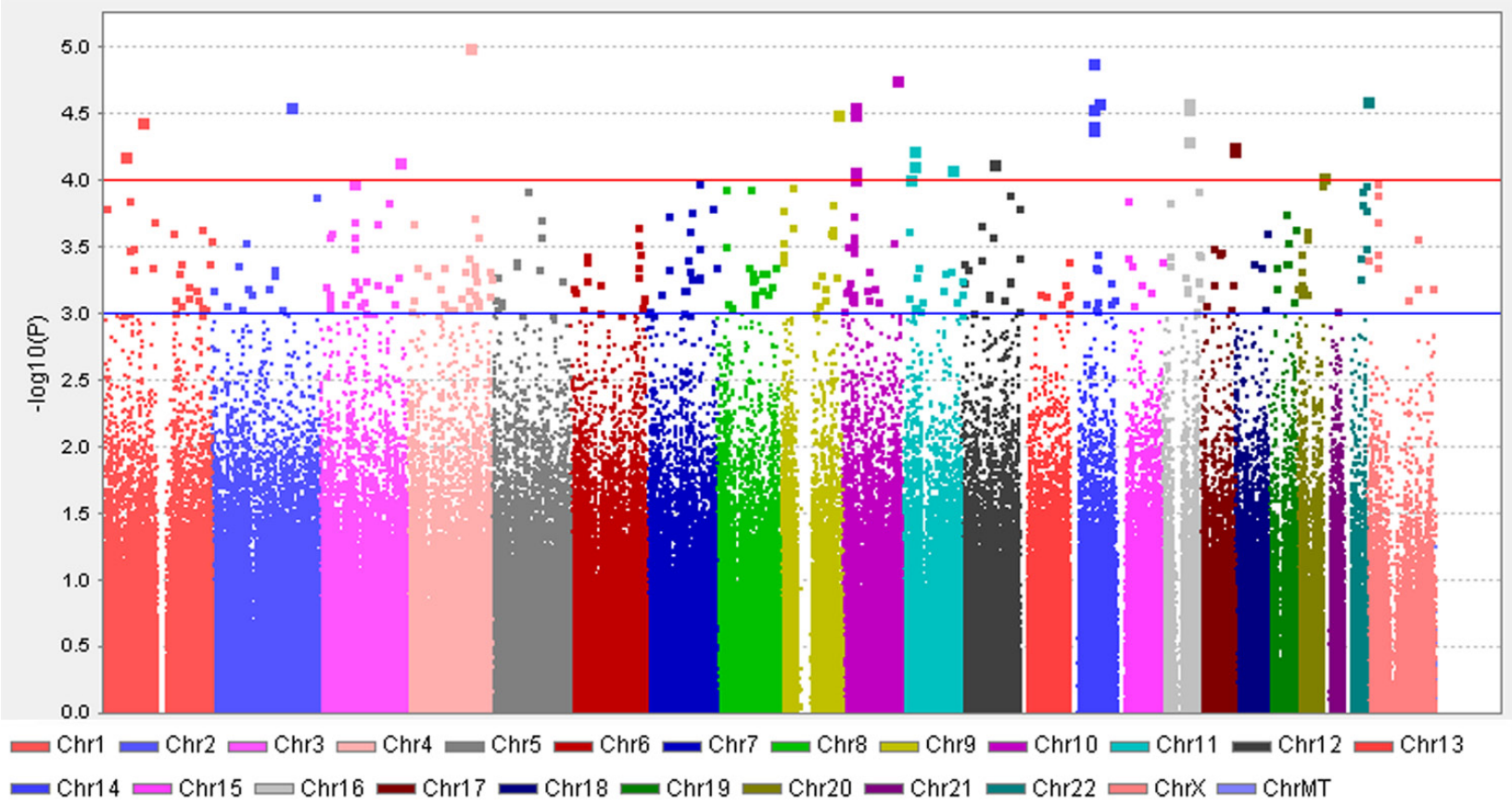

Figure 1. The Manhattan plot shows a genome-wide view of the *p* values [log10 (P)] for association between SNPs tested and week 4 neutropenia in cervical cancer patients. 32 SNPs were discovered with $P<10^{-4}$, with some SNPs clustered in some loci.

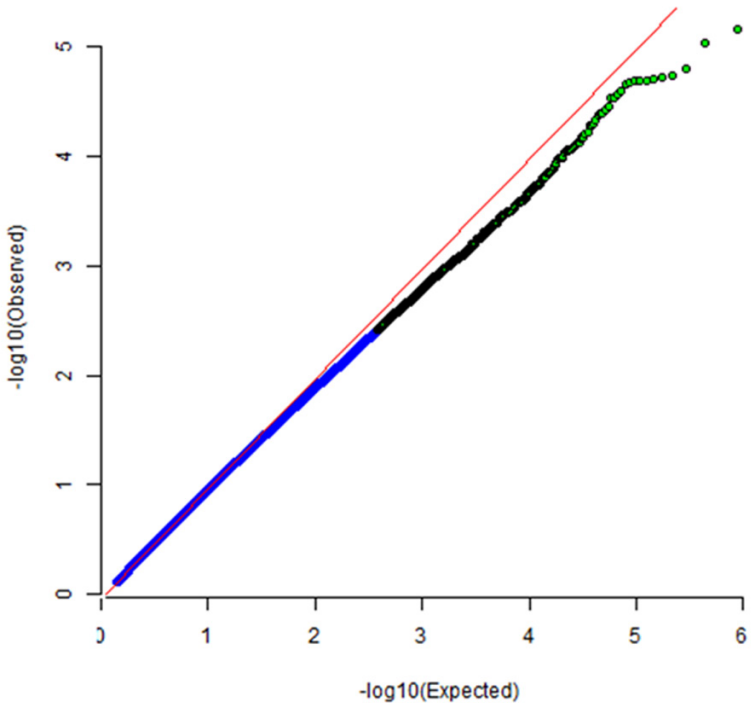

Figure 2. QQ plot for cases and controls study. The plot shows inflation factor for this genome-wide association study; and the effect of SNPs on the occurring of neutropenia.

ANN: training and internal validation

ANN model was constructed using Matlab software (version 8.5). The model was trained with back propagation. Samples from Tongji hospital and ZhejiangUniversity were used asinternal group for training and internal validation. Data from three quarters of the first internal group were used to train the ANN. Data from the rest one quarter of internal group were used for internal validation of the ANN. During training, the input variables were entered as either categorical or continuous data into the ANN, while the output variables were entered as 0 or 1. Instructed by the pre-designed code, the programme was allowed to run and a prediction was made, then the output value was correlated with the actual outcome value. If the output was not correct, a process of back propagation readjusted weights within the hidden layer until the correct prediction result was achieved. This process was repeated thousands of times until the training completed. During the validation process, the actual outcome value was concealed from the networks, and output value was compared with the actual outcome value. During validation, all the input variables for training were used by the ANN to predict outcome.

ANN: external validation

Clinical data on a group of 60 patients from a cohort study (ClinicalTrials. gov Identifier: NCT01628757), were used for external validation of the model. Between 2008 and 2012, they were admitted to XiangfanHospital with chemotherapy treatment and the samples were genotyped by BeadChip. Compared with the internal validation group, the patients in the external validation group were from an independent hospital from central area of China. And the area has not so good economy and health care as internal group. Just like before, all the input variables for training and internal validation were entered in the external validation database.

Statistical analyses

The associations of single SNPs with cervical cancer were analyzed using PLINKv1.04 software. The genomic inflation factor (λ=1.013) in our analyses suggested that cases and controls matched well, indicating no population-stratification, and ourresults were based on the uncorrected *P* values. A quantile-quantile plot created by the R Programming Language was then used to evaluate the overall significance of the GWAS results (**Figure 3**). The per-allele ORs were calculated and presented for the minor allele of each SNP, unless stated otherwise. A genome-wide association analysis was carried out using an additive model in a logistic regression analysis with chemotherapy regimen as a covariate. Accuracy of the predictive model wascalculated by sum of correct prediction divided by totalpredictions. Sensitivity, specificity, positive predictivevalue, negative predictive valuewere also calculated. Also, receiver-operating characteristic (ROC) curves for the ANN were generated for the outcome variables in internal validation sample and external validation sample. The statistical methods used included the χ^2 test and Fisher's

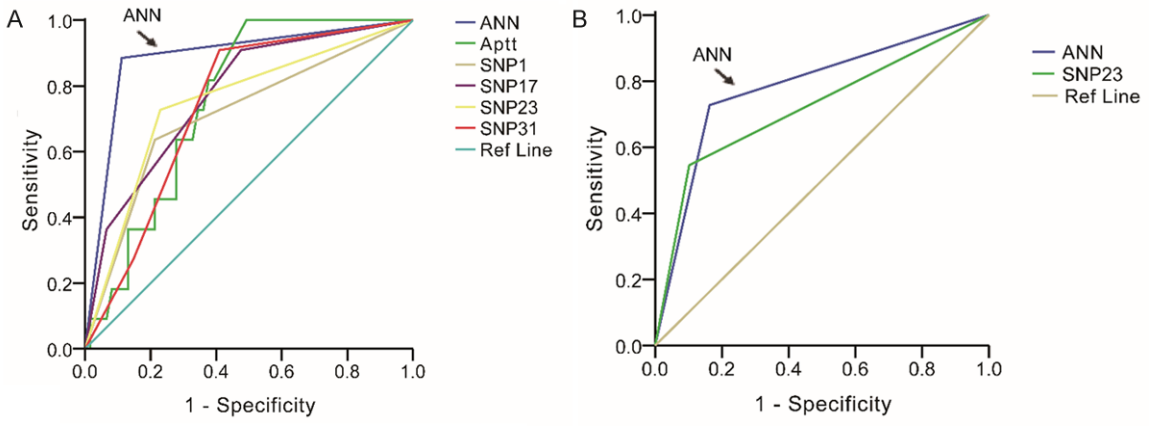

Figure 3. ROC for factors in internal validation and external validation groups. The largest area under the curve was covered by the ANN model in both the groups. The factors with low level of predicting were excluded, and only factors with area more than 0.7 were included in our analysis.

Table 1. Patient Characteristics

Characteristic	Control	Case	*P*-Value
All patients	227	59	
Age, years			
Medium	44	45	
Q1-Q3	40-49	40-50	
Weight, kg			
Medium	56	55	
Q1-Q3	51-61	49-60	
Height, cms			
Medium	158	159	
Q1-Q3	155-161	154-161	
Menarche, years			
Medium	14	14	
Q1-Q3	13-16	13-15	
Menstruation, days			
Medium	5	5	
Q1-Q3	4-6	4-7	
Menstrual cycle, days			
Medium	30	30	
Q1-Q3	29-30	29-30	
Gravidity			
Medium	3	3	
Q1-Q3	2-5	3-5	
Produce			
Medium	2	2	
Q1-Q3	1-3	1-3	
Abortion			
Medium	1	1	
Q1-Q3	0-2	0-3	
Basic Fg value			
Medium	3.43	3.47	

exact test forcategorical variables. All *P*-values were two-tailed, and values<0.05 were considered statistically significant. These statistical analyses werecarried out using the SPSS13.0 statistical software package.

Results

The main characteristics for cases and controls are listed in **Table 1**. ALL patients were Han ancestry. Most of basic clinical variables were similar in both case and control data sets except chemotherapy regimens. Neutropenia was more frequent in CP arm than TP arm (P<0.001).

Genomic association analysis

Assuming the additive mode, the top32 SNPs ($P \leq 1 \times 10^{-4}$) were chosen, whichwere most highly associated with neutropenia with adjustment of chemotherapy regimens. The selected SNPs were listed in **Table 2**. All genotype distributions followed the Hardy-Weinberg equilibrium law. The quantile-quantile plots showed some evidence for inflation due to population stratification (genomic inflation factor ($\lambda = 1.013$) (**Figure 2**).

Predictive factors (either clinical or genetic) for neutropenia

The details of the chemotherapy regimens and neutropenia cases in each

Q1-Q3	3.06-3.98	3.05-4.04	
Basic APTT value			
Medium	30.2	31.0	
Q1-Q3	27.7-32.8	28.8-33.4	
Basic PT value			
Medium	11.0	11.6	
Q1-Q3	10.3-12.2	10.5-12.4	
Basic WBC value			
Medium	6.27	5.0	
Q1-Q3	5.17-7.70	4.10-6.21	
Basic N value			
Medium	3.94	3.08	
Q1-Q3	3.02-5.11	1.99-3.96	
Basic Hb value			
Medium	111.0	105.0	
Q1-Q3	98.8-125.0	96.0-119.0	
Basic PLT value			
Medium	274	220	
Q1-Q3	219-345	187-310	
Chemo-regimens			
TP	158	25	<0.001
CP	59	32	
Missing	10	2	
Patients location			
Urban	83	22	0.87
Rural	123	31	
Missing	21	6	
Pathology			
Squamous carcinoma	196	49	0.72
Adenocarcinoma	19	5	
Adenosquamous carcinoma	4	2	
FIGO stage			
IA1	5	0	0.10
IA2	3	0	
IB1	71	19	
IB2	47	10	
IIA	37	17	
IIB	54	9	
IIIA	1	1	
IIIB	8	2	
IV	0	1	
Missing			
Surgery			
Pre-surgery	121	29	0.57
Post-surgery	106	30	
Menstrual bleeding			
Excessive	13	6	0.49
Moderate	204	51	
Inadequate	4	1	

group are shown in **Table 2**. Data from three quarters of the first internal group were used to train the ANN. Data from the rest one quarter of internal group were used for internal validation of the ANN. The two groups were similar in terms of chemotherapy regimens and clinical presentation (**Table 2**). All clinical and genetic variables shown in the **Table 1** were used to construct the ANN model.

ANN model accuracy

The predictive accuracy of the ANN model was similar in the internal and external validation groups. The ANN model selected for analysis had an accuracy of 88.9% in predicting neutropenia (WHO grade 2-4) in the internal validation group, and 81.7% in the external validation group. Although the positive predictive value (PPV) of the ANN was not high in either validation group, the negative predictive value (NPV) was high in both the internal and external validation groups (98.2 and 93.2%, respectively) (Details was shown in **Table 3**).

ROC curve

The ROCcurves for clinical factors as well as each individual genomic marker and the ANN classifier illustrate the maximum area under the curve (AUC) for each factor. In the subset of evaluated cases in each validation cohort, the AUC for the ANN classifier (0.897 in training cohort, **Figure 1A**; 0.782 in validation cohort, **Figure 1B**) was greater than the AUC for all other factors considered in **Table 1**.

Discussion

As we know, this is the first study toinvestigate genetic determinants forchemotherapy-related cytopenia using a genome wide association approach in cervical cancer patients. In this study, we have identified a group of genetic variants causing neutropenia, SNPs with *P* value less than 10^{-4}. In the drug of CPT-11 treated individuals, some genetic variants have been identified

Missing			
Dysmenorrhea			
Yes	198	49	0.13
No	19	9	
Missing	10	1	
Menopause			
Yes	48	16	0.44
No	137	35	
Missing	42	8	
Smoking status			
No	193	51	1.00
Yes	2	0	
Missing	32	8	
Drinking status			
No	192	49	0.28
Yes	3	2	
Missing	32	8	
HIV			
Negative	170	51	none
Positive	0	0	
Missing	57	8	
HBV			
Negative	154	49	0.25
Positive	15	2	
Missing	49	6	

to associate with neutropenia,but no such studies for taxanes treated cervical cancer patients [14, 15].

Screeningthe patients genetically predisposed to severe toxicity of classical cytotoxic agents is a critical issue. To analyze the role of genetic variants may shed light on the determinant factors of chemotherapy side effects. Understanding the genetic predisposition of cancer patients for developing severe toxicity has major clinical consequences. Our data indicate that platinum-based chemotherapy is relatively safe in patients with certain genetic background. On the contrary, severe and potentially life-threatening toxicity may occur and should be avoided in patients with suchbackground. This patient subset should probably reduce doses or, alternatively, with prepared Granulocyte Colony-Stimulating Factor (G-CSF), no matter where they are, in hospital or at home.

ANN-based modeling techniques were used in an attempt to predict the occurrence of moderate and serious adverse events. As predictive instruments for making a clinical decision, one of the important features is that only data that are readily available to the clinician at the time of occurrence of neutropenia are used. Our ANN predictive model combined data derived from genomic andclinical variantsby initial laboratory investigation, clinical history, and physical examination. We also emphasize that our ANN-based model is not meant to surrogate an experienced doctor. However, we advise that the ANN can be used as a decision aid for the clinical doctors. False positive rate andfalse negative rate were calculated to evaluate the model. The ANN model performed well in predicting the adverse events in both the internal and external validation groups.

This feature suggests that ANN may have a role in identifying patients who are at low risk of neutropenia and unlikely need therapeutic intervention during the treatment period. These patients could conceivably be discharged from the department with close outpatient follow-up, which may help save patients' time and money. Such management strategy has obvious advantage on efficient utilization of health-care resources.

When our ANN model was tested in the independent, external cohort of patients, its performance was also impressive. Considering external cohort differs from those of the internal group, the clinical features between the two groups of patients were also different. Although the positive predictive value of the ANN was not high in either validation group, the negative predictive value was high in both the internal and external validation groups. This result was similar to the study made by doctors in University Hospitals of Cleveland [24]. Compared with conventional predictive models like multiple logistic regression model, ANN-based models are more universally applicable [20]. This kind of computer-based systems have generated exciting result for bettering care of patients [25].

Although the ANN software (usually Matlab) allowed researchers to identify the specific input parameters that improve predictive accu-

Table 2. Top 32 SNPs identified by genome-wide association study

CHR	SNP	RS	BP	A1	OR	L95	U95	P
4	SNP1	NA	136871784	T	3.178	1.907	5.295	9.06E-06
14	SNP2	NA	47140657	G	4.307	2.241	8.277	1.18E-05
10	SNP3	rs1638410	118515516	T	4.454	2.24	8.857	2.05E-05
14	SNP4	rs9323332	58313237	G	2.919	1.776	4.799	2.40E-05
22	SNP5	rs1108364	46341876	G	2.686	1.697	4.25	2.46E-05
10	SNP6	rs11011962	20651003	C	2.867	1.757	4.678	2.50E-05
2	SNP7	NA	170944892	T	4.986	2.359	10.54	2.59E-05
16	SNP8	rs4436775	54281857	A	2.66	1.686	4.198	2.63E-05
16	SNP9	rs4564560	54282265	A	2.618	1.672	4.102	2.63E-05
14	SNP10	NA	47073011	T	6.163	2.636	14.41	2.71E-05
10	SNP11	rs10764221	20649311	G	2.859	1.748	4.675	2.86E-05
1	SNP12	rs4351663	83855776	C	6.458	2.676	15.58	3.32E-05
14	SNP13	rs10137341	46905759	A	3.639	1.972	6.714	3.58E-05
14	SNP14	NA	47135262	T	4.028	2.077	7.812	3.73E-05
14	SNP15	NA	47138095	T	4.028	2.077	7.812	3.73E-05
9	SNP16	rs72759216	125894343	T	3.07	1.791	5.263	4.53E-05
17	SNP17	NA	70069186	T	2.668	1.661	4.284	4.92E-05
16	SNP18	rs11862589	54281443	C	2.53	1.614	3.966	5.17E-05
11	SNP19	rs340986	22463511	T	0.2363	0.1174	0.4755	5.26E-05
17	SNP20	rs55981110	70069449	G	2.594	1.634	4.119	5.35E-05
1	SNP21	rs34200648	44328065	G	3.372	1.863	6.103	5.94E-05
3	SNP22	NA	177154093	G	3.142	1.791	5.513	6.57E-05
11	SNP23	rs1399096	22452671	G	0.2414	0.12	0.4858	6.79E-05
12	SNP24	rs11176925	66568062	C	2.981	1.741	5.102	6.84E-05
10	SNP25	rs11011954	20638640	A	2.89	1.708	4.893	7.73E-05
11	SNP26	rs1509728	108418533	G	2.494	1.583	3.93	8.11E-05
20	SNP27	rs7264385	57223196	G	2.94	1.717	5.037	8.59E-05
10	SNP28	rs987548	20639027	G	2.74	1.657	4.532	8.63E-05
11	SNP29	rs12799890	12490718	G	0.347	0.2043	0.5893	8.95E-05
23	SNP30	rs7051411	22358259	A	2.492	1.575	3.944	9.65E-05
7	SNP31	rs7796627	115873952	T	4.389	2.085	9.239	9.81E-05
3	SNP32	rs9822882	73736463	A	7.577	2.733	21.01	9.93E-05

A1, minor alleles. BP, Base pair position of the SNP. OR,odds ratio. OR and P werecalculated by an additive model in logistic regression analysis adjusted for chemo-regimens. MAF, minor allele frequency. L95, OR's lower limitation for 95% CI. U95, OR's upper limitation for 95% CI.

racy, these parameters should not be regarded as independent prognostic factors as perceived by a physician. Black-box approach was used in ANN-based models, while direct cause and effect relations between independent and dependent variables are usually unclear. Generally speaking, ANN-based models process input parameters in a non-linear style, and the network logic of calculation cannot be broken down into simple factors of clinical reasoning [26]. In the future, besides artificial neural network (ANN), other data-mining methods such as decision tree, logistic regression and support vector machines (SVMs) should be employed to analyze the massive data [27, 28]. And pharmacogenetic studies also made contribution to identify biomarkers [29], which include genetic variants for evaluation of drug response [29-31]. Thus, the most suitable one can be applied. Prospective cohort studies are also needed to test the ability of ANN prediction models to improve management of chemotherapeutic patients in daily clinical practice.

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Table 3. Predictive performance of the ANN model in the internal and external validation group

Model Validation	Accuracy (%)	Sensitivity (%)	Specificity (%)	AUC	PPV (%)	NPV (%)
Internal Validation	88.9	90.9	88.5	0.897	58.8	98.2
External Validation	81.7	72.7	83.7	0.782	50	93.2

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Disclosure of conflict of interest

None.

Abbreviations

TP, Taxanesplus platinum; CP, CPT-11plus platinum; LACC, Locally advanced cervical cancer; NACT, Neoadjuvant chemotherapy; FIGO, International Federation of Gynecology andObstetrics; DFS, Disease-free survival; OS, Overall survival; pCR, Pathological completeresponse; OPT, Optimal pathologic response.

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