Two-stage approach for risk estimation of fetal Trisomy 21 and other aneuploidies using computational intelligence systems

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ABSTRACT

Objective: To estimate the risk for fetal trisomy 21 (T21) and other chromosomal abnormalities at 11-13 week's gestation using computational intelligence classification methods.

Methods: As a first step, we train the artificial neural networks with 72054 euploid pregnancies, 295 cases of T21 and 305 of other chromosomal abnormalities (OCA). Then, we sort the cases into two categories of "no-risk" and "risk". The cases of "no-risk" are no further examined, while the cases with "risk" are forwarded in Stage 2 for further examination where we classify them in three types of risk, namely "no-risk", "moderate-risk" and "high-risk".

Results: Of a total of 36328 unknown to the system pregnancies, in the first Stage, 17512 euploid, 2 T21 and 18 other chromosomal abnormalities are classified as "no-risk". The remaining 18796 (51.4% FPR) cases are reassigned in Stage 2 where 7895 euploid, 2 T21 and 2 OCA are classified as "no-risk", 10464 euploid, 83 T21 and 61 OCA as "moderate-risk" and 187 euploid, 50 T21 and 52 OCA as "high-risk". The sensitivity and the specificity for T21 in Stage 2 are 97.1% and 99.5% respectively, assuming that cell-free DNA test can identify all the euploid and aneuploid cases.

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Conclusion: We propose a method for the early diagnosis of chromosomal abnormalities, which ensures that most of the T21 are classified as “high-risk” at any Stage. At the same time, we minimize the euploid cases that have to undergo invasive or cell-free DNA examinations through a routine procedure offered in two Stages. Our method is minimally invasive and of relatively low cost, highly effective on T21 identification and it performs better than other existing statistical methods.

Introduction

First-trimester screening for trisomy (T21) by a combination of maternal age, fetal nuchal translucency (NT) and serum free β-human chorionic gonadotropin (β-hCG) and pregnancy associated plasma protein-A (PAPP-A) can detect about 90% of affected pregnancies at a false positive rate (FPR) of 5%[1,2]. The performance of the first-trimester combined test can improve with increase in detection rate (DR) to >95% and decrease in FPR to <3% with the addition of the ultrasonographic markers of absent nasal bone, and abnormal flow in the ductus venosus and across the tricuspid valve.[1,3-5]

A recent major improvement in performance of screening for T21 has been achieved with analysis of cell-free DNA (cfDNA) in maternal blood with DR of >99% and FPR of <0.1%.[6] However, universal screening by cfDNA testing as an alternative to the combined test, would be expensive and ignore the other benefits of the combined test, including early detection of many major fetal defects, diagnosis of multiple pregnancies and their chorionicity, and early prediction of pregnancy complications, such as preeclampsia, with the potential of prevention through prophylactic pharmacological interventions. The alternative to universal screening by the cfDNA test is a strategy of cfDNA testing contingent on the results of first-line screening by the combined test. This approach retains the major advantages of cfDNA testing in increasing DR and decreasing FPR, but at considerably lower cost than offering cfDNA testing to the whole population.

Artificial neural networks (ANNs), which are a specific simplified version of computational intelligence, are being increasingly applied in medicine and biological research[7-10]. Essentially, it deals with mathematical algorithms implemented in software that learn from historical data and capture the knowledge and the internal dynamics that are contained in the data. Suitably trained models of computational intelligence approach the functionality of small biological neural clusters in a very fundamental manner that mimics human-like behavior. They constitute the digitized model of the biological brain and can detect complex non-linear relationships between dependent as well as independent variables in a dataset which are undetectable by the human brain. Artificial neural networks can learn from their input data, also known as training data. Such learning is achieved because ANN models can infer a function from observations and can subsequently use this function.

The objective of this study is to examine the potential value of ANN schemes in the stratification of risk for fetal T21 and other chromosomal abnormalities (OCA) incorporating the use of the combined test, the additional first-trimester ultrasonographic markers and cfDNA testing; it is aimed to achieve the highest possible DR at the minimum possible number having the cfDNA test and invasive testing.

Methods

Study population

The study population was derived from women with singleton pregnancies attending the Fetal Medicine unit at Kings' College Hospital, London, (March 2006 to May 2015), Obstetric ultrasound unit at University College London Hospital, London (April 2009 to July 2013) and Fetal Medicine unit at Medway Maritime Hospital, Gillingham (April 2010 to May 2015) for screening for aneuploidies at 11^{+0} to 13^{+6} weeks' gestation. Maternal age and other demographic characteristics were recorded and transabdominal ultrasound examination was performed for measurement of fetal crown–rump length (CRL) and nuchal translucency (NT) thickness and assessment for presence or absence of the fetal nasal bone, reversed a-wave in the ductus venosus and tricuspid regurgitation by sonographers who had received the appropriate Fetal Medicine Foundation Certificates of Competence.[1-4] The pregnancy was dated according to the measurement of fetal CRL.[11] Maternal blood was collected and automated machines that provide reproducible results within 30 min were used to measure serum PAPP-A and free β-hCG concentrations (Delfia Express System, Perkin Elmer, Waltham, MA, USA).

The best available method for establishing the presence of T21 and OCAs was prenatal fetal karyotyping by chorionic villous sampling or amniocentesis, postnatal karyotyping from neonatal blood; absence of the target condition was established by either prenatal karyotyping or clinical examination of a phenotypically normal neonate.

The artificial neural network diagnostic system

A feed forward network of neurons consisting of a number of layers that are connected to each other was build. The first layer is called input layer and it contains as many neurons as the input parameters. The last layer is called output layer and it contains one neuron. Other added layers, placed between the first and the last, are called hidden layers. A typical ANN architecture has one or two hidden layers. In every connection, there is a weight and an activation function that represent the process in the synapses of cells in a biological brain. The weights are optimized in the training procedure by presenting all the examples several times and calculating the error which is then used for adjusting the weights through a learning algorithm. The number of repetitions is a parameter and is called epochs. The number of layers and neurons, the activation functions and the epochs are parameters that are pre-defined, in most cases empirically by the system designer.

An unknown case is evaluated by an artificial neural network by presenting the parameters to the input layer. This information is passed through the layers by applying the appropriate weights and the transfer functions. The output value of the neural network in the last layer takes values in the range between 0 and 1 due to the sigmoid transfer function. To classify a case into positive or negative we use a cut-off point that is applied to the output of the neural network, which was optimized to achieve the highest detection of T21 at the lowest FPR.

Data reduction for handling the class imbalanced effect

The vast majority of cases in our dataset are euploid, creating a highly imbalanced situation between the two classes, normal and abnormal. This is known as the "class imbalanced problem"[12]. More precisely, from the total number of 72654 cases used in

the training set, 72054 are euploid and 600 are aneuploid. In medical data, this is a typical finding since abnormalities are less common, thus resulting in an imbalanced situation between the normal and abnormal classes[13-15].

In machine learning, most of the supervised techniques for classification are not able to generalize the data and the performance of the classifiers is low when the training set consists of imbalanced data. For instance, the Bayesian classifier uses the population of each class to estimate the posterior probabilities. In the case of class imbalanced populations, the probability of an unknown case will be biased towards the majority class. Similarly, the popular classifier of Support Vector Machines performs poorly with imbalanced populations[16]. ANNs adjust their weights based on a classification error, as explained above. Since the error is calculated globally for both normal and abnormal cases, a false negative classification has equal impact with a false positive classification. In practice however, a false negative classification has higher importance due to the small population. In other words in our dataset, a false positive classification has a percentage of 1 / 72054 = ~0% while a false negative has a percentage of 1 / 600 = 0.1%.

In the literature, a lot of work has been done for generating a balanced set from an imbalanced dataset for classification purposes[17-19]. This is typically done either by oversampling the minority class[20] or downsampling the majority class[21]. For the problem under study, we applied both approaches and we found that downsampling the majority class yields better results.

We thus first generated a cluster map of the entire euploid population, using the k-means[22] algorithm with five prototypes, assuming that the normal cases have their own sub-clusters. We then computed the prototype vector for each sub-cluster using the k-means algorithm and selected representative cases around this vector. In this way, we ended up with a reduced training set for the euploid class which represents the entire euploid population. For a more detailed explanation of this approach, we refer to our previous work[23].

Cross validation

We followed a standard procedure for testing the performance of our system. Typically in machine learning, three different datasets are used for building and verifying a system namely the training set, the validation set and the test set. The training set consists of known cases that are used for the learning procedure of the ANN and the validation set are known cases that are used to assessing the learning performance of the system during the training phase. The test set is usually another dataset that the labels are only known to the doctor. When the test set is given for testing without knowing the state of each case, we call it blind set.

The training and test sets are randomly chosen from a dataset with a percentage of 70% and 30% for training and validation sets respectively, and the experiments are repeated three times with different sets. This procedure is called three-fold cross validation. Similarly, the ten-fold cross validation is typically done by using the 90% of the dataset for training and 10% for validation. Another popular procedure is the “leave one out cross validation” where the training set consists of the total population and only one case is used for validation. This procedure is repeated as many times as the size of the dataset and the results are often presented with statistical terms such as average and standard deviation. This is done for making sure that the results are consistent, even though the training sets are different.

The training set used in this study is consisted of euploid, T21, trisomy 18, trisomy 13, triploidy, Turner and other chromosomal abnormalities. We applied a three-fold cross validation with random selection and in each run we achieved 100% true positive rate for T21 at a FPR of less than 5%, for both training and validation sets. After the development of the appropriate ANNs, we used another blind set to evaluate the performance of our system and produced the results presented in this paper.

Stratification of risk

In this study we used a two-Stage approach for stratification of risk and diagnosis of fetal T21 and OCA (Figure 1).

In Stage 1, we used four neurons in the input layer, representing the maternal age in years, the serum free β-hCG, the PAPP-A and the NT in mm. The output was binary: “no-risk” and “risk” for aneuploidy. The “no-risk” group from Stage 1 should not require any further testing and the group at “risk” for aneuploidy was subjected to Stage 2 screening.

In Stage 2, we used seven neurons in the input layer, representing the maternal age, the free β-hCG, the PAPP-A, the NT, the nasal bone (present or absent), the ductus venosus flow (positive or negative a-wave) and the tricuspid flow (present or absent tricuspid regurgitation). The output was “no-risk”, “moderate-risk” and “high-risk” for aneuploidy. The “no-risk” group should not require any further testing, the “moderate-risk” group should only had cfDNA testing and the "high-risk" group would be strongly suggested to undergo invasive testing without going through cfDNA test.

Results

Study population

The composition of the whole population of the training and validation sets used in the study is shown in Table 1. In total there were 108112 euploid and 870 aneuploid pregnancies, including 432 cases of T21, 166 of trisomy 18, 56 of trisomy 13, 35 of triploidy, 63 of Turner syndrome and 118 of other aneuploidies. The training set contained 72054 euploid pregnancies that were reduced to 5002 as explained in the Methods Section, 295 cases of T21 and 305 of other aneuploidies and the validation set contained 36058 euploid pregnancies, 137 cases of T21 and 133 of other aneuploidies.

Stratification of risk

In Stage 1, we used four markers (maternal age, PAPP-A, β-hCG and nuchal translucency) as inputs to the ANN. From the 36328 pregnancies in the blind set, 17532 (48.3%) were classified as “no-risk” and 18796 (51.7%) were allocated to the risk group that was subsequently assessed in Stage 2 (Figure 2).

In Stage 2, we include three additional markers, the ductus venosus, the tricuspid flow and the nasal bone and from the 137 T21 cases, 50 cases were allocated to the “high-risk” group, 83 to the “moderate-risk” group and 2 to the “no-risk” group. Furthermore, 187 of the euploid pregnancies were allocated to the “high-risk” group, 10464 to the “moderate-risk” group and 7895 to the “no-risk” group (Table 2). The FPR from Stage 1 to Stage 2 is reduced from 51.7% to ~1%, assuming that cell-free DNA test can identify all the euploid and aneuploid cases.

In addition to the diagnosis of T21, our method achieves high accuracy in detecting the OCA. The validation set contained 133 pregnancies with aneuploidies other than T21; 18 of these were allocated to the “no-risk” group in Stage 1, 2, 61 and 52 were allocated respectively to the “no-risk”, “moderate-risk” and “high-risk” groups in Stage 2.

Discussion

The findings of this study demonstrate the potential value of artificial neural network schemes in the prediction of T21 and other aneuploidies from ultrasonographic and biochemical markers at 11-13 weeks' gestation. We used multilayer feed forward neural systems because these are considered to be the most suitable from the point of view of satisfactory generalization and diagnostic yield[24]. Essentially, a multilayer network of neurons was build and adjusted according to a set of parameters for each case of either aneuploidy or euploid fetus in order to maximize the correct identification of each group.

Artificial neural networks have the ability to handle non-linear structures by using multiple hidden layers. Furthermore, assumptions about statistical concepts such as distributions, mean and standard deviation values are not needed. In addition to the above advantages, they can learn to recognize patterns in data and they have been used widely for medical tasks such as image recognition for several diseases.

In this work we present a two-Stage approach for the estimation of the risk for aneuploidy. In both Stages, we make sure, by adjusting the cut-off point accordingly, that only a minimum number of T21 cases are classified as euploid (i.e. 97% sensitivity). At the same time, we focus our studies to minimize the FPR to the lowest possible. We have validated our results using a test set of a total number of 36058 euploid, 137 T21 and 133 OCA cases. In Stage 2, 10626 pregnancies were allocated to the "moderate-risk" group and consequently 29% of the total population of 36328 pregnancies would require cfDNA testing. However, the percentage of the euploid that will be suggested to perform an invasive test is less than 1% of the total population. The values here assume that cfDNA detects accurately all euploid and aneuploid cases. Although cfDNA testing performance for T21 approaches this assumption, for OCAs, this is not accurate.

We report higher classification results than the state-of-the-art statistical mixture model that is currently used as a classifier. For making an accurate comparison between our method and the standard first trimester serum plus US screening test, we compare the 95% DR for T21 that is reported in the literature at the 5% FPR. We adjust a cutoff point at the value of 0.45, as explained in the section Methods, and achieved 94.2% and 79.5% DRs for T21 and the OCA respectively at a FPR of 1.2%. Therefore, for the same DR of 95% for T21, we achieve significantly lower FPR.

Our proposed methodology has the potential to be used in a real time application in medical centers, since it returns immediate results during a regular visit of the pregnant woman, thus reducing time and cost for additional examinations. Moreover, it can have a built-in learning mechanism, which will add continually to the knowledge acumen of the system while new identified cases are added into the system. Currently this updating is done manually at certain intervals. Since the application is installed in a normal computer, the doctor could easily use it and the validation of the cases will be done with no cost.

One drawback of our method is that it does not classify correctly, at both stages, the euploid cases as no-risk. About 51% of the euploid population after the Stage 1 will be requested to access sonographers with training in the assessment of certain markers that are not routinely assessed by everyone. The access to such doctors is relatively limited for the whole advantaged population. Furthermore, in a percentage of patients, some of those markers cannot be successfully obtained even by experienced doctors,

due to the fetal position for example. About 30% of the euploid population in Stage 2 is classified as “moderate-risk”, thus resulting in many cases requiring further testing such as cfDNA testing. This fact makes the proposed method weak in terms of cost effectiveness, but it is better than having the entire euploid population undergoing cfDNA testing.

Certainly, more work needs to be done for improving further the DR of the OCA. Our outcome assessments in Stage 2 are based on prenatal karyotype of fetuses that screened positive and postnatal karyotype of fetuses that are not phenotypically normal. Several OCAs will not be picked up by that type of assessments. The cfDNA testing does not target, and thus detect several OCAs, while some of them even not diagnosable by phenotype assessment at birth. Furthermore, none of the low risk and moderate risk cases was phenotypically considered as normal at birth. Several of which, here considered as euploid, would actually be carriers of OCAs not targeted by cfDNA and/or with apparently normal phenotype at birth. In the supplementary material, we describe each of the OCAs that were allocated to the “no-risk” group in stage 1 and to the "no-risk" and "moderate-risk" in stage 2. The first ones would only get a karyotype if they had a phenotype at birth and the second ones would get only cfDNA testing, which does not target all OCAs.

In this study we made sure that only 3% of Trisomy 21 cases will be born unexpectedly (detection rate was 97%). Still we were able to limit the number of cfDNA tests in comparison with a clinical approach offering this test as a first tier to every woman, while keeping the number of invasive tests as low as what is obtained with such approach.

For future work, we will focus our research to build models that will associate the risk for aneuploidy with preeclampsia and other pregnancy complications. We currently have preliminary results that we aim to publish in another paper.

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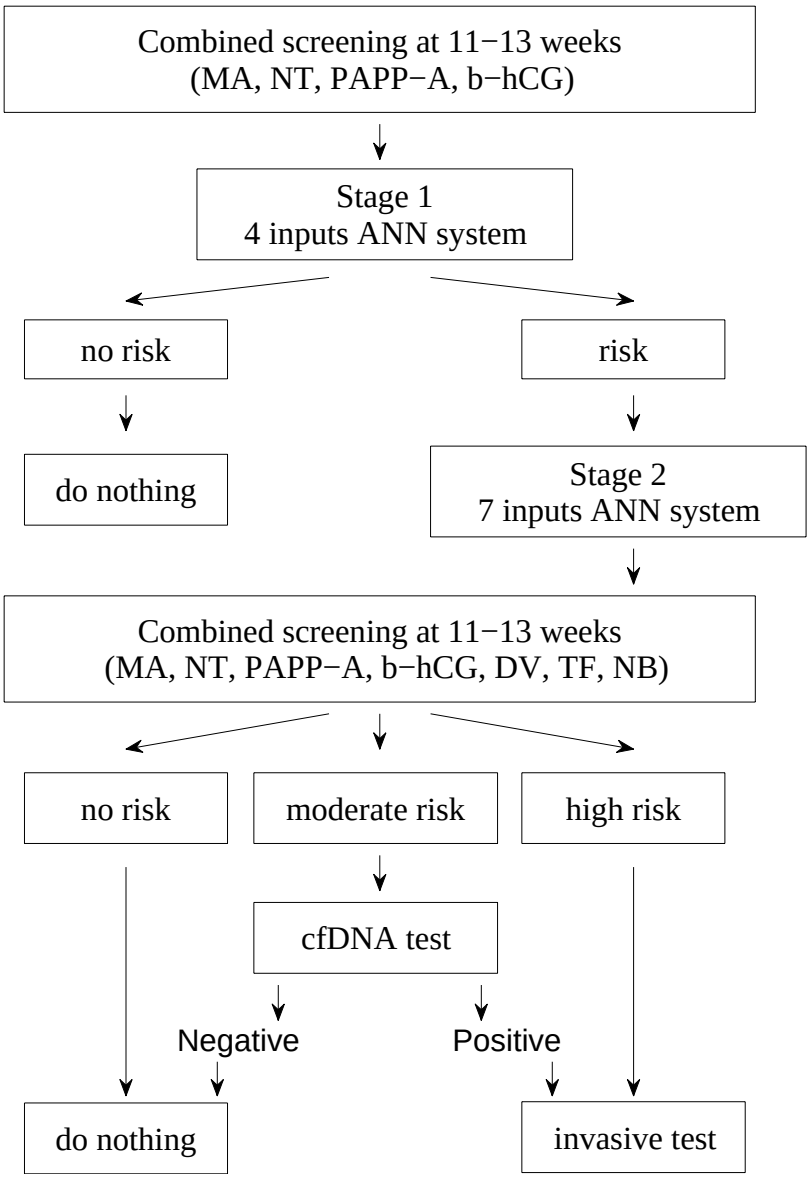
Figure Legends

Figure 1 Overview of the proposed methodology. Every case is suggested to perform the first Stage of the prenatal examination for estimating the risk for fetal aneuploidy. The "risk" cases are reassigned in Stage 2 that are finally classified in "no-risk" and continue the pregnancy, "moderate-risk" and suggested to perform the cfDNA test or "high-risk" and suggested for an invasive test for reaching diagnosis. The abbreviations MA, NT, DV, TF, NB stand for maternal age, nuchal translucency, ductus venosus, tricuspid flow and nasal bone respectively.



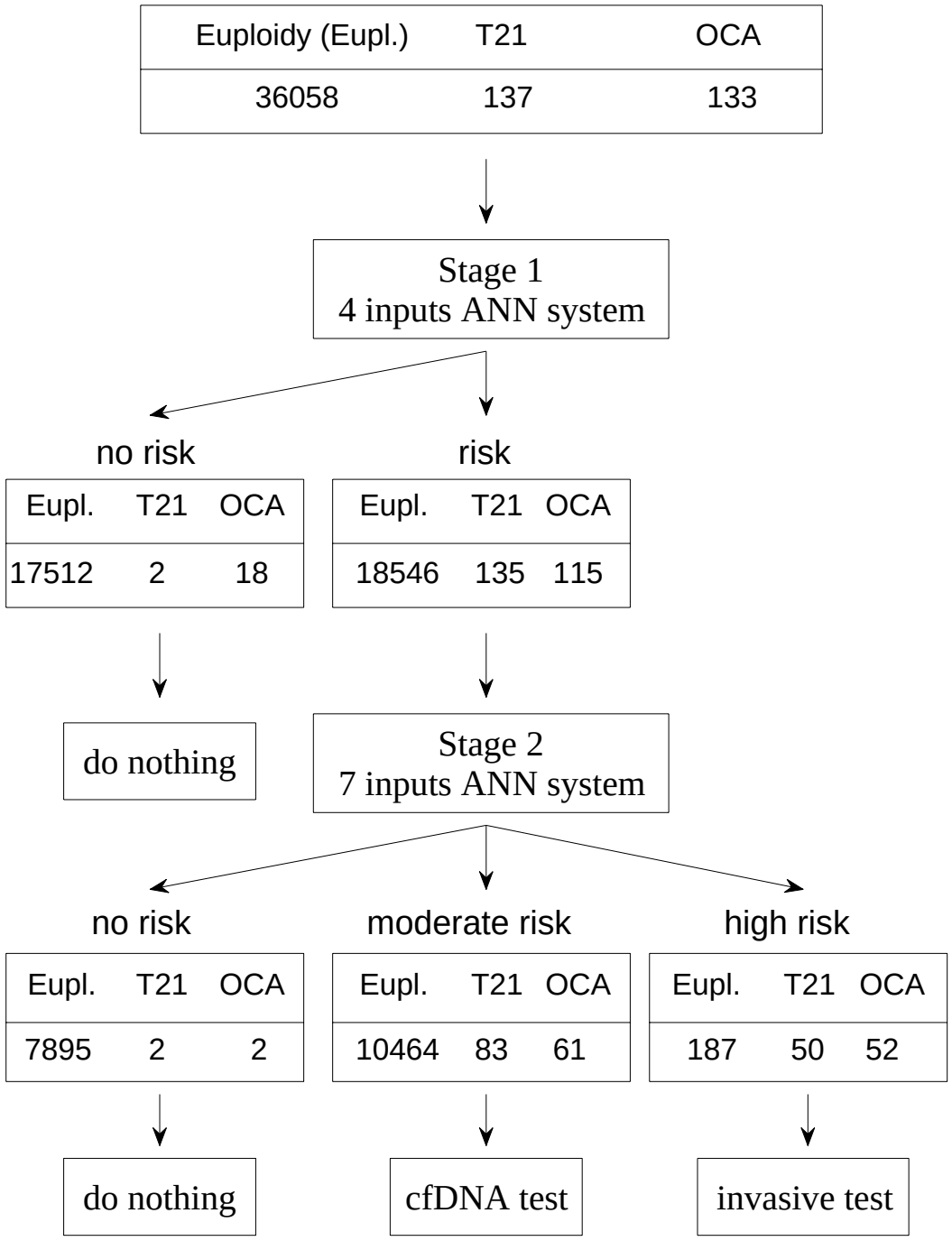


Figure 2 The results of the artificial neural network for Stages 1 and 2.

Table 1. Composition of the whole population and the training and blind sets used in the study.

Dataset	Euploid	Trisomy 21	Trisomy 18	Trisomy 13	Triploidy	Turner	Other
Total	108112	432	166	56	35	63	118
Training	72054 (reduced to 5002)	295	115	37	29	45	79
Blind	36058	137	51	19	6	18	39

Table 2. The results of the proposed system for the "blind set" for Stages 1 and 2.

Results of Stage 1: Risk category	*Blind set* (n=36328)
Euploid (n=18546)	N=36058 (51%)
Trisomy 21 (n=135)	N=137 (99%)
Other aneuploidy (n=115)	N=133 (87%)
Results of Stage 2: no-risk category	
Euploid (n=7895)	N=18546 (43%)
Trisomy 21 (n=2)	N=135 (2%)
Other aneuploidy (n=2)	N=115 (2%)
Results of Stage 2: moderate-risk category	
Euploid (n=10464)	N=18546 (56%)
Trisomy 21 (n=83)	N=135 (62%)
Other aneuploidy (n=61)	N=115 (53%)
Results of Stage 2: moderate-risk category	
Euploid (n=187)	N=18546 (1%)
Trisomy 21 (n=50)	N=135 (37%)
Other aneuploidy (n=52)	N=115 (45%)