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# First Trimester Noninvasive Prenatal Diagnosis: A Computational Intelligence Approach

Andreas C. Neocleous, Kypros H. Nicolaidis, and Christos N. Schizas, *Senior Member, IEEE*

**Abstract**—The objective of this study is to examine the potential value of using machine learning techniques such as artificial neural network (ANN) schemes for the noninvasive estimation, at 11–13 weeks of gestation, the risk for euploidy, trisomy 21 (T21), and other chromosomal aneuploidies (O.C.A.), from suitable sonographic, biochemical markers, and other relevant data. A database<sup>1</sup> consisted of 51,208 singleton pregnancy cases, while undergoing first trimester screening for aneuploidies has been used for the building, training, and verification of the proposed method. From all the data collected for each case from the mother and the fetus, the following 9 are considered by the collaborating obstetricians as the most relevant to the problem in question: maternal age, previous pregnancy with T21, fetal crown-rump length, serum free  $\beta$ -hCG in multiples of the median (MoM), pregnancy-associated plasma protein-A in MoM, nuchal translucency thickness, nasal bone, tricuspid flow, and ductus venosus flow. The dataset was randomly divided into a training set that was used to guide the development of various ANN schemes, support vector machines, and k-nearest neighbor models. An evaluation set used to determine the performance of the developed systems. The evaluation set, totally unknown to the proposed system, contained 16,898 cases of euploidy fetuses, 129 cases of T21, and 76 cases of O.C.A. The best results were obtained by the ANN system, which identified correctly all T21 cases, i.e., 0% false negative rate (FNR) and 96.1% of euploidies, i.e., 3.9% false positive rate (FPR), meaning that no child would have been born with T21 if only that 3.9% of all pregnancies had been sent for invasive testing. The aim of this work is to produce a practical tool for the obstetrician which will ideally provide 0% FNR and to recommend the minimum possible number of cases for further testing such as invasive. In conclusion, it was demonstrated that ANN schemes can provide an effective early screening for fetal aneuploidies at a low FPR with results that compare favorably to those of existing systems.

**Index Terms**—Bioinformatics, chromosomal abnormalities, computational, intelligence, non-invasive prenatal diagnosis.

## I. INTRODUCTION

THE identification of chromosomal abnormalities in the early stages of pregnancy can be achieved, with high confidence, by performing an amniocentesis test or a chorionic

villus sampling test (CVS) [1]. These methods, however, are invasive and carry high risk for miscarriage or cause medical side effects to the pregnant woman.

### A. Invasive Methods

In the work of Tabor and Alfirevic [2], it is reported that amniocentesis and CVS have a miscarriage rate of 0.5–1.0%. Furthermore, the amniocentesis test should not be performed prior to the 15 weeks of pregnancy since the miscarriage rate increases significantly, while there is also increased risk of developing talipes equinovarus [3].

Therefore, the amniocentesis or the CVS test should not be performed unless there are serious indications of high risk for chromosomal aneuploidy in the fetus. It is also emphasized that both of these methods carry additional costs to the family, which is not a trivial issue.

### B. Noninvasive Methods

There has been an increased interest and need of exploring noninvasive methods for the prediction of chromosomal aneuploidies in the first trimester, or earlier, of pregnancy. In the literature, several methods that were aiming at a noninvasive prediction of chromosomal abnormalities had been reported. These methods can be classified into three main categories, based on their methodology.

1) *First-Trimester Prenatal Statistical Screening*: Statistical methods for appraising the probability of aneuploidy are properly applied on several markers that are obtained by an antenatal test. Typically, the markers used are the fetus crown ramp length (CRL), the fetal nuchal translucency (NT), the maternal age (MA), the pregnancy-associated plasma protein-A (PAPP-A), the serum free  $\beta$ -hCG, the ductus venosus flow (DV), tricuspid valve flow, and others [4]–[6]. The risk for aneuploidies increases with MA, and it is higher in women with previous affected pregnancies. It also increases with fetal NT thickness and is higher in those with absence of the fetal nasal bone and with abnormal flow through the ductus venosus and across the tricuspid valve. The maternal serum concentration of the placental products free  $\beta$ -human chorionic gonadotropin and PAPP-A also influence the risk for aneuploidy [4]. Serum PAPP-A is decreased in trisomies 21, 18, 13 and the Turner syndrome, while serum free  $\beta$ -hCG is increased in T21, decreased in trisomies 18 and 13 and not altered in the Turner syndrome.

Most of these methods use posterior probabilities based on the median and the standard deviation of the markers, or by using a suitable multivariate statistical approach. Typically, the models output a probability on the risk of fetus aneuploidy. In the work of Nicolaidis *et al.* [7], a multivariate likelihood

<sup>1</sup>The dataset can become available for academic purposes by communicating directly with the authors. Digital Object Identifier 10.1109/JBHI.2015.2462744

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approach is described for the identification of the T21 in the first trimester of pregnancy. A multiplication of the MA-related risk and each likelihood ratio (LR) derived from the fetal NT and maternal weight-adjusted serum free  $\beta$ -hCG and PAPP-A outputs the patient-specific risks for aneuploidy. More on this method can be found in [8].

Traditionally, screening for aneuploidies is done by estimating the patient-specific risk for each aneuploidy. This is done by multiplying the *a priori* risk, by a factor of approximately 1.75 in cases with a previous history of aneuploidy. The LR for each ultrasonographic and biochemical marker is used to calculate the detection and false positive rates (FPRs) by taking the proportions with risks above a given risk threshold [4]. The LRs for the categorical variables, such as the absence or presence of nasal bone and the abnormality of flow in the tricuspid or ductus venosus valves, are ratios of prevalence of each marker in each type of fetal aneuploidy to the prevalence in euploidy fetuses. In the case of continuous variables, such as fetal NT thickness and maternal serum free  $\beta$ -hCG and PAPP-A, the LRs are derived from the overlap of the Gaussian distribution of each marker in each type of fetal aneuploidy with the respective Gaussian distribution in euploidy fetuses.

2) *Maternal Cell-Free DNA Screening*: Schmorl's experiment with women who died of eclampsia was the first study to suggest that fetal cells circulate in the mother's blood [9]. It is estimated that 1 in 1000 to 1 in 10 000 000 nucleated cells in maternal blood are fetal [10]. The isolation of fetal DNA from the maternal DNA could give insights for studying genetic diseases. For the problem under study, the isolation of the fetus DNA is still extremely hard to achieve; it is labor intensive and requires highly skilled operators. While the majority of the studies focused on the identification of the T21 [11], [12], recently, the trisomies 18 and 13 are also identified. In [13], Papageorgiou *et al.* claim a very good prediction (100%) of 26 euploidy and 14 cases of T21 in the validation set, by determining the DNA methylation ratio of 12 selected differentially methylated regions.

Other studies used similar approaches, mainly for T21, trisomy 18, and trisomy 13 [14]–[16]. Palomaki *et al.* [16] report a detection rate of 100% for trisomy 18 and 91.7% for trisomy 13 with an FPR of 0.28% and 0.97%, respectively. However, the test was done in the late first and early second trimester of the pregnancy, which can be considered relatively late for abortion. Most importantly, the circulating cell-free DNA fragments are being presented as differentially methylated markers and not identified under microscope. Then, the procedure for the prediction of the risk for aneuploidies is done with simple statistical analysis of the methylated markers.

The above-described noninvasive methodologies have their relative advantages but at the same time have their disadvantages. The first methodology suffers in the sense that one cannot combine and examine simultaneously all the relevant parameters of the case or visualize them in a multidimensional space. Visualization of more than three parameters at a time is extremely difficult and not practical in a medical environment. Also, parameters that are correlated can lead to unreliable conclusions. In the second methodology, the parameters used (called

markers) have no relation to the phenotype of the fetus, such as, for instance, the CRL or the fetal heart rate which for the certain problem is important. In the majority of the published studies, the population of their databases is very small for drawing reliable conclusions for such a complex problem. For instance, in [10], they are dealing with 40 cases (euploidy and T21). Even though this work was published in *Nature Medicine*, one can identify two important limitations of the proposed method, which imply lack of scientific confirmation. For such a complex problem, it is not convincing whether a perfect prediction of a population of 40 cases is satisfactory for drawing robust conclusions. In contrast to this database, we studied more than 51 000 cases of pregnant women and validated 16 898 cases of euploidy fetuses, 129 cases of T21, and 76 cases of O.C.A. Also, there is no information in [13] whether the results were cross validated. It is important to present cross-validated results such as tenfold or leave-one-out cross validation. In this scenario, one can have a better insight about the generalization ability of the method. Therefore, it is not explicit if their method will yield similar results by randomly selecting different training and test sets. Another serious limitation of the study [10] is the fact that only euploidy and T21 can be handled; what will the system predict if the unknown case in question is trisomy 13 or O.C.A. Furthermore, even though the whole analysis is based on the existence of free DNA cells of the fetus in the mother's blood, no such cell is isolated for singling out the pathological gene. The determination of a pathological existence in the genes is done statistically and probabilistically.

3) *Computational Intelligence*: In this study, we report a computational intelligent approach for the noninvasive estimation of the risk of aneuploidies. This approach involves the development of a system predictor, which takes as input a number of parameter values. These values have different origins and source, and they are collected at certain prespecified times during pregnancy. For example, during the first-trimester screening for fetal T21 and for O.C.A., certain parameter values are recorded, which are a combination of maternal and fetoplacental nature [4].

The computational artificial neural networks (ANNs), which are a specific paradigm of computational intelligence, had been used as effective classifiers and predictors for the last 25 years. Indeed, they had been extensively applied in medical and biological research and applications [17]–[20].

ANNs are essentially mathematical algorithms implemented in software that learn from data and capture the knowledge and the internal dynamics that are contained in the data. Suitably trained models approach the functionality of small biological neural systems in a very fundamental manner that mimics human-like behavior. Thus, once they are properly trained, they exhibit computational intelligence in a simplistic mimicry of the biological intelligence. They constitute a very simple digitized model of the biological brain and, in some cases, can detect complex nonlinear relationships between dependent and independent variables in a dataset which may be undetectable by a human brain. Indeed, they can execute certain tasks, especially in classification and recognition that would be extremely

difficult to be done by the traditional and conventional computing techniques. They can learn from data, even in self-organized manners.

In the medical field, ANNs proved to be a powerful method for medical diagnosis. As an example, Al-Shayea [21] reports a medical diagnosis system for acute nephritis disease and heart disease using feedforward ANN. A correct classification of 99% has been reported. Hayashia and Setiono [22] used a two-level approach combining two ANN systems for the diagnosis of hepatobiliary disorders. In this study, the database consisted by 536 samples with 9 input features describing four hepatobiliary disorders: Alcoholic Liver Disease, Primary Hepatoma (PH), Liver Cirrhosis, and Cholelithiasis. Their best ANN models classified 95% of the four diseases. A chest disease diagnosis system is reported in [23]. The database in this work consisted of chest disease measurements of 357 samples and 6 classes, namely Tuberculosis, COPD, Pneumonia, Asthma, Lung Cancer, and Normal. All samples had 38 features. The authors report 90.2% average classification accuracy for all the 6 classes. Other studies that used ANN are referenced in [24]–[26].

The objective of our study is to examine the potential value of ANNs and other computational intelligence techniques in the prediction of the risk for T21 and O.C.A. from ultrasonographic and biochemical markers at 11–13 weeks of gestation.

In Section II, computational intelligence approach and the proposed method are presented and discussed. Furthermore, statistical analysis has been applied to the nonbinary features. This analysis is presented including the visualization of the feature distributions and the testing of the separability of the features in pairs. In Section III, we present our experiments and results, and in Section IV, we discuss further the results of the present work and how it is compared with other methods. Finally, in Section V, we report our conclusions.

## II. METHODS

In this section, we present the computational intelligence approach that has been adopted, the procedure for data collection, the data grouping into cross-validation sets, and two schemes for aneuploidy risk prediction. We have implemented models with ANNs, support vector machines (SVM) (kernel 1 and 2), and k-nearest neighbors (k-NN).

### A. ANN Diagnostic System

Many different ANN structures had been proposed and used by researchers in different fields. The most widely used ANN structure is the fully connected multilayer feedforward structure. Mathematically, this is represented by Eq. 1 as:

$$y_{iL}^{[L]} = f_{iL}^{[L]} \left( \sum_{j_{L-1}=1}^{n_{L-1}} y_{j_{L-1}}^{[L-1]} W_{L-1,L}^{[L]} \right) \quad (1)$$

where  $y_{iL}^{[L]}$  is the output value of each neuron  $i$  of layer  $L$  that has a total of  $n_L$  neurons. Typically, the function has a squashing form such as the logistic or the hyperbolic tangent.  $W_{L-1,L}^{[L]}$  is the set of weights associating neurons in layer  $L - 1$  to neurons in layer  $L$ .

In this study, we used fully connected feedforward ANNs as described in Eq. 1. The first layer is typically called input layer and it has as many neurons as the input parameters. The weights in the ANN represent the intensity of the processes in the synapses of the biological neural network. In a practical implementation of ANNs, the initial values of the weights are typically set to random values.

Once the ANN topology is decided, an effective training and tuning procedure needs to be implemented, so that the network will achieve the capability for generalization as a risk estimator. Many training procedures had been proposed and are available for implementation. The most widely used for feedforward networks is the backpropagation algorithm [27]. In this study, we implemented fully connected feedforward neural networks with backpropagation learning. The justification for selecting this simple network is discussed further down in the paper. The activation function for the input layer and the hidden layer was set to logistic. We have trained and validated a large number of networks by changing different parameters in the training procedure. The best results were obtained with the networks built with 20 to 40 neurons in the hidden layer as will be explained in the results.

### B. Database and Parameters Used

The dataset used was collected from women having singleton pregnancies while attending the Fetal Medicine Centre at Kings' College Hospital and University College London Hospital in London, for aneuploidy screening at 11+0 to 13+6 weeks of gestation. The MA and the previous history of the pregnancy, in particular on whether there was a previous case of T21, were recorded. Also, a transabdominal ultrasound examination was performed for measurement of the fetal CRL and the NT thickness, as well as an assessment of the fetal nasal bone and the flow in the DV and across the tricuspid valve. These were done by sonographers who had received the appropriate Fetal Medicine Foundation Certificates of Competency. The pregnancy was dated according to the measurement of the fetal CRL [28]. Additionally, maternal blood was collected and used to measure serum PAPP-A and serum free  $\beta$ -hCG concentrations through automated machines that provide reproducible results within 30 min (Delfia Express System, Perkin Elmer). The measured PAPP-A and serum free  $\beta$ -hCG were converted into multiples of the median (MoM). The measured fetal NT was expressed as a difference from the expected normal mean for the specific CRL [29]. These maternal demographic characteristics, ultrasonographic measurements, and the biochemical results were recorded in a structured database.

The following 9 parameters were used as suitable markers that could help in establishing the risk for aneuploidies: MA in years, history of previous pregnancy with T21, fetal CRL in mm, serum free  $\beta$ -hCG in MoM, PAPP-A in MoM, delta NT in mm, nasal bone (present or absent), tricuspid flow (regurgitation or normal), and DV (reversed a-wave or normal).

### C. Statistical Analysis of the Data

In this section, we present the results of a statistical analysis that has been applied to the data. The aim of this analysis

TABLE I  
RESULTS FROM THE KOLMOGOROV-SMIRNOV TEST (NORMALITY TEST). “MA” STANDS FOR MATERNAL AGE, “N” STANDS FOR NORMAL DISTRIBUTION, WHILE “NN” STANDS FOR NON-NORMAL DISTRIBUTIONS.

	MA	CRL	PAPP-A	$\beta$ -hCG	delta NT
Euploidy	NN	NN	NN	NN	NN
T13	N	N	NN	N	N
T18	N	N	NN	NN	N
T21	NN	N	NN	NN	NN
Triploidy	N	N	NN	NN	NN
Turner	N	N	N	NN	N

is to discover the significance of separability between pairs of distributions for each feature, by means of their medians, that is, to test for the statistical null hypothesis whether data that belong to same category share equal medians. Moreover, we have tested the normality of the distribution of each feature. This was done to create a better understanding of our data, as well as to avoid using methods that require normal distributions in the input feature space, such as the Gaussian Mixture Models and the student’ t-test. The histogram of each nonbinary feature has been computed for all the classes of our database, namely euploidy, T13, T21, T18, Triploidy, and Turner. The normality of the data was tested with the Kolmogorov–Smirnov test [30].

In Table I, the results of the normality test are shown. In the first row, the names of the five nonbinary markers are shown, while in the first column, the names of the classes (euploidy or aneuploidies). It should have been expected that the data used in this study will not follow a normal distribution since the population under study is not normally distributed by nature, since pregnancy occurs in a certain age range which is naturally skewed to the right; in addition to this, the large database used in this study contains proportionally much more trisomy cases than reported statistically. For example, T21 occurs 1 in about 800 pregnancies, and thus, in our database, we should have had 65 cases instead of 408. Similarly, trisomy 18 occurs 1 in 5,000 pregnancies, and thus, we should have had ten instead of 145 cases, trisomy 13 1 in about 16,000 pregnancies, etc.

The results of the normality test confirm this reality. Another factor that may contributed to this is the fact that our database is highly unbalanced, i.e., euploidies are by far more than aneuploidies. In large sample sizes, the probability that a normality test will reject the null hypothesis that the sample comes from a normal distribution increases due to samples that depart from normality, which are statistically significant. Furthermore, the population in the classes of T13, Triploidy, and Turner is relatively small ( $< 50$ ), and therefore, the rejection of the null hypothesis may be because of insufficient number of samples.

A widely used method to measure the separability between two classes for a given feature is the student’s t-test. The student’ t-test assumes that the testing data follow a normal distribution and the population of the two classes is equal. The t-test rejects the null hypothesis at a confidence level of 95% ( $p < 0.05$ ), where  $p$  is the estimated probability of rejecting the null hypothesis.

TABLE II  
THE SIGNIFICANCE OF THE DIFFERENCE BETWEEN THE MEDIANS OF EVERY CLASS COMPUTED BY THE PAIRED-DIFFERENCE USING THE WILCOXON RANK SUM METHOD

	MA	CRL	PAPP-A	$\beta$ -hCG	delta NT
Eupl. - Triploidy	0.89 True	$<<0.05$ False	$<<0.05$ False	$<<0.05$ False	0.99 True
Eupl. - Turner	0.08 True	0.14 True	$<<0.05$ False	0.18 True	$<<0.05$ False
T13 - T18	0.14 True	0.28 True	0.02 False	$<<0.05$ False	0.0763 True
T13 - T21	$<<0.05$ False	$<<0.05$ False	$<<0.05$ False	$<<0.05$ False	0.06 True
T13 - Triploidy	0.04 False	0.30 True	0.03 True	0.05 True	$<<0.05$ False
T13 - Turner	$<<0.05$ False	0.19 True	$<<0.05$ False	$<<0.05$ False	$<<0.05$ False
T18 - T21	0.13 True	$<<0.05$ False	$<<0.05$ False	$<<0.05$ False	$<<0.05$ False
T18 - Triploidy	$<<0.05$ False	0.79 True	0.07 True	0.35 True	$<<0.05$ False
Tripl. - Turner	0.32 True	0.04 False	$<<0.05$ False	0.025 False	$<<0.05$ False

Taking into consideration that a subset of the features does not follow a normal distribution and the population between the classes is not equal, the t-test could not be applied.

The significance of the difference between the medians of every class for the nonbinary features was computed by the paired-difference Wilcoxon Rank Sum method [31]. The Wilcoxon Rank Sum method is a nonparametric method in contrast with the widely used t-test method. It has been proposed by Frank Wilcoxon in 1945 and popularized by Sidney Siegel in 1956 in applications to behavioral sciences. The p-values of the Wilcoxon Rank Sum method are summarized in Table II. The pairs for testing the null hypothesis are shown in the first column in Table II. The word True signifies rejection of the null hypothesis and False acceptance. The null hypothesis that the medians of the data being compared have no statistical significance is rejected with a p-value that is less than 0.05. The method rejected the null hypothesis for all the features for the pairs “euploidy & T13,” “euploidy & T18,” “euploidy & T21, T18 & Turner,” “T21 & Triploidy,” and “T21 & Turner,” and therefore, the results were excluded from Table II.

In Figs. 1 to 5, we use the box plots to present the median, the standard deviation, the normality of the distribution, and the outliers of each feature. The total range of each feature is shown with a vertical dashed line, while the lowest and the highest values are shown with small horizontal lines at its ends. The box in the middle represents the distribution of the feature in the range between the points of the first quartile (Q1) and the third quartile (Q3), where Q1 is defined as the middle number between the smallest number and the median of the dataset. Similarly, Q3 is defined as the middle value between the median and the highest value of the dataset. The median of each feature is shown with a small horizontal line within the box. In each figure, a box plot of a feature is plotted against all the 6 classes. A dashed and a solid horizontal lines show the median of the euploidy and the median of the T21, respectively. These two lines were placed manually for visualization purposes.

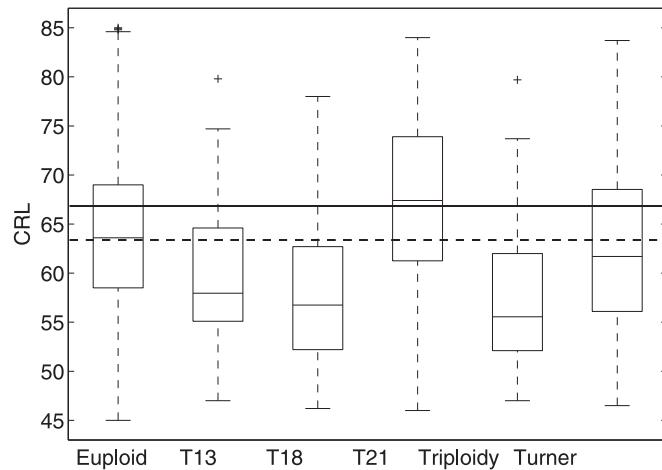


Fig. 1. Visualization of statistical properties of the CRL feature.

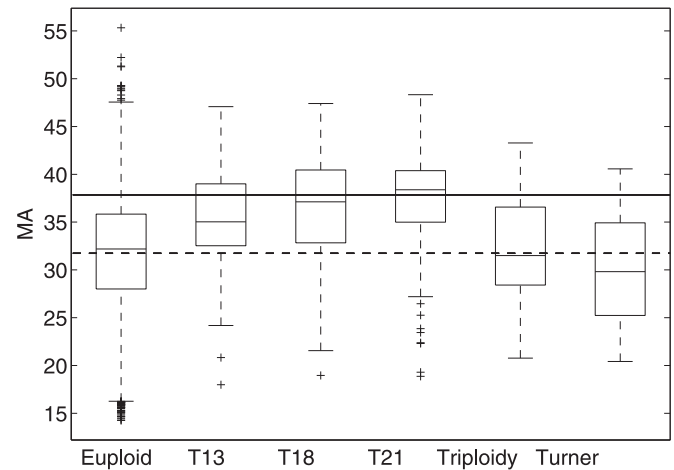


Fig. 4. Visualization of statistical properties of the Mother's age feature.

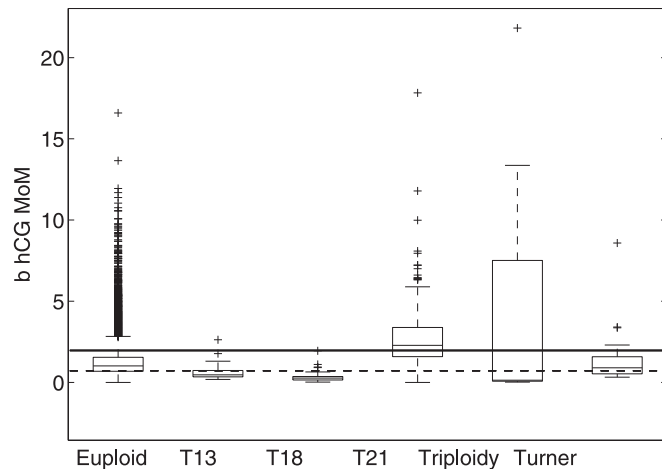
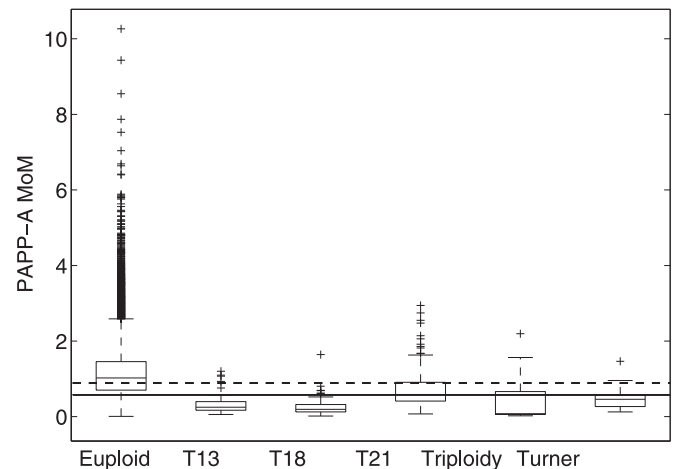
Fig. 2. Visualization of statistical properties of the  $\beta$ -hCG MoM feature.

Fig. 5. Visualization of statistical properties of the PAPP-A MoM feature.

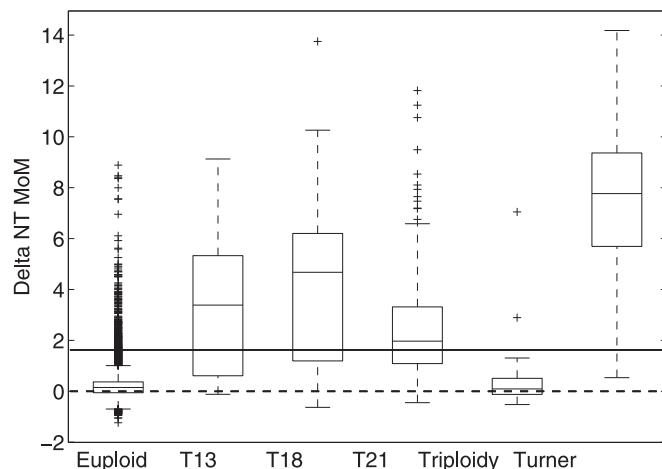


Fig. 3. Visualization of statistical properties of the Delta NT feature.

The purpose of this analysis is to gain additional knowledge about the statistical properties and the significance of the separability of every feature. For instance, the medians of the populations of each class can be roughly compared by simply observing the distance between them. Also, it is interesting to

visualize how the distribution around the median is spread. This can be done by observing the area enclosed in the box around the median. The normality of the distribution can also be observed if the median lies in the middle of the box. The outliers are also marked by a cross allowing a rapid way to estimate their population and their statistical significance with respect to the distance from the median.

Fig. 1 shows statistical information of the fetal CRL parameter. The value of the CRL is extracted during the ultrasound screening. The doctor is manually annotating the preferred positions of the crown ramp by looking at the fetus on the screen. The manual annotation of the CRL creates a possibility for human errors in the measurement. Indeed, it may be that the high variance of the data for all the classes can be explained due to this observer error. It is worth mentioning that the median of the T21 is significantly higher from the euploidy, while the median of the O.C.A. is lower. This fact also explains that a system which in the training procedure takes all the trisomies as a single class may not achieve a robust generalization.

In Fig. 2, it is shown that the variance of the data for the serum free  $\beta$ -hCG feature is narrow with an exception on the data of the triploidy. As can be seen, there is one extreme outlier that

TABLE III  
NUMBER OF CASES THAT WERE USED FOR TRAINING IN THE  
CROSS-VALIDATION PROCEDURE

Training	Euploidy	T21	T18	T13	Triploidy	Turner
Fold 1	33,619	279	106	38	22	41
Fold 2	33,840	278	105	39	23	40
Fold 3	33,982	277	104	37	24	39

forces the data to a non-Gaussian distribution. This is reasonable, taking into account the small population of in this class. The feature serum-free  $\beta$ -hCG has high separability by means of their median, for the pair “euploidy & T21” and low separability for the pairs “euploidy & Turner,” “T13 & Triploidy,” and “T18 & Triploidy.”

The analysis of the feature Delta NT is shown in Fig. 3. The variance of the data in the classes T13, T18, and Turner is relatively higher with respect to the data in the euploidy and triploidy classes. It is also shown that the separability between the euploidy and the trisomies T13, T18, T21, and Turner has statistical significance.

It is well known that the MA plays a significant role for the classification of the euploidy or T21. This can also be observed in Fig. 4. The mean MA of the euploidy is 33 years, while for the T21, it is 38 years. It can also be seen that the means of euploidy and triploidy do not have significant statistical differences. While the mean of the MA of T21, T13, and T18 is significantly higher, the mean for Turner is significantly lower. This can be seen in Fig. 4 and, mathematically, by the Wilcoxon Rank Sum method, shown in Table II.

There is a statistical significance in the difference of the means between euploidy and the rest of the trisomies for the feature PAPP-A. In Fig. 5, it is shown that the mean of the euploidy has a higher value with respect to the means of the O.C.A. The variance of all the classes for this feature is relatively low. In addition to the statistical analysis done in these data and the useful information regarding the nature of the data, an expert obstetrician can use these graphs while examining a new case. It can provide to him a tool for visualizing a new case with respect to thousands of previously confirmed cases.

#### D. Cross Validation

The systems and approach described in this paper were tested with a threefold cross validation. This was done by randomly dividing the data of 51,208 (50,517 euploidy, 408 T21, and 283 O.C.A.) pregnancies into three training and evaluation sets containing proportionally the same numbers of euploidy and aneuploidy cases, as shown in Tables III and IV.

#### E. System 1: Classification Into Two Classes: Euploidy and T21

The dataset was split into a training and an evaluation set. The training dataset consisted only of euploidy and T21 pregnancies (Fig. 6), whereas the evaluation set also contained cases with O.C.A. Various supervised models with ANN, SVM (kernel 1

TABLE IV  
NUMBER OF CASES THAT WERE USED FOR VALIDATION IN THE  
CROSS-VALIDATION PROCEDURE

Validation	Euploidy	T21	T18	T13	Triploidy	Turner
Fold 1	16,898	129	39	14	10	13
Fold 2	16,677	130	40	13	9	14
Fold 3	16,535	131	41	15	8	15

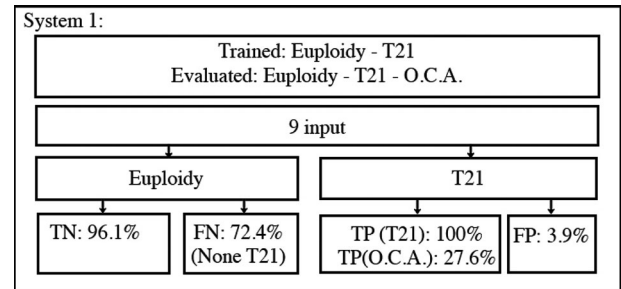


Fig. 6. System 1, distinguishing between euploidy and T21 for the 9-input ANN. In this system, the training set contained only euploidy and T21 cases. The evaluation set contained euploidy, T21, and O.C.A.

TABLE V  
PARAMETERS USED AS INPUT VECTORS FOR THE TRAINING MODELS

Parameter	Model of 6 inputs	Model of 9 inputs
Maternal age	Used	Used
History of previous T21	Used	Used
Crown ramp length	Used	Used
Delta nuchal translucency	Used	Used
Serum PAPP-A	Used	Used
Serum free $\beta$ -hCG	Used	Used
Nasal bone	Not Used	Used
Ductus venosus flow	Not Used	Used
Tricuspid flow	Not Used	Used

and 2), and k-NN were developed and the best results in separating euploidy from T21 pregnancies were achieved by using a standard multilayer feed-forward neural network with one hidden layer. There were 9 neurons in the input layer, representing the 9 markers that were used for training the networks (see Table V). The network output target was set to 0 for T21 and 1 for euploidy.

After completion of the learning process through the use of the training dataset, the system was tested by the evaluation dataset, which consisted of euploidy, T21, and O.C.A. It is important to mention that system 1 can be considered as an autonomous system where T21 cases are detected with considerably low FPR. The drawback of this system is that the O.C.A. are mostly predicted as euploidy. These false negative predictions of the O.C.A. are important to be identified as abnormalities. While generally the O.C.A. have an increased possibility of miscarriage during pregnancy, and therefore, the early diagnosis of such abnormalities are not considered to have equal importance to the T21, in some cases, the embryo survives until the late stages of pregnancy, or it is born and die few days later. This

fact may cause health complications to the mother and generate additional psychological damage to the relatives.

In order to predict correctly the O.C.A., we propose system 2 that minimizes the false negative rate (FNR) with a cost of increasing the FPR and creating false alarm to some families. The doctors may use system 1 or system 2, having in mind the cost and the risk of sending a euploidy for further invasive examinations or considering an abnormal case as euploidy and let the pregnancy continue naturally.

#### F. System 2: Separate Classification of Three Classes: Euploidies, T21, and All the O.C.A.

The same approach followed in developing system 1 had been followed for generating suitable classification models that could separate not only T21 from euploidy cases but also to test for the capability to separate the O.C.A. from the euploidy cases.

In a first attempt, we tried to build a neural network that could predict 6 situations. These were set as the outputs of the ANN. They were the euploidy, T21, trisomy 18, trisomy 13, Turner syndrome, and triploidy. Although the cases of T21 were separable from euploidy, when the O.C.A. were involved during the training phase of the network, this separation deteriorated and most of the O.C.A. were classified as euploidy. Thus, this approach was abandoned.

A second approach that we tested was to build neural networks that were trained to separate euploidy from T21, euploidy from trisomy 18, euploidy from trisomy 13, euploidy from Turner syndrome, euploidy from triploidy, and T21 from O.C.A. This approach was also abandoned since it failed to successfully separate the groups. It was observed, however, that most of the false positive cases (i.e., euploidy cases classified as aneuploidy) were the same for all the models examined. Thus, it was decided to exclude the models “euploidy & T13” and “euploidy & triploidy” from the overall system due to very low performance and combine the results of the tree systems in a logical way (“euploidy & T21,” “euploidy & T18,” and “euploidy & Turner syndrome”).

System 2 was, therefore, decided to involve two stages for distinguishing between euploidy, T21, and aneuploidy pregnancies. In stage 1, each case is assessed independently by the three models explained above and classified as euploidy or aneuploidy (T21, trisomy 18, or Turner syndrome). This is done with the logical statement: “If a case is classified as euploidy by all three models, then this case is given a final classification as euploidy, otherwise is send to stage 2 for further examination.” In Stage 2, any case given an aneuploidy result by any of the three models is reassessed by a 9-input model of “T21 & O.C.A.” with a binary output (0 for T21 and 1 for O.C.A.) and reclassified into T21 or O.C.A. as shown in Fig. 7.

### III. RESULTS

We present our results in terms of detection rate, the accuracy, and Matthews correlation coefficient (MCC) [32]. A correct classification of an abnormal case is called true positive prediction (TP), while a false classification of an abnormal case is called false negative prediction (FN). True negative (TN) and false positive (FP) are the correct and false classification,

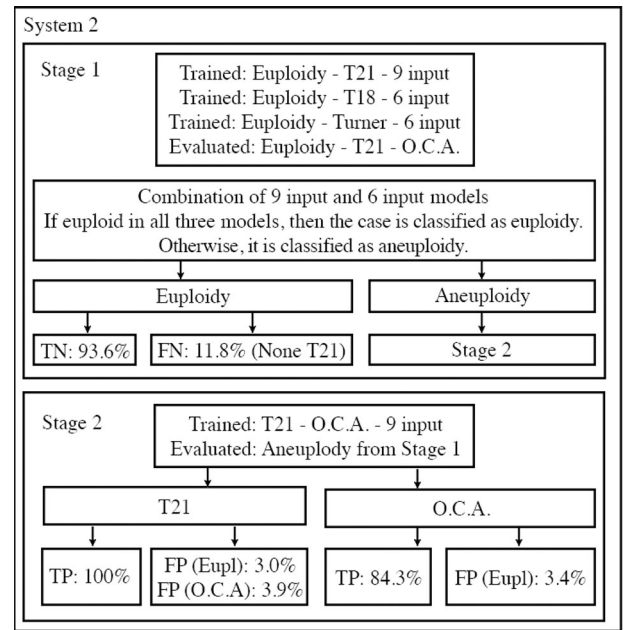


Fig. 7. System 2, distinguishing between euploidy, T21, and O.C.A. It is a combination of four ANN trained with 1) euploidy and T21, 2) euploidy and T18, 3) euploidy and Turner, and 4) T21 and O.C.A.

TABLE VI  
ACCURACY AND THE MATTHEWS CORRELATION COEFFICIENT (MCC) OF THE ANN, SVM AND k-NN OF SYSTEM 1 FOR THE FIRST VALIDATION DATASET. SYSTEM 1 WAS TRAINED WITH EUPLOIDY AND T21 CASES. IT WAS VALIDATED FOR THE ENTIRE DATABASE INCLUDING EUPLOIDY, T21 AND O.C.A.

System 1	Accuracy	MCC
ANN	0.96	0.40
SVM 1	0.93	0.31
SVM 2	0.92	0.28
k-NN	0.92	0.28

respectively, for a normal case. The detection rate is defined as the correctly classified instances divided by the total population of each class. The accuracy is defined as the sum of true positives and true negatives divided by the total population. The MCC is a balanced measure of the quality of binary classifications in the range  $-1$  and  $1$ , and it is commonly used to describe the results of highly unbalanced class populations. A value of  $-1$  represents a complete error of classification, while a value of  $1$  represents perfect classification. A value of  $0$  shows random classification. It was introduced by the biochemist Brian W. Matthews in 1975, and it is defined as

$$MCC = \frac{TP * TN - FP * FN}{\sqrt{(TP + FP)(TP + FN)(TN + FP)(TN + FN)}}. \quad (2)$$

#### A. System 1: Classification Into Two Classes: Euploidy and T21

The results of System 1 for fold 1 and for ANN, SVM (kernel 1 and 2), and k-NN are summarized in Table VI. The results of the threefold cross validation for ANN are shown in Table VII. In Table VIII, we present the histogram of the 9-input



TABLE VII  
DETECTION RATES OF ANN OF SYSTEM 1 FOR THE THREE VALIDATION DATASETS. SYSTEM 1 WAS TRAINED WITH EUPLOIDY AND T21 CASES. IT WAS VALIDATED FOR THE ENTIRE DATABASE INCLUDING EUPLOIDY, T21, AND O.C.A.

System 1	Euploidy	T21	O.C.A.
Fold 1	96.1%	100.0%	27.6%
Fold 2	97.1%	93.9%	57.9%
Fold 3	97.2%	90.1%	65.8%

TABLE VIII  
PERFORMANCE OF SYSTEM 1, THE 9-INPUT MODEL “EUPLOIDY & ANEUPLOIDY,” WHERE EACH CASE WAS QUANTIFIED TO A CLASS BETWEEN 0 AND 1

Output	Euploidy $n = 16,898$	T21 $n = 129$	O.C.A. $n = 76$
0 to 0.1	104 (0.6%)	115 (89.2%)	12 (15.8%)
> 0.1 to 0.2	80 (0.5%)	8 (6.2%)	3 (3.9%)
> 0.2 to 0.3	129 (0.8%)	3 (2.3%)	1 (1.3%)
> 0.3 to 0.4	138 (0.8%)	2 (1.6%)	2 (2.6%)
> 0.4 to 0.5	203 (1.2%)	1 (0.8%)	3 (3.9%)
> 0.5 to 0.6	192 (1.1%)	–	2 (23.6%)
> 0.6 to 0.7	217 (1.3%)	–	2 (23.6%)
> 0.7 to 0.8	231 (1.4%)	–	5 (6.6%)
> 0.8 to 0.9	123 (0.7%)	–	7 (9.2%)
> 0.9 to 1.0	15481 (91.6%)	–	39 (51.3%)

network output where in the range 0 to 0.5, the system correctly identified as abnormal all 129 cases of T21 with an FPR of 3.9% (Fig. 6). In the case of T21 pregnancies, the average output value was 0.029 (standard deviation 0.074) and in 123 (95.4%) of cases the output was in the range of 0 to 0.2. In the euploidy pregnancies, the average output value was 0.95 (standard deviation 0.15) and in 15,604 (92.3%) of cases the output was in the range of >0.8 to 1. The overall diagnostic yield of the system was true negative rate (TNR) of 96.1%, FNR of 0%, true positive rate of 100%, and FPR of 3.9%. Table VIII has a particular importance. It is shown that the FPR can be reduced considerably to 1% by changing the threshold to 0.2, if the society is ready to support 4.6% of the T21 which will be undetected (FN). This can be interpreted as about 50 births of T21 in 1,000,000 births.

### B. System 2: Classification Into Three Classes: Euploidy, T21, and O.C.A.

System 2 is consisted of two subsystems (Stage 1 and Stage 2). In Stage 1, the system classifies an unknown case as euploidy or aneuploidy. In Stage 2, the aneuploidies are being further classified as T21 or O.C.A. (Fig. 7).

1) *Classification Into Euploidy or Aneuploidy by Three Models.*: The network combined the 9-input model “euploidy & T21” (used in system 1) and the 6-input models “euploidy & trisomy 18” and “euploidy & Turner” syndrome. This network correctly classified as euploidy (concordance in all three systems) 15,820 (93.6%) of the 16,898 euploidy cases and as aneuploidy (in any one of the three systems) 196 (95.6%) of the 205 aneuploidy cases, including all 129 cases of T21 (100%),

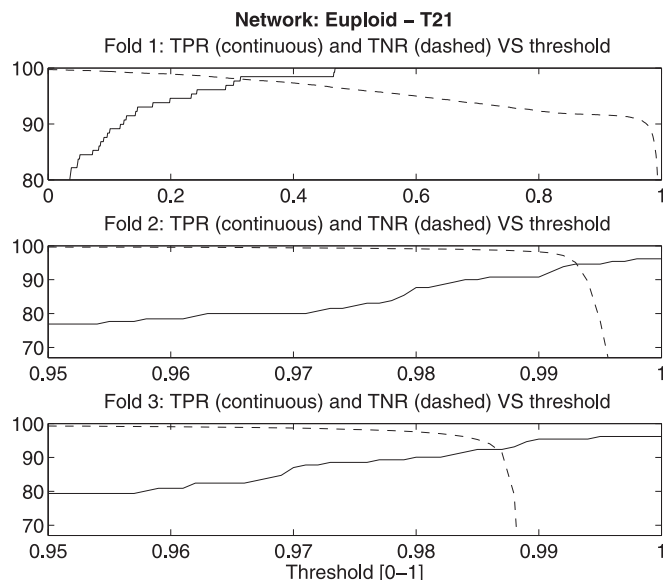


Fig. 8. TPR (filled line) and TNR (dashed line) for the network “euploidy & T21.” The three plots show the results for the three validation sets (folds 1, 2, and 3).

34 (87.2%) of the 39 cases of trisomy 18, 11 (78.6%) of the 14 cases of trisomy 13, eight (80%) of the ten cases of triploidy, and 11 (84.6%) of the 13 cases of Turner syndrome (see Fig. 7).

Therefore, at the end of Stage 1, 1,274 cases were classified as aneuploidy, including 1,078 euploidy pregnancies (FPR 6.4%), and 15,829 cases were classified as euploidy, including 15,820 (99.9%) which were truly euploidy and 9 of the O.C.A. The detection rate of the euploidy of SVMs with kernel 1 and 2 in Stage 1 is 93.4% and 92.2% and for the aneuploidies 73.2% and 72.68%, respectively. The k-NN classified correctly 91.7% of the euploidy and 74.2 of the aneuploidies.

The TPR and TNR for the networks trained with “euploidy & T21,” “euploidy & T18,” and “euploidy & Turner” are shown in Figs. 8–10 for the three evaluation datasets, labeled as fold 1, fold 2, and fold 3. We present the values of the TPR and TNR for different values of the threshold that were used to quantize the outputs of the networks and classify instances into the desired classes.

We tested several values of the threshold in the range between 0 and 1 with a step of 0.1. The first plot in Fig. 8 shows the results of the network “euploidy & T21.” The TPR for the T21 aneuploidies is plotted with black continuous line and with black dashed line the TNR for the first evaluation dataset (fold 1). This network was used individually to construct system 1, while it is also used as a subpart in system 2.

The TPR reaches maximum rate 100.0% at a 0.48 threshold. The FPR at the maximum TPR is 3.9%. The results of the second and the third evaluation datasets are shown in the next two plots, respectively, in Fig. 8.

The results of the network “euploid & T18” for the three validation datasets are shown in Fig. 9. The TPR of the trisomy 18 cases reaches maximum rate 89.8% at an FPR of 2.6% and threshold 0.98 for the first evaluation dataset. The TPR of the

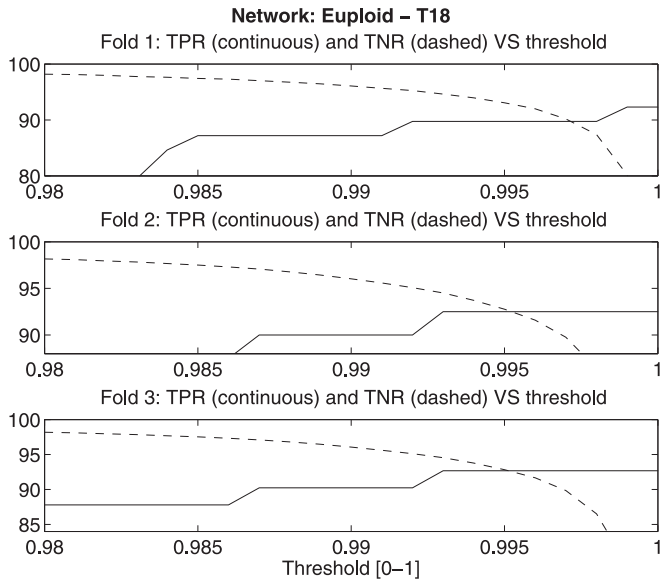


Fig. 9. TPR (filled line) and TNR (dashed line) for the network “euploidy & T18.” The three plots show the results for the three validation sets (folds 1, 2, and 3).

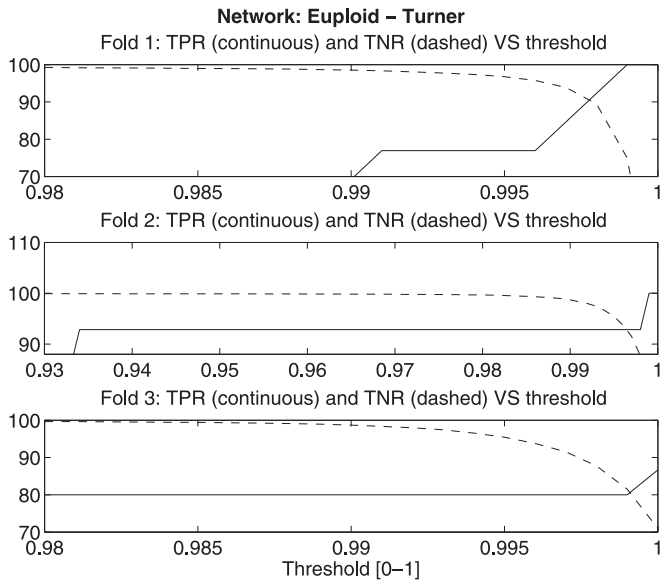


Fig. 10. TPR (filled line) and TNR (dashed line) for the network “euploidy & Turner.” The three plots show the results for the three validation sets (folds 1, 2, and 3).

second and third evaluation dataset at around 3% FPR is 92.5% and 90.2%, respectively.

The results of the network “euploidy & Turner” for the three validation datasets are shown in Fig. 10. The TPR for the first evaluation dataset of the Turner cases reaches maximum rate 76.9% at an FPR of 1.3% and threshold 0.98. The TPR of the second evaluation dataset reaches maximum rate 100.0% at an FPR of 1.7%. The TPR of the third evaluation dataset reaches maximum rate 66.7% at an FPR of 3.0%.

2) *Classification Into T21 or O.C.A.*: The 1,274 cases classified as aneuploidy in Stage 1 were examined by the 9-

TABLE IX

ACCURACY AND THE MATTHEWS CORRELATION COEFFICIENT OF THE ANN, SVM AND K-NN OF SYSTEM 2, STAGE 2 FOR THE FIRST VALIDATION DATASET. SYSTEM 2 IN STAGE 2 WAS TRAINED WITH T21 AND O.C.A. IT WAS VALIDATED FOR THE ENTIRE DATABASE INCLUDING EUPLOIDY, T21 AND O.C.A.

System 2	Accuracy	MCC
ANN	0.94	0.88
SVM 1	0.64	0.13
SVM 2	0.64	0.11
k-NN	0.51	-0.05

TABLE X

DETECTION RATES OF THE ANN OF THE SYSTEM 2 IN STAGE 2. SYSTEM 2 IS CONSISTED BY ONE NEURAL NETWORK BUILT WITH T21 AND O.C.A.

System 2 stage 2	Euploidy	T21	O.C.A.
Fold 1	93.7%	100.0%	84.2%
Fold 2	96.7%	90.0%	57.9%
Fold 3	96.2%	87.8%	44.3%

input model “T21 & O.C.A.” in Stage 2 (Fig. 7). The output values for all cases of T21 were 0 to 0.1, whereas the values of the O.C.A. were mostly distributed near to 1. In 64 (95.5%) of the 67 O.C.A., the output was more than 0.1, and therefore, three of these cases were wrongly classified as T21. SVMs with kernel 1 and kernel 2 classified 98.5% and 97.7% of the T21 and 6.6% of the O.C.A. k-NN classified 62.0% of the T21 and 32.9% of the O.C.A.

The accuracy and MCC of the ANN, SVM, and k-NN for the System 2 in Stage 2 for the first fold validation are summarized in Table IX. The detection rates of the first, second, and the third validation sets for the ANN are summarized in Table X. It is shown from Table IX that only ANNs were able to separate T21 from the O.C.A., while SVM and k-NN failed.

#### IV. DISCUSSION

The findings of our studies demonstrate the value of ANN schemes in the prediction of T21 and O.C.A. from ultrasonographic and biochemical markers at 11–13 weeks of gestation.

A multitude of ANN structures, training procedures, and evaluation strategies have been tried. In this study, we used multi-layer feedforward neural systems because these are proved to be the most suitable from the point of view of satisfactory generalization and diagnostic yield for such predictive systems. This was confirmed empirically by the authors after running several ANN models with different structures and parameters and observing their performance. The various multilayer networks of neurons were built and adjusted according to a set of parameters for each case of either euploidy or aneuploidy fetus in order to maximize the correct identification of each group. We have carried out a comparative study by using other classification techniques such as the SVMs and the k-NNs. The higher accuracy on the classification of euploidy, T21, and O.C.A. was achieved with neural networks structures.

In the traditional approach to first trimester screening for T21, the *a priori* risk is multiplied with the LR of each sonographic and biochemical marker. Algorithms combining 6 parameters, including MA, previous history of aneuploidy, delta NT, serum-free  $\beta$ -hCG MoM, and PAPP-A, MoM have been successfully applied to screening for T21 achieving a detection rate of about 90.0%, at an FPR of 5.0% [4]. In specialist fetal medicine centers, the performance of screening can be improved further, with an increase in detection rate to about 95.0% and a decrease in FPR to less than 3.0%, by the inclusion the additional sonographic markers of presence or absence of the fetal nasal bone and normal or abnormal blood flow across the tricuspid valve and in the ductus venosus [4].

In this study, we initially attempted to develop a supervised ANN systems with 6 outputs: one each for the euploidy pregnancies and the five chromosomal abnormalities. However, this was unsuccessful because the cases of T21 were separable, but when the O.C.A. were in the system, this separation was destructed, and most of the aneuploidies were classified as euploidy.

Subsequently, we concentrated our efforts at developing neural network models with the intention of separating euploidy from T21 pregnancies. One model utilized 6 neurons representing the basic parameters of MA, previous history, and the other 9 neurons representing the parameters shown in Table V columns 1 and 2 respectively. A large database consisting of 33,619 euploidy and 279 T21 pregnancies was used to train the systems, which were then used for testing the totally unknown database which included 16,898 euploidy and 129 T21 and 76 OCA pregnancies. The 6-input network correctly identified 92.3% of the cases of T21 at an FPR of 3.9%, and the 9-input network detected all cases of T21 at an FPR of 3.9%.

The 6-input and 9-input “euploidy & T21” models recognized that 63.2% and 27.6%, respectively, of the aneuploidies other than T21 were different from euploidy pregnancies. Subsequently, various attempts were made to improve the detection rate of the O.C.A. by building neural networks that were trained to separate euploidy from each type of aneuploidy. The performance of neural networks attempting to separate trisomy 13 and triploid from euploidy pregnancies was low, and these models were abandoned. A two-stage approach involving four neural networks was then used to achieve the best overall performance. In Stage 1, each case was assessed independently by three models (“euploidy & T21,” “euploidy & trisomy 18,” and “euploidy & Turner syndrome”). This two-stage approach correctly identified all cases of T21 and 84.2% of the O.C.A. but at an overall FPR of 6.4%.

Like every methodology, ANN and the computational intelligence approach have relative advantages and disadvantages. Some of the significant advantages, when compared to the above methodologies, the proposed approach are as follows.

- 1) Every case is seen by the system as a string of parameter values and are thus processed and assessed simultaneously. At the same time, data related to the fetus are seen and assessed together with data that are collected from the mother.
- 2) Each new case that has been observed during pregnancy can become a new definite case once the child delivery

takes place. This new case can add to the acumen of the existing knowledge base by simply running the learning algorithm once the new case enters the database.

- 3) In addition to the above, advantages such as fault tolerance, generalization handling, missing data handling, learning, and inference mechanisms, are advantages inherited from computational intelligence.

At present, most medical centers providing first trimester screening for T21 measure fetal NT and CRL and maternal serum free  $\beta$ -hCG and PAPP-A. In such centers, the use of the proposed combined 6-input system could correctly identify as aneuploidy about 93.0% of the cases of T21 and 63.0% of those with O.C.A., at an FPR of 3.9%. This performance of screening compares favorably with the 90.0% detection rate of T21, at an FPR of 5.0%, achieved by the traditional algorithms for screening [4]. Nevertheless, in all cases classified by the neural network as being suspicious of T21, invasive testing by chorionic villus sampling or amniocentesis would still be necessary to distinguish between the euploidy and aneuploidy pregnancies and in the diagnosis of the exact type of aneuploidy.

In fetal medicine centers, with expertise in assessing the fetal nasal bone and Doppler flow across the tricuspid valve and in the ductus venosus in addition to the measurements of fetal NT and CRL and maternal serum free  $\beta$ -hCG and PAPP-A, there are two options on the use of ANNs. The first is to use a 9-input “euploidy & T21” network which could correctly identify as aneuploidy all cases of T21 and 27.6% of those with other major aneuploidies, at an FPR of 3.9%. Alternatively, a two-stage approach involving four neural networks can be used, which could also correctly identify as aneuploidy all cases of T21 and 84% of those with O.C.A., but at an increased FPR of 6.4%. Since in all cases classified by the neural networks as being suspicious of T21, invasive testing would be necessary to distinguish between the euploidy and aneuploidy pregnancies, it is likely that the first option, with a substantially lower FPR, would be preferred by the parents and would also be more cost effective. Although the second option identifies more of the O.C.A., unlike T21, these conditions are highly lethal either in utero or in the neonatal period, and they are associated with abnormalities that can be easily detected by ultrasonography. These include holoprosencephaly, exomphalos and megacystis in trisomies 18 and 13 [33], large cystic hygromas in Turner syndrome [34], and either an enlarged partially molar placenta or small placenta but severely growth restricted fetus with pronounced wasting of the body and sparing of the head in triploidy. Since the prevalence of these defects is less than 0.1%, the effect on the overall proportion of pregnancies requiring an invasive test would be minimal.

## V. CONCLUSION

We have presented a noninvasive prenatal diagnosis of chromosomal abnormalities in the first trimester of the pregnancy. The collection of the database took several years, and it covers a wide area of population such as age range, ethnicity, information whether the mother is alcoholic, drug addicted, cases with

previous history. The population of the cases in our database ensures statistical confidence of our results, compared to databases used in similar work of other groups. We believe that medical diagnosis systems that classify instances based on statistical methods should provide representative databases with a convincing number of population. Also, the results should be presented with cross validation, ensuring their robustness. The most important, however, is the fact that our system identifies and predicts O.C.A. than T21, such as trisomy 18, trisomy 13, Turner syndrome, and Triploidy. The diagnosis is done in the presence of a pregnant woman with a computer system in the doctor's office by basically using routine examination data within a negligible time and with low financial cost.

We achieved with ANN a 100.0% detection rate of T21 with FPR of 3.9% and 84.2% of the O.C.A. with an FPR of 6.4%. We note that these results do not yield perfect classification, neither accuracy of 1 since there is still an FPR of 6.4%. We have repeated the same experiments with SVM and k-NN, and it was experimentally concluded that the best results for this problem would be achieved with the ANN. More precisely, SVM and k-NN yield similar results with lower accuracy than the proposed ANN structure of both systems 1 and 2. It is also prominent that both SVM and k-NN were not able to distinguish the T21 from the O.C.A.

For future work, we need to emphasize our investigations on exploring other neural network schemes such as recurrent networks and the possibility of using parameters from the father. Preliminary results show that it is worth investigating the substitution of the parameter values in MoMs with the actual raw values. It is also worth mentioning that the maternal cell-free DNA screening method does not use any information from the mother or the father; such as the age. This important information should be included in their methodology and the analysis of their results.

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#### REFERENCES

- [1] B. Brambati, G. Simoni, C. Danesino, A. Oldrini, E. Ferrazzi, L. Romitti, G. Terzoli, F. Rossella, M. Ferrari, and M. Fraccaro, "First trimester fetal diagnosis of genetic disorders: Clinical evaluation of 250 cases," *J. Med. Genet.*, vol. 22, pp. 92–99, Apr. 1985.
- [2] A. Tabor and Z. Alfirevic, "Update on procedure-related risks for prenatal diagnosis techniques," *Fetal Diagn. Ther.*, vol. 27, no. 1, Jan. 2010.
- [3] K. Sundberg, J. Bang, S. Smidt-Jensen, V. Brocks, C. Lundsteen, J. Parner, N. Keiding, and J. Philip, "Randomised study of risk of fetal loss related to early amniocentesis versus chorionic villus sampling," *Lancet*, vol. 350, no. 9079, pp. 697–703, Sep. 1997.
- [4] K. H. Nicolaides, "Screening for fetal aneuploidies at 11 to 13 weeks," *Prenat. Diag.*, vol. 31, no. 2, pp. 7–15, Jan. 2011.
- [5] K. Spencer, "Aneuploidy screening in the first trimester," *A. J. Med. Genet. Part. C Sem. Med. Gen.*, vol. 145C, pp. 18–32, Feb. 2007.
- [6] L. Dugoff, "First-and second-trimester maternal serum markers for aneuploidy and adverse obstetric outcomes," *A. Coll. Obst. Gynecol.*, vol. 115, no. 5, pp. 1052–1061, May 2010.
- [7] K. H. Nicolaides, K. Spencer, K. Avgidou, S. Faiola, and O. Falcon, "Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: Results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening," *Ultrasound Obstet. Gynecol.* vol. 25, no. 3, pp. 221–226, Mar. 2005.
- [8] T. Reynolds and M. Penney "The mathematical basis of multivariate risk analysis: With special reference to screening for down syndrome associated pregnancy," *Ann. Clin. Biochem.*, vol. 27, pp. 452–458, Sep. 1990.
- [9] G. Schmorl, *Pathologisch-Anatomische Untersuchungen Uber Puerperal-Eklampsie*. Leipzig, Germany: Verlag FCW Vogel, 1893.
- [10] D. Bianchi, A. Flint, M. Pizzimenti, J. Knoll, and S. Latt, "Isolation of fetal DNA from nucleated erythrocytes in maternal blood," *Proc. Natl. Acad. Sci. USA*, vol. 87, pp. 3279–3283, May 1990.
- [11] M. Ehrlich, C. Deciu, and T. Zwiefelhofer, "Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: A study in a clinical setting," *Am. J. Obstet. Gynecol.*, vol. 204, pp 205–211, Mar. 2011.
- [12] R. W. Chiu, R. Akolekar, Y. W. Zheng, T. Y. Leung, H. Sun, K. C. Chan, F. M. Lun, A. T. Go, E. T. Lau, W. W. To, W. C. Leung, R. Y. Tang, S. K. Au-Yeung, H. Lam, Y. Y. Kung, X. Zhang, J. M. van Vugt, R. Minekawa, M. H. Tang, J. Wang, C. B. Oudejans, T. K. Lau, K. H. Nicolaides, and Y. M. Lo, "Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: Large scale validity study," *Brit. Med. J.*, vol. 342, p. c7401, Jan. 2011.
- [13] E. Papageorgiou, A. Karagrigoriou, E. Tsaliki, V. Velissariou, N. Carter, and F. Patsalis, "Fetal-Specific DNA methylation ratio permits noninvasive prenatal diagnosis of trisomy 21," *Nat. Med.*, vol. 17, pp. 510–513, Mar. 2011.
- [14] C. Myers, M. Dunham, S. Kung, and O. Troyanskaya, "Accurate detection of aneuploidies in array CGH and gene expression microarray data," *Bioinformatics*, vol. 20, no. 18, pp. 3533–3543, Jul. 2004.
- [15] G. Ashoor, A. Syngelaki, M. Wagner, C. Birdir, and K. Nicolaides, "Chromosome-Selective sequencing of maternal plasma cell free dna for first-trimester detection of trisomy 21 and trisomy 18," *Am. J. Obstet. Gynecol.*, vol. 206, no. 4, pp. 322–326, Jan. 2012.
- [16] G. Palomaki, E. Kloza, G. L. Messerlian, J. Haddow, L. Neveux, M. Ehrlich, D. Boom, A. Bombard, C. Deciu, W. Grody, S. Nelson, and J. Canick, "DNA sequencing of maternal plasma to detect down syndrome: An international clinical validation study," *Genet. Med.*, vol. 13, no. 11, pp. 913–920, Nov. 2011.
- [17] J. Patel and R. Goyal, "Applications of artificial neural networks in medical science," *Curr. Clin. Pharmacol.*, vol. 2, no. 3, pp 217–226, Sep. 2007.
- [18] F. Schnorrenberg, C. Pattichis, K. Kyriacou, and C. Schizas, "Computer-aided detection of breast cancer nuclei," *IEEE Trans. Inf. Technol. Biomed.*, vol. 1, no. 2, pp. 128–140, Jun. 1997.
- [19] C. Schizas and C. Pattichis, "Learning systems in biosignal analysis," *BioSystems*, vol. 41, no. 2, pp. 105–125, Jan. 1997.
- [20] C. Neocleous, K. Nicolaides, K. Neokleous, and C. Schizas, "Artificial neural networks for non-invasive chromosomal abnormality screening of fetuses," in *Proc. Int. Joint Conf. Neural Netw.*, 2010, pp. 1–4.
- [21] Q. Al-Shayea, "Artificial neural networks in medical diagnosis," *Int. J. Comput. Sci. Issues*, vol. 8, no. 2, pp. 150–154, Mar. 2011.
- [22] Y. Hayashia and R. Setiono, "Combining neural network predictions for medical diagnosis," *Comput. Biol. Med.*, vol. 32, no. 4, pp. 237–246, Jul. 2002.
- [23] O. Er, N. Yumusak, and F. Temurtas, "Chest diseases diagnosis using artificial neural network," *Expert Syst. Appl.*, vol. 37, no. 12, pp. 7648–7655, Dec. 2010.
- [24] I. Khan, P. Zope, and S. Suralkar, "Importance of artificial neural network in medical diagnosis disease like acute nephritis disease and heart disease," *Int. J. Eng. Sci. Innovative Technol.*, vol. 2, no. 2, pp. 210–217, Mar. 2013.
- [25] A. Maithili, K. Kumari, and S. Rajamanickam, "Neural networks towards medical diagnosis," *Int. J. Modern Eng. Res.*, vol. 1, no. 1, pp. 57–64, Sep./Oct. 2011.
- [26] C. Stephan, H. Cammann, H. Meyer, C. Muller, S. Deger, M. Lein, and K. Jung, "An artificial neural network for five different assay systems of

prostate-specific antigen in prostate cancer diagnostics," *Brit. J. Urology*, vol. 102, no. 7, pp. 799–805, Jun. 2008.

- [27] P. Werbos, "Beyond regression: New tools for prediction and analysis in the behavioral sciences," Ph.D. dissertation, Dept. Appl. Math., Harvard Univ., Cambridge, MA, USA, Nov. 1974.
- [28] H. Robinson and J. Fleming, "A critical evaluation of sonar crown rump length measurements," *Brit. J. Obstet. Gynaecol.*, vol. 82, no. 9, pp. 702–710, Sep. 1975.
- [29] D. Wright, K. Kagan, F. Molina, A. Gazzoni, and K. Nicolaides, "A mixture model of nuchal translucency thickness in screening for chromosomal defects," *Ultr. Obstet. Gynecol.*, vol. 31, no. 4, pp. 376–383, Apr. 2008.
- [30] A. Kolmogorov, *Grundbegriffe der Wahrscheinlichkeitsrechnung*. Berlin, Germany: Springer, 1933.
- [31] F. Wilcoxon, "Individual comparisons by ranking methods," *Biomet. Bull.*, vol. 1, no. 6, pp. 80–83, Dec. 1945.
- [32] B. W. Matthews, "Comparison of the predicted and observed secondary structure of t4 phage lysozyme," *Biochimica et Biophysica Acta (BBA) Protein Struct.*, vol. 405, no. 2, pp. 442–451, 1975.
- [33] K. O. Kagan, I. Staboulidou, A. Syngelaki, J. Cruz, and K. H. Nicolaides, "The 11-13-Week scan: Diagnosis and outcome of holoprosencephaly, exomphalos and megacystis," *Ultr. Obstet. Gynecol.*, vol. 36, no. 1, pp. 10–14, Jul. 2010.
- [34] K. Kagan, D. Wright, K. Spencer, F. Molina, and K. Nicolaides, "First-Trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: Impact of maternal and pregnancy characteristics," *Ultr. Obstet. Gynecol.*, vol. 31, no. 5, pp. 493–502, May 2008.



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