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Marker Selection for the Detection of Trisomy 21 Using Generalized Matrix Learning Vector Quantization

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Abstract—In this work we explore the relevance of markers that are used for the early detection of fetal chromosomal abnormalities. For medical applications, it is important to optimize the number of used markers with respect to the number of necessary clinical examinations. We use the Generalized Matrix Learning Vector Quantization (GMLVQ) method to identify the most relevant markers from a set of 18 clinical examinations. We cross-validated our results using ten different training and test sets and we repeated our experiments using different parameters of GMLVQ. We identified the seven most relevant markers and we found that with these seven markers we obtain results that are comparable with the results that can be achieved with the full set of 18 markers. The results are in line with previous work that is found in the literature.

I. INTRODUCTION

The early detection of fetal chromosomal abnormalities in the first trimester of pregnancy can prevent several pregnancy or social complications. In the literature, it is reported that women carrying a fetus with chromosomal abnormality have increased risk of miscarriage [1]. Additionally, fetuses with trisomies 13 (T13) and 18 (T18), triploidy and Turner syndrome usually die during pregnancy. It is important to identify such abnormalities in an early stage of the pregnancy in order to take action if it is necessary.

The most accurate methods for the identification of fetal chromosomal abnormalities are the Chorionic Villus Sampling (CVS) and the amniocentesis tests. Both methods return the result with almost 100% confidence, however they are invasive and there is a danger of harming the fetus, or causing infections to the mother and increased risk of miscarriage. [2].

For the estimation of the risk of the trisomy 21 (T21), more widely known as Down syndrome, a standard non-invasive pre-natal examination is performed to pregnant women between the 11th and the 13th week of pregnancy. Information about the fetus is extracted from an ultrasonographic examination. For instance, some important markers that have significant discriminative power are the presence or absence of the nasal bone (NB), the physical distance between the crown and the rump of the fetus (CRL), and the nuchal translucency (NT) that is the size of the translucent space behind the neck of the fetus. From the maternal blood, the serum free β -hCG and the pregnancy associated plasma protein-A (PAPP-A) are measured. The statistical significance of the β -hCG and the PAPP-A values is explained in [3]–[5]. Other information is recorded regarding the maternal age, weight, ethnicity, if the mother is a smoker and others. Two additional markers that are related to the cardiovascular system of the fetus, the ductus venosus flow and the tricuspid flow, are captured by a specialized ultrasonographic examination using Doppler techniques [6], [7]. However, the full examination is not performed to all pregnant women due to the fact that special equipment is required.

Currently, hospitals and private doctors that are approved by the Fetal Medicine Foundation to perform such pre-natal examinations, estimate the risk of T21 by a sequential screening as suggested by Snijders and Nicolaides in [8]. For every binary marker mentioned above, a likelihood ratio is defined as the ratio between the chromosomally abnormal fetuses and the normal fetuses. For the markers with graded values, such as the PAPP-A, the β -hCG and the NT, the values are split into a number of ranges creating categories, and the likelihood ratio

is calculated for each range. All likelihood ratios are multiplied with the maternal age related risk to yield a final result.

Another non-invasive method for the detection of T21 and other chromosomal abnormalities such as T13 and T18 is the *Cell-Free Fetal DNA Test* [9]–[12]. The fetal DNA is partially isolated from the maternal blood via double centrifugation. Then, further processing on the sequences of the fetal DNA is carried out to detect chromosomal abnormalities. This method has high accuracy but also some drawbacks, such as increased cost of the test, the necessity of special equipment and a delay in the delivery of the result. Therefore, it is practical to first make the above mentioned non-invasive tests and estimate the risk of aneuploidy and to refer only the high risk cases to further examinations such as the *Cell-Free Fetal DNA Test* or the amniocentesis test.

In this paper, we use Generalized Matrix Learning Vector Quantization (GMLVQ) [13] to explore the relevance of every marker in our dataset. The GMLVQ is a prototype based classification method that uses distances between data points and prototypes for classification. Moreover, the distance metric that leads to optimal classification results is determined in the course of classifier training. Its elements can be interpreted to infer which markers are most effective. We applied GMLVQ to the entire set of 18 markers and we identified the seven markers that are most effective for classification. This information is of practical interest because it allows to reduce the number of tests needed.

The rest of the paper is organized as follow: in Section II we explain our dataset, we give an overview of our methodology and we explain in short the GMLVQ method. In Section III we provide our results and in Section IV we analyze and discuss the findings of this study. Finally, we draw conclusions in Section V.

II. METHODS

A. Data

The dataset we use consists of 50517 euploid and 408 T21 cases. The data have been collected by the Fetal Medicine Centre at King's College Hospital and University College London Hospital in London. In Table I we list the 18 markers that are included in the dataset. The information whether a woman had a previous pregnancy with trisomy is abbreviated as “previous T21, T13 or T18”. The markers conception, smoking, parity, ethnicity and weight concern the mother. The parity is defined as the number of times that the mother has given birth to a fetus with a gestational age of 24 weeks or more. The ethnicity includes the classes “white”, “black”, “south Asian” and “mixed”. The ethnicity is a categorical feature and it could be considered irrelevant for the GMLVQ since it does not contribute to the distance computation, and therefore, could be removed.

B. Adaptive distance measures

1) *Learning vector quantization*: The Learning Vector Quantization (LVQ) method was initially proposed by Kohonen and Somervuo [14]. It has been used for various

Fig. 1. The pipeline of our proposed method. We first apply a Z-score normalization to the individual features, then we train the GMLVQ algorithm ten times using a different randomized training set. We repeated our experiments for different number of prototypes in each class.

TABLE I
THE 18 MARKERS THAT ARE AVAILABLE IN THE USED DATASET.

Marker #	Name
1	Gestational age in days
2	Maternal age
3	Previous T21
4	Previous T13
5	Previous T18
6	Crown rump length
7	Nuchal translucency
8	Fetal heart rate
9	Nasal bone
10	Tricuspid flow
11	Ductus venosus flow
12	Conception
13	Smoking
14	Parity
15	Ethnicity
16	Weight
17	PAPP-A
18	β -hCG

problems [15]–[18]. The K prototypes are represented as vectors $w^1, w^2, \dots, w^K$ with $w^j \in \mathbb{R}^N$ (N - number of features) together with their associated labels according to the given classes $S^1, S^2, \dots, S^K$ with $S^j \in \{1, 2, \dots, C\}$. In the training procedure, all training cases are “seen” by the algorithm one by one. In one iteration, the following processes are performed: From the dataset, a single example ξ^t is chosen randomly and the distances between the sample and all prototype vectors are calculated. The minimum distance is identified as shown in Eq. (1), and the corresponding “winner” prototype is updated using Eq. (2). The winner prototype w^{i*} is moved towards the data point if the function $\psi(S^{i*}, \sigma^t)$ returns 1 which indicates that the prototype is labeled with

the same class as the data point. If they belong to different classes, the prototype is moved in the opposite direction of the data point. The step size that the prototype is moved is proportional to the distance between the prototype and the data point, multiplied by a factor $\eta_w < 1$ which is called the “learning rate”. One epoch is considered when all the training examples are “seen” by the LVQ algorithm. The stopping criterium can be for instance a target training error or a pre-defined number of epochs. In the classification mode, a test case ξ^t is assigned to the class of the closest prototype.

$$w^{i*} = \underset{j}{\operatorname{argmin}} \{d[w, \xi^t]\} \quad (1)$$

$$w^{i*} = w^{i*} + \eta_w \psi(S^{i*}, \sigma^t)(\xi^t - w^{i*})$$

$$\psi(S, \sigma) = \begin{cases} +1 & \text{if } S = \sigma \\ -1 & \text{if } \text{else} \end{cases} \quad (2)$$

2) *Generalized matrix learning vector quantization:* The training in the GMLVQ [19] is done in a similar way as the LVQ algorithm by optimizing a cost function [13] which is related to the classification performance and is based on a generalized quadratic distance measure of the form:

$$d^\Lambda(w, \xi) = (\xi - w)^T \Lambda (\xi - w) \quad (3)$$

Here, Λ is a full $N \times N$ matrix and accounts for the interplay of the dimensions. The similarity measure of Eq. (3) results in a meaningful distance only if Λ is positive semi-definite and symmetric. This can be achieved by substituting $\Lambda = \Omega^T \Omega$ where Ω is an arbitrary real $N \times N$ matrix. The update equations for the elements of Ω are obtained by computing the derivatives of the cost function given in [13] with respect to w and Ω .

3) *Canonical representation:* In the training process, GMLVQ can yield different transformation matrices Ω e.g. as a result of different initialisations of Ω . A given matrix Λ can be parameterized by many different Ω as there is no unique solution to $\Lambda = \Omega^T \Omega$. A unique transformation $\hat{\Omega}$ can be defined in a canonical way as follows:

$$\hat{\Omega} = ([\sqrt{\lambda_1}v_1, \sqrt{\lambda_2}v_2, \dots, \sqrt{\lambda_N}v_N])^T \in \mathbb{R}^{N \times N} \quad (4)$$

where v_i and λ_i , $i = 1 \dots N$, denote the eigenvectors and eigenvalues of Λ respectively. Each row of the matrix $\hat{\Omega}$ is a row-eigenvector of the matrix Λ that defines a transformed feature as a linear combination of the original features. The eigenvector of Λ corresponding to its largest, dominating eigenvalue, represents the leading contribution of original features to the distance measure. Detailed information about the mathematical explanation of GMLVQ can be found in [13], [20].

We explore the relevance of the markers in our dataset as follows: We use the values of the markers listed in Table I to form a feature vector for each individual case. As shown in Fig. 1, we first split the data into a training and a test set

and then we apply a Z-score transformation based on training set statistics. We use the function *nFoldCrossValidation* from the GMLVQ MATLAB toolbox¹ that is freely available, to select randomly 500 euploid and 370 (90%) of the T21 cases to form the training set. The remaining cases are used as a test set. Then, we choose the number of prototypes for each class and finally we train the classifier.

III. RESULTS

We present our results in Figs. 2, 3, 4 and Table II. For performance evaluation, we use the true positive rate (TPR), the false positive rate (FPR) and the Matthews correlation coefficient (MCC). A true positive (TP) is observed when a true T21 case is identified as such by GMLVQ. Similarly, a true negative (TN) is observed when an euploid case is assigned to the negative class correctly. A classification of a T21 case as euploid is considered a false negative (FN) while a false positive (FP) assignment occurs when an euploid case is classified as T21. As a single quality measure computed from TN, FN, TP, and FP we employ MCC:

$$MCC = \frac{TP \cdot TN - FP \cdot FN}{\sqrt{(TP+FP)(TP+FN)(TN+FP)(TN+FN)}} \quad (5)$$

which is frequently used for highly imbalanced class populations. A value of -1 represents a maximum error of classification while a value of 1 indicates perfect classification. A value of 0 corresponds to random classification. The statistics of the MCC values across many runs of the method for different initialisations and training/test sets is shown in Fig. 2 using bar plots.

Fig. 2 displays the results of the GMLVQ classifier for 16 combinations of different numbers of prototypes that we used. The labels on the X-axis represent the number of prototypes for the two classes. In each combination of prototypes, we repeat the learning procedure 10 times by choosing randomly different training sets. For instance, the label [1 - 3] indicates that there is one prototype in class euploid and three prototypes in class T21. We achieved the best results with the combination [1 - 2] which indicates one prototype in the euploid class and two prototypes in the T21 class. However, the differences between the first 4 cases are not statistically significant and for simplicity, results discussed in the following correspond to the configuration of [1 - 1] prototypes.

In Fig. 3 we present the eigenvalues of the matrix Λ after a number of training epochs that is sufficient to allow one of the eigenvalues to become much larger than all other eigenvalues. The corresponding leading eigenvector, is displayed in Figure 4. The inner product of this eigenvector with a feature vector defines the transformed feature that has the by far largest contribution in the distance computation. Thus the elements of the leading eigenvector with the largest absolute values are the most important ones for determining the distance and can be interpreted as relevances of the corresponding markers. These are markers #2, #7, #9, #10, #11, #17 and #18. In Table II

¹<http://matlabserver.cs.rug.nl/gmlvqweb/web/>

Fig. 2. MCC statistics over 10 randomly selected training sets for 16 combinations of numbers of prototypes. The first number in the labels in the x-axis indicate the number of prototypes that are defined in class 1 (euploid) and the second, the number of prototypes in class 2 (T21).

Fig. 3. The eigenvalues of the matrix Λ as obtained in the system with the full panel of markers.

we compare the results on the test set for the two cases that GMLVQ was trained with the full set of 18 markers and with the seven most relevant markers. In the last row of Table II we also show the results of the classifier for an arbitrary selection of seven markers (#1, #6, #8, #12, #13, #14 and #15).

IV. DISCUSSION

In practice one aims at the identification of all positive cases (100% TPR) with the lowest FPR possible. According to the literature different methods achieve a TPR for T21 between 90% and 100% at an FPR 3% - 5%, while GMLVQ achieves a TPR of 90.5% at a FPR of 2.3%. It is shown in Table II that

Fig. 4. Leading eigenvector of Λ for the system with 1 prototype in class 1 and 1 prototype in class 2. The seven most relevant markers are β -hCG (#18), nuchal translucency (#7), PAPP-A (#17), nasal bone (#9), ductus venosus flow (#11), tricuspid flow (#10) and maternal age (#2).

TABLE II

RESULTS OF THE GMLVQ TRAINED WITH THE ENTIRE MARKER SET, THE PROPOSED 7 MARKER SET (#2, #7, #9, #10, #11, #17 AND #18) AND A RANDOM SELECTION OF 7 MARKERS (#1, #6, #8, #12, #13, #14 AND #15).

	TPR (%)	FPR (%)	MCC
18 Markers	90.5	2.3	16.5
7 suggested Markers	80	0.8	24.7
7 random Markers	50	1.5	11.3

the model trained with the proposed seven markers achieves lower TPR and lower FPR than the one that was trained with the full marker set. However, to make a fair comparison, we need to compare the TPR at the same FPR of 2.3%. In other words, if we increase the FPR from 0.8% to 2.3%, then the TPR will reach higher score than 90.5%. As shown in the last row of Table II, the case where the classifier was trained using a random selection of seven markers achieves a TPR of only 50%. In future studies, more sophisticated frameworks for performance evaluation like AUC or Precision-recall should be employed.

The observations reported in this paper are in line with previous research by Nicolaides et. al. [21], [22] which suggest using the suggested seven markers in two stages. In the first stage, a standard pre-natal examination is performed, and the markers #2, #7, #17 and #18 are extracted. The cases from stage 1 that are ranked as high risk are suggested to proceed further with stage 2 where three additional markers, the #9, #10 and #11 are recorded.

V. CONCLUSIONS

Using GMLVQ we showed that the seven (out of 18) most relevant markers for the detection of trisomy 21 are: maternal

age, tricuspid flow, ductus venosus flow, nasal bone, PAPP-A, nuchal translucency and β -hCG. The GMLVQ relevance profile of the markers verifies our previous suggestions for the significance of these markers [23], [24]. What differentiates this work from [23], [24] is that here we use a computational method to come to our conclusions in contrast with the heuristic approach used in other previous work.

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REFERENCES

- [1] R. J. M. Snijders, K. Sundberg, W. Holzgreve, G. Henry, and K. H. Nicolaides, "Maternal age and gestation-specific risk for trisomy 21," *Ultrasound Obstet Gynecol*, vol. 13, p. 167170, December 1999.
- [2] C. Enzensberger, C. Pulvermacher, J. Degenhardt, A. Kawacki, U. Gerner, U. Gembruch, M. Krapp, J. Weichert, and R. Axt-Flidner, "Fetal loss rate and associated risk factors after amniocentesis, chorionic villus sampling and fetal blood sampling," *Ultraschall in der Medizin (Stuttgart, Germany : 1980)*, vol. 33, no. 7, p. E759, December 2012.
- [3] K. Kagan, D. Wright, A. Baker, D. Sahota, and K. Nicolaides, "Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-a," *Ultrasound in Obstetrics & Gynecology*, vol. 31, no. 6, pp. 618–624, 2008.
- [4] 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," *Ultrasound in Obstetrics & Gynecology*, vol. 31, no. 5, pp. 493–502, 2008.
- [5] K. Spencer, V. Souter, N. Tul, R. Snijders, and K. Nicolaides, "A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free β -human chorionic gonadotropin and pregnancy-associated plasma protein-a," *Ultrasound in obstetrics & gynecology*, vol. 13, no. 4, pp. 231–237, 1999.
- [6] N. Maiz, C. Valencia, K. Kagan, D. Wright, and K. Nicolaides, "Ductus venosus doppler in screening for trisomies 21, 18 and 13 and turner syndrome at 11–13 weeks of gestation," *Ultrasound in Obstetrics & Gynecology*, vol. 33, no. 5, pp. 512–517, 2009.
- [7] I. Huggon, D. DeFigueiredo, and L. Allan, "Tricuspid regurgitation in the diagnosis of chromosomal anomalies in the fetus at 11–14 weeks of gestation," *Heart*, vol. 89, no. 9, pp. 1071–1073, 2003.
- [8] R. Snijders and K. Nicolaides, "Sequential screening," *Nicolaides KH, editor. Ultrasound markers for fetal chromosomal defects.*, vol. 44, no. 9, pp. 109–113, 1996.
- [9] G. Ashoor, A. Syngelaki, M. Wagner, C. Birdir, and K. H. Nicolaides, "Chromosome-selective sequencing of maternal plasma cell-free dna for first-trimester detection of trisomy 21 and trisomy 18," *American journal of obstetrics and gynecology*, vol. 206, no. 4, pp. 322–e1, 2012.
- [10] E. A. Papageorgiou, A. Karagrigoriou, E. Tsaliki, V. Velissariou, N. P. Carter, and P. C. Patsalis, "Fetal-specific DNA methylation ratio permits noninvasive prenatal diagnosis of trisomy 21," *Nature medicine*, vol. 17, no. 4, pp. 510–513, 2011.
- [11] H. Zhang, Y. Gao, F. Jiang, M. Fu, Y. Yuan, Y. Guo, Z. Zhu, M. Lin, Q. Liu, Z. Tian *et al.*, "Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146 958 pregnancies," *Ultrasound in Obstetrics & Gynecology*, vol. 45, no. 5, pp. 530–538, 2015.
- [12] T. Sahoo, S. W. Cheung, P. Ward, S. Darilek, A. Patel, D. Del Gaudio, S. H. L. Kang, S. R. Lalani, J. Li, S. Mcadoo *et al.*, "Prenatal diagnosis of chromosomal abnormalities using array-based comparative genomic hybridization," *Genetics in Medicine*, vol. 8, no. 11, pp. 719–727, 2006.
- [13] P. Schneider, M. Biehl, and B. Hammer, "Adaptive relevance matrices in learning vector quantization," *Neural Computation*, vol. 21, no. 12, pp. 3532–3561, 2009.
- [14] T. Kohonen and P. Somervuo, "Self-organizing maps of symbol strings," *Neurocomputing*, vol. 21, no. 1, pp. 19–30, 1998.
- [15] E. Mwebaze and M. Biehl, "Prototype-based classification for image analysis and its application to crop disease diagnosis," in *Advances in Self-Organizing Maps and Learning Vector Quantization*. Springer, 2016, pp. 329–339.
- [16] D. Mudali, M. Biehl, K. L. Leenders, and J. B. Roerdink, "LVQ and SVM classification of FDG-PET brain data," in *Advances in Self-Organizing Maps and Learning Vector Quantization*. Springer, 2016, pp. 205–215.
- [17] S.-D. Wang and C.-J. Lee, "Fingerprint recognition using directional micropattern histograms and lvq networks," in *Information Intelligence and Systems, 1999. Proceedings. 1999 International Conference on*. IEEE, 1999, pp. 300–303.
- [18] P. Hájek and V. Olej, "Air quality modelling by kohonens self-organizing feature maps and lvq neural networks," *WSEAS transactions on environment and development*, vol. 4, no. 1, pp. 45–55, 2008.
- [19] M. Biehl, B. Hammer, and T. Villmann, "Distance measures for prototype based classification," in *International Workshop on Brain-Inspired Computing*. Springer, 2013, pp. 100–116.
- [20] M. Kaden, M. Lange, D. Nebel, M. Riedel, T. Geweniger, and T. Villmann, "Aspects in classification learning-review of recent developments in learning vector quantization," *Foundations of Computing and Decision Sciences*, vol. 39, no. 2, pp. 79–105, 2014.
- [21] K. H. Nicolaides, A. Syngelaki, G. Ashoor, C. Birdir, and G. Touzet, "Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population," *American journal of obstetrics and gynecology*, vol. 207, no. 5, pp. 374–e1, 2012.
- [22] K. 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 in obstetrics & gynecology*, vol. 25, no. 3, pp. 221–226, 2005.
- [23] A. C. Neocleous, K. H. Nicolaides, and C. N. Schizas, "First trimester non-invasive prenatal diagnosis: a computational intelligence approach," *IEEE Journal of Biomedical and Health Informatics*, vol. 20, 2015.
- [24] A. C. Neocleous, K. H. Nicolaides, and C. N. Schizas, "Intelligent non-invasive diagnosis of aneuploidy: Raw values and highly imbalanced dataset," *IEEE Journal of Biomedical and Health Informatics*, vol. 20, 2016.