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# **PhD THESIS**

## **SUMMARY**

**EVALUATION STRATEGIES OF GENETIC  
PATHOLOGY CORRELATED TO TECHNOLOGIC  
AND INFORMATIC PROGRESS**

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**Timișoara  
2019**

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## INTRODUCTION

A genetic disorder is any pathology caused by an abnormality in an individual's genome, from large-scale chromosomal changes to point mutations (variants).

Genetic disorders can be caused by a pathogenic or probably pathogenic variant in one gene (Mendelian inherited monogenic disorders, present since conception), in multiple genes (polygenic or multifactorial inheritance disorder), by a combination of gene variants and environmental factors (acquired mutations in a gene or group of genes that randomly occur during a person's life or due to some toxic or irradiant environmental exposure), by chromosomal changes (ex. number or structure of entire chromosome, the structure that carry genes; copy number losses (or microdeletions) and copy number gains (or microduplication syndromes), or uniparental disomies (UPD)) or by mitochondrial inheritance (4).

Around 3-4% of all born individuals are affected by congenital or early onset disorders which generally generate chronic disabilities with critical influence on the lives of affected people and their families and also on the health-care system. Despite outstanding advances in policies, technology and bioinformatics, the burden of genetic rare diseases is spread worldwide, raising specific issues in relation to their rarity. There are now around 8,000 such gene-related disorders catalogued in the OMIM (9), Orphanet (10) and DECIPHER (11) databases. For about 5856 of these disorders an associated gene has been discovered, of which 3,573 are characterized as clinically actionable to some degree (10,12). Rare disorders represent a broad and heterogeneous group, but with wide phenotypic spectrum, therefore rare diseases will represent the first direction this work will address to, a particular focus being offered to epidemiological and diagnosis aspects in western Romania and to the state of the art in rare genetic diseases in Romania.

On the other hand, a particular direction of the thesis will be the approach of a multifactorial disease, type 2 diabetes mellitus. Despite global efforts in medicine and research for the prevention of T2DM, it was estimated a total number of 422 million adults living with diabetes in 2014 (90% with T2DM), compared to 108 million in 1980, reflecting an increase in associated risk factors such as obesity, and environmental and lifestyle factors (21,22). As multifactorial disorder with critical increasing prevalence, and because the genetic testing to confirm a predisposition to develop T2DM is not possible at the actual knowledge, T2DM should benefit from a rigorous preventive approach and tools to achieve into the decrease of modifiable risk factors as overweight and obesity.

As already shown, the visceral fat accumulation or percent body fat (VFA) contribute to adipose tissue dysfunction and T2DM (24–26). VFA can represent a simple measurable index by bioelectrical impedance analysis (BIA), even if this technology's utility in medical practice is controversial. Maybe this is the rationale for not taking into consideration that VFA can be a reliable item in assessing the risk for developing T2DM. FINDRISC score is one of the most used risk-scoring algorithms for T2DM in many countries around Europe and beyond and it comprises 8 items: age, BMI, waist circumference measured below ribs, daily physical activity, the frequency of eating vegetables, fruit or berries, frequency of taking medication for high blood pressure, history of hyperglycemia, familial history of diabetes (type 1 or type 2) (27,28). Our study aims to determine whether body fat percentage association with FINDRISC score leads to a better prediction of type 2 diabetes mellitus.

## **RESEARCH QUESTIONS AND SPECIFIC OBJECTIVES**

As commented above in statement of the problem, 2 main research direction emerge:

- I. Evaluation strategies of genetic pathology correlated to technologic and informatic progress in Romania
- II. Risk assessment of type 2 diabetes mellitus as a multifactorial disorder with a complex genetic component

The specific objectives to sustain the main research directions are the following:

- I.1. To determine the addressability of patients and their geographical provenience to a Regional Center of Medical Genetics in Romania during almost 4 years of activity
- I.2. To contribute to epidemiological data on genetic disorders in Romania by describing the cohort of patients presenting with a suspicioned diagnostic
- I.3. To estimate the prevalence of different categories of genetic disorders from the entire cohort of patients according to the new ICD-11 for Mortality and Morbidity Statistics
- I.4. To provide the diagnostic yield of genetic testing in Timis Regional Center of Medical Genetics together with Center for Genomic Medicine in the University of Medicine and Pharmacy "Victor Babes" Timisoara
- I.5. To propose evaluation strategies of genetic disorders correlated to technologic and informatic progress, applicable in Romania



- I.6. To summarize Romania's position regarding the implementation of the key needs of the RD community, from the point of view of Timis Regional Center of Medical Genetics and of the Center for Genomic Medicine in the University of Medicine and Pharmacy "Victor Babes" Timisoara
- II.1. To contribute to epidemiological data on overweight and obesity in young healthy Romanian population, as an important risk factor to develop type 2 diabetes mellitus
- II.2. To provide the landscape of FINDRISC score application to a young cohort of Romanian healthy individuals
- II.3. To determine whether body fat percentage association with FINDRISC score leads to a better prediction of type 2 diabetes mellitus

## **EVALUATION STRATEGIES OF GENETIC PATHOLOGY CORRELATED TO TECHNOLOGIC AND INFORMATIC PROGRESS**

### ***MATERIAL AND METHODS***

The retrospective cohort study assessed 1038 patients referred for genetic evaluation to Timis Regional Center of Medical Genetics (RCMGT), affiliated to "Louis Turcanu" Emergency Hospital for Children, between 2015 and November 2018.

All referred patients were included into the assessment group, but not also into the data analysis because of insufficient information.

Comprehensive clinical assessment data was collected for each individual, as requested in the medical genetics consultation chart of RCMGT including: patient demographics and general information, family history of diseases, data about the antenatal and perinatal period, personal physiological history, symptoms and pathological medical history, clinical findings in physical examination, documentation of relevant investigation results, medication, other information.

Patients presenting with dysmorphic features were asked to fill in a consent to allow photographs in order to facilitate diagnosis.

Investigation plan for each patient is personalized, following one of the five possible scenarios:

- 1) recommendation of additional tests and expert evaluations needed before genetic testing to sustain the suspicioned diagnosis,

- 2) when presenting with a specific phenotype for a genetic disease that may be confirmed by genetic testing, patients are asked to fill in the informed consent for genetic testing and a biological sample is taken,
- 3) when a genetic test is not available for the moment, patient's DNA may be stored for further research, with informed consent,
- 4) necessity of clinical genetics reevaluation in a defined period of time if suspected a disorder but with no sufficient features for undergoing the diagnosis process,
- 5) a genetic disease is excluded after comprehensive evaluation.

Patients underwent specific tests chosen by the clinical geneticist. Genetic testing services in Romania are commissioned and delivered in line with current national policy, free of charge for both children and adults enrolled in the National Program of Health of Women and Child, Subprogram VI.3 Prevention of congenital malformations by pre and postnatal diagnosis (69). Genetic testing was performed at the Center for Genomic Medicine from "Victor Babes" University of Medicine and Pharmacy of Timisoara, POSCCE Project ID: 1854, cod SMIS: 48749, contract 677/09.04.2015 (classic karyotype; FISH (10 specific regions), PCR (50 variants Single nucleotide base change), Fragile X Syndrome; SNP array (molecular karyotype); next generation sequencing (NGS) panels: TruSight Cardio (174 genes) and TruSight One panel (4813 genes)). Tests that were not available in our Center, were performed in collaboration with other Romanian Regional Centers for Medical Genetics (Dolj, Iasi, Bucuresti, Cluj).

A standard written informed consent was signed by children parents/guardians or by the patients if over 18 years old.

If a diagnosis was confirmed, the patient or his parent/guardians were asked to present for another consultation in the outpatient clinic to be informed about the global management of the disease, possible treatment approaches, complications prevention, about the initial needed clinical work-up and regular follow-up and for genetic counselling.

Descriptive statistics for this retrospective cohort study included all individuals who had a genetic consultation in RCMGT and was performed using IBM SPSS Statistics v23. Descriptive statistics were run on selected group variables and presented as percentages and means.

Prevalence of different categories of genetic diseases was calculated from the cohort of patients and according to ICD-11 for Mortality and Morbidity Statistics (ICD-11 MMS), version 2018(99).

Diagnostics yields (positive predictive value for different genetic tests) were calculated as the proportion of positive findings in each test for all tested patients for that specific test.

## ***RESULTS AND DISCUSSIONS***

RCMGT local patient's registry includes almost 1000 unique patients who received a genetic consultation in the last 4 years of activity, having a tripled number of patients in 2018, compared to 2015. Patients from whole Romania presented for genetic consultation, the majority from the 4 assigned counties (TM-45%, AR-11.8%, CS-9.9%, HD-7.7%), but also 6.2% of Mehedinti County and 19.5% of other 30 Romanian counties. Although RCMGT serves inhabitants from 4 counties, 25.6% of the addressed patients are from the rest of the country. Considering the number of patients having a diagnostic suspicion of a genetic disorder, for the 4 assigned counties the prevalence of genetic disorders was estimated at 0.0436%, and for Timis county at 0.0668%. This fact demonstrates a national improvement of access to information for both specialists and patients, and also of the medical services concerning rare genetic disorders.

Regarding the form of presentation in the Clinics, 429 unique patients were admitted into the Medical Genetics Clinical Department, 411 were seen in the outpatient clinic, 140 were referred for consultation by other hospital units and 58 patients were addressed from Bucharest, Cluj-Napoca, Craiova, Iasi and Oradea hospital units, with a complete clinical work-up, for Next generation sequencing only.

60% of patients are established in urban areas, while 40% in rural Romanian areas. 467 individuals were examined from Timis county, 58% coming from urban areas and 42% from villages and communes.

Increasing number of patients were evaluated in RCMGT in the 4 years of activity 2015-2018. In 2018, the number of new unique patients receiving a genetic consultation per month was in average 35.

It was estimated a very low prevalence of population affected by genetic disorders comparing to international epidemiological data, certainly due to underdiagnosed individuals and to the aggregation in our Center of a small number of patients presenting developmental malformations and/or intellectual disability because of our ERN-ITHACA membership. For instance, oncological field and, unfortunately some others are not covered by our expertise yet. Our cohort is dominated by male patients and urban area establishment, distributions maintained higher in all further characteristics. Male predominance could be due to a higher number of patients with intellectual disability in males due to X-linked mental retardation syndromes. Concerning age at first presentation for diagnosis, children and adolescents were the majority, most from the 1 to 7 years

old subgroup (32%), followed by 7 to 14 years old subgroup and infants, but also 16% adult patients. These late presentations sustain the “diagnostic odyssey” widely recognized in the field of rare diseases, together to increased morbidity rate, imposing for earlier referring to specialists (17,18).

The most frequent were chromosomal anomalies, including micro-deletion/duplication syndromes (203 patients, also with trisomy 21), followed by conditions with disorders of intellectual development as a relevant clinical feature (195 patients), multiple developmental anomalies or syndromes (179 patients), and unspecified developmental anomalies (172 patients). 5.5% of patients were referred having a suspicion of genetic hypertrophic cardiomyopathy, most of them at an adult age, and this high percentage is due to a close corroboration of RCGMT with the Center of expertise for rare diseases in the field of rare cardiovascular diseases from the Cardiology Section III, structure of the Emergency Institute for Cardiovascular Diseases “Prof. C.C. Iliescu” Bucharest. We also analyzed the prevalence of some specific diseases in our cohort of patients. Down syndrome had a prevalence of 7.7% in our cohort, and one of 39.8% in the group of chromosomal anomalies. Down syndrome was followed by Fragile X syndrome, Noonan and Marfan syndrome.

In our cohort what we firstly observed was a consistent decrease in karyotyping over years and increase of diagnostic yields (17.9% in 2016 and 26.4% in 2018), as SNP array offers better chances in diagnosing incomplete chromosomal deletions and duplications. Patients with intellectual disability, with or without malformations, had a diagnostic yield of 20.5% by SNP array analysis, compared to literature (8-12% (103)). As for NGS panels, molecular diagnostic yields were high for both Cardio and extended “Clinical exome” panels compared to literature (11.3% (104), 26% (105)), showing also an appropriate clinical assessment in guiding investigation. Novel disease-associated variants were also discovered (data not detailed in this work), needing supplementary investigations to be confirmed and to establish a better phenotype and management strategy for these patients.

High diagnostic yield show a good phenotype-genotype correlation both in clinics and laboratory, an accuracy at the bench and a systematic interpretation of data, according to current research trends. These numbers offer more certainty and courage when working with rare genetic disorders. Also, they give to Romanian patients, similar chances for a diagnostic at home, and not abroad. Currently, RCGMT dispose for Whole Exome Sequencing for wider pathology coverage, but the lack in human and budget resources is coordinating the test allocation/patient. Whole Genome Sequencing should improve the rate

of diagnostic in patients for whom all previous genetic testing was not conclusive.

Evaluation strategy for each pathology and for each clinical case, especially, is particular: a clear phenotype allows rapid diagnosis suspicion, but unspecific ones require a thorough approach and further clinical investigations work-up in collaboration with different specialists, such as pediatricians, cardiologists, neurologists, metabolic specialists, nephrologists, gastroenterologists, endocrinologists, immunologists, oncologists, and others.

For some cases, repeated clinical/ dysmorphology and developmental assessments over time are more informative than one-off assessments in planning investigations and management. Also, online resources and access to them is an important tool for difficult phenotyping (106). Also, as discussing about rare diseases, even after exhaustion of all available genetic tests, we may meet unsolved genetic diagnostic.

Future priorities for RCMGT are to shorten the turnaround time by supplementing human and financial resources, to extend the tests offered to whole genome sequencing (WGS), as the whole exome sequencing (WES) has just been added to our list and to improve research pipelines in rare disease in collaboration with ERN ITHACA.

It is important to take into account several limitation of the study. The geographical area for the studied population was restricted and results cannot be generalized for the entire country, but nevertheless, this could be the start for a national wide study.

Testing for all patients addressing the center was not performed due to limited funding. Priority was given to patients with diseases, and not to check carrier status unless needed. For these patients, clinical diagnosis criteria and further evaluation remained a possibility.

Also, as WES was not performed to any of these patients prior to this study, the percentage of yet undiagnosed disorders was higher than the present one.

Limitations with national resonance are related to the national networks that do not function as proposed, yet: each county should have at least a contact person for genetics field, at least until a geneticist would have his place in the county hospital. Nowadays, only 1 county has a geneticist apart from the university centers. The linkage with our study is that we did not have Romanian terms of comparison concerning a Center's approach and cohort of patients, many patients are mistreated for different other diseases than their real cause of health issues and also that patients

across the country come to RCGMT or one of the other five Regional Genetic Centers for diagnosis and management.

## **CONCLUSIONS**

Despite outstanding advances in policies, technology and bioinformatic, the burden of rare diseases is spread worldwide, raising specific issues in relation to their rarity. Nowadays, thorough clinical assessment is no longer the only available tool for diagnosis, but it is crucial in guiding towards different genetic investigations, restricting our focus to a specific organ, system or phenotype component.

In our cohort, it was estimated a very low prevalence of population affected by genetic disorders comparing to international epidemiological data, fact certainly due to underdiagnosed individuals and to the aggregation in our Center of a small number of patients presenting developmental malformations and/or intellectual disability because of our ERN-ITHACA membership. The highest prevalence estimated for our cohort of patients was for unspecified developmental anomalies, followed by chromosomal anomalies, including microdeletion/microduplication syndromes, conditions with intellectual development as a relevant clinical feature, multiple developmental anomalies or syndromes and neuromuscular disorders.

RCMGT was successful to reach a diagnosis (sometimes using more than one type of test/per patient), with higher yields compared to those in literature, however with longer turnaround time due to limited human and financial resources.

Further improvements are needed to bring forward the health care strategies for patients with genetic rare diseases in Romania, ultimately for improving their quality of life. Currently, RCGMT dispose for Whole Exome Sequencing for wider pathology coverage, but the lack in human and budget resources is coordinating the test allocation/patient. Whole Genome Sequencing should improve the rate of diagnostic in patients for whom all previous genetic testing was not conclusive.

# **RISK ASSESSEMENT OF TYPE 2 DIABETES MELLITUS AS A MULTIFACTORIAL DISORDER WITH A COMPLEX GENETIC COMPONENT**

## ***MATERIALS AND METHOD***

This cross sectional study performed in 2016, carried out within the Cardiology Department/Preventive Medicine and Cardiovascular Rehabilitation, Angiogenesis Research Center, Victor Babes University of Medicine and Pharmacy from Timisoara, enrolled 341 young healthy medical voluntary students from “Victor Babes” University of Medicine and Pharmacy, Timisoara, Romania, who agreed to join the study and gave written informed consent. All procedures were approved by “Victor Babes” University ethics committee and complied with Declaration of Helsinki.

Exclusion criteria were represented by pregnant participants, those who had a history of major surgery on their extremities, malignancies, chronic kidney disease stage IV or renal replacement therapy, liver cirrhosis with ascites, heart failure with peripheral edema, or severe hypothyroidism, fever resulting from an active infection or inflammation, those receiving systemic steroid treatment, those suffering severe dehydration and those having chronic medication (e.g. statins, diuretics, and other medication that might affect water distribution in body). As there are contraindications related to the measurement of the body percent fat and combination with other medical devices (pacemaker, portable electrocardiograph, etc), for every individual measured these aspects were carefully checked.

Anthropometric measurements were performed by a single examiner. Weight, height, waist circumference and hip circumference were measured with footwear removed and in light clothing, using the same devices. Waist circumference was measured at the midpoint between the iliac crest and the rib cage. BMI was calculated as weight (kg) divided by height squared ( $m^2$ ) and WHR as waist measurement divided by hip measurement ( $W \div H$ ) (125). All measurements fulfilled quality control criteria.

Abdominal VFA was measured using a tetrapolar multifrequency BIA (Bioelectrical Impedance Analysis) machine (InBody720®) for each individual. The device uses 1, 5, 50, 250, 500 kHz, and 1 MHz frequencies to analyze intracellular and extracellular fluid values and water content. Three consecutive readings were obtained for each individual with the average of the 3 used for statistical analysis. The most frequently used cutoff points for PBF defining

overweight (20.1–24.9% for men and 30.1–34.9% for women) and obesity ( $\geq 25\%$  for men and  $\geq 35\%$  for women) were applied (128,129).

Participants were asked to fill in the FINDRISC Score assessment questionnaire after all items were explained. The items (8) were the classic ones from FINDRISC T2DM risk assessment form: age, BMI, waist circumference measured below ribs, daily physical activity, the frequency of eating vegetables, fruit or berries, frequency of taking medication for high blood pressure, history of hyperglycemia, familial history of diabetes (type 1 or type 2). The final score is the sum of the scores from 8 questions and ranges from 0 to 26. The interpretation of the assessment form was performed after cumulating the total number of points corresponding to each item, FINDRISC score being considered as a continuous and categorical variable, as following:

- Lower than 7: Low- estimated 1 in 100 will develop disease
- 7–11: Slightly elevated- estimated 1 in 25 will develop disease
- 12–14: Moderate- estimated 1 in 6 will develop disease
- 15–20: High- estimated 1 in 3 will develop disease
- Higher than 20: Very high- estimated 1 in 2 will develop disease (27).

Statistical analysis was performed using IBM SPSS Statistics 23 program and a two-tailed p value  $< 0.05$  was considered significant. To describe the cohort, data was tested for normal distribution. Results were compared between females and males using independent samples t- test. The expected value was calculated and a cut-off point of 5 was considered. Spearman's correlation coefficients were applied to establish the correlations between variables.

## ***RESULTS AND DISCUSSIONS***

A total of 341 healthy medical students, adults, 143 females and 198 males, aged between 18 to 44 years old were recruited into the study.

The variables of the anthropometric measures did not have normal distribution and were presented using median and quartiles. There were no differences between males and females in mean ages (20 years old). 27.6% of the entire cohort was determined as being overweighed and 12% obese (significantly lower prevalence when compared to the global prevalence).

Sex distribution was the following: 13.9% of the female's group presented overweight, and 7% obesity, while 37.4% of the males presented overweight, and 15.7% obesity. The median BMI was 25.18 kg/m<sup>2</sup> for males and 21.04 kg/m<sup>2</sup> for females, p-value 0.002. Generally, men had also a larger WHR: the calculated median WHR for males was 0.86 while for females 0.79, p-value 0.015. Women had higher PBF (29% compared to 20.9%). A research in



Romanian population subgroup 20-39 years old, published in 2016, found a prevalence of 27.20% for overweight and 20.90% for obesity overall. Males had a prevalence of overweight at 40.20% and of obesity at 20.70%, and overweight in females was lower at 14.80%, but obesity higher at 21.10%. Our results were similar to the other Romanian young cohort just regarding male and female overweight, but lower regarding obesity. These differences can be however explained by the extension of the age-group to 39 years old and by the higher number of subjects (131).

The FINDRISC score had an average of 5.05 for the whole cohort; 76.2% of the students have a low risk (estimated 1 in 100 will develop disease), 18.8% have a slightly elevated risk (estimated 1 in 25 will develop disease), 2.9% have a moderate risk (estimated 1 in 6 will develop disease), 1.8% have a high risk (estimated 1 in 3 will develop disease) and 0.3% have a very high risk for developing T2DM in the following 10 years (estimated 1 in 2 will develop disease), according to the assessment form criteria. Individuals found with moderate and high risk were advised to measure fasting blood glucose and for subsequent follow-up.

We also attributed FINDRISC score to BMI and we could show that the number of individuals with slightly elevated risk to develop T2DM was quite equally distributed among all categories of the nutritional status, excepting underweight. But if we take into consideration that a percent of 5.86% from the entire cohort, representing individuals with underweight and normal BMI, have an estimated risk of 1 in 25 will develop disease, this percent becomes quite important because it is addressed to the target population of this specific study. As regarding a moderate and high FINDRISC score among obese individuals, it was expected to dominate the picture (14 obese individuals from 18 with moderate and high FINDRISC score).

In a cross-sectional study conducted at Hashemite University in Zarqa from Jordan in 2014, it was reported a percentage of 66.9% students with low risk, 26.2% corresponding to a slightly elevated risk, 5.2% indicating a moderate risk and 1.8% at a high risk of diabetes. The minimum differences between our data and the compared study may be due to our relatively small cohort comparing to the one from Jordan, and also to ethnic particularities (133).

Therefore, this limitation in the applicability of FINDRISC score is debatable because of increasing prevalence of T2DM in the young. Also, the literature has not provided yet many studies regarding risk score noninvasive calculation in the young.

We analyzed the correlation between FINDRISC score and WTR for the entire cohort as control and we found a statistical high and positive correlation – 0.477,  $p < 0.001$  (WTR is an item of FINDRISC score). Further, we have analyzed the correlation between FINDRISC score and Percent Body Fat for the whole group of students and it was a statistical higher and positive correlation than the one with WTR (0.561,  $p < 0.001$ ). As for the subgroup of both sexes, the correlation between FINDRISC score and both WTR and PBF was direct, very strong and statistically significant ( $p < 0.001$ ). Interestingly, our study found a stronger correlation between FINDRISC score and PBF compared to FINDRISC score and WHR for the entire cohort, but also for both males and females.

One limitation is linked to the FINDRISC score's validation among individuals below 34 years old and above 64 years old. This issues from the primary study design of FINDRISC score which was applied to this range of ages and, even if used worldwide in various research works, it was not extended below (27,28,132).

Also, a limitation is that BIA measurement of PBF is still controversial, therefore these results may not value much for those thinking that it is not a reliable method of quantification.

An important limitation of this study from genetics point of view was that the initial goal was to propose candidate genes for T2DM for the cohort of patients, or to test those with a high FINDRISK score. This was not possible because of founding and time limitations.

## **CONCLUSIONS**

Considering the worldwide increasing prevalence of Type 2 Diabetes Mellitus, along with overweight and obesity, despite global efforts for its prevention, all additional measures which could improve results should be considered.

Epidemiological data in the analyzed young and healthy cohort show a lower prevalence of main modifiable risk factor, obesity, when compared to both national and international studies, for both sexes, but still double in males compared to females. Lower prevalence of overweight and obesity can be due to the 20 years old mean of age in our cohort compared to more extensive studies and it should not be taken as a favorable risk factor for not developing T2DM.

This study emphasizes on the reliability of body fat percentage measurement by simple bioelectrical impedance analysis when assessing

T2DM risk. Our outcomes support a significant correlation between FINDRISC assessment prediction model and PBF, even stronger than between FINDRISC and waist-to-hip ratio, one of its partial items. Thus, we recommend PBF measured by BIA (respecting quality control procedures) as a potential parameter to be considered into the risk model predictions for T2DM as it is an accessible and affordable tool to use in the primary level of healthcare and also because it may improve risk assessment in young population.

### ***CRITICAL ASSESSMENT OF OWN WORK***

This thesis provides main international and national approaches regarding genetic disorders. The corroboration of global standards in rare disorder's field to our current national "state of the art" guides for adjusted policies and stepwise strategy elaboration. As there is no other national paper or scientific publication to gather all key points regarding the comprehensive assessment of rare genetic disorders, the contribution brought by this work is important.

The author presents results regarding the prevalence of genetic diseases in Timis County and the group of 4 counties assigned to Timis Regional Center of Medical Genetics (TM, HD, CS, AR) and showing an apparent very low prevalence compared to the one presumed, type of data not reported before.

The author presents the prevalence of main genetic categories of diseases and also for some specific disorders, providing reference percentages with applicability on Romanian patients, and especially to the ones coming from one of the 4 assigned counties.

Concerning type 2 diabetes mellitus, as a multifactorial disorder with global and Romanian increasing of prevalence also in young population, our community is demanding efficacy in its prevention. The author applies FINDRISC assessment form to determine T2DM risk in a young healthy population and is the first to propose the percent body fat measured by bioelectrical impedance analysis as a potential parameter to be considered into the risk model predictions for T2DM as it is an accessible and affordable tool to use in the primary level of healthcare.

## **FURTHER WORK**

Further work to complete and improve results from presented thesis could look into extending the analysis of rare genetic diseases based on a more comprehensive database. Classification of disorders could be more in detail presented and applied for clear prevalence among a national extended studied population of patients. Also, we plan a data gathering from all Regional Genetic Centers and similar or more complex analysis which will provide more confident disorders' prevalence and with applicability for the entire country.

A questionnaire for the quality of life and one regarding the diagnosis odyssey and special needs are planned to be used in RCGMT starting next year, and this data will serve to a better care-giving to our patients.

This type of work is suitable for continuation into elaborating Guidelines for Genetic Tests and Diagnoses in Medical Practice.

Novel disease-associated variants, also present in our study population but not detailed in this work, needs supplementary investigations to be confirmed and to establish a better phenotype and management strategy for these patients.

Concerning multifactorial disorders, and type 2 diabetes mellitus in particular, a contribution to candidate gene findings from Romanian population along with measurement PBF also by other methods and including it into FINDRISC score could be further objectives to complete the present work.

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