BURDEN OF RARE GENETIC DISEASES –EXPERIENCE OF TIMIS REGIONAL CENTRE OF MEDICAL GENETICS, ROMANIA

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Abstract

Introduction. A rare disease is a low prevalence health condition, affecting a small number of people in general population, however raising specific issues in relation to their rarity. We aim to evaluate the diagnostic approach strategy in Timis Regional Centre of Medical Genetics (RCMGT), in collaboration with the Centre for Genomic Medicine in the University of Medicine and Pharmacy "Victor Babes" Timisoara. Material and method. Patients addressing the RCMGT have a comprehensive clinical assessment and different genetic testing, in accordance to their phenotype. Diagnostics yields were calculated for each test performed. Results. 1038 unique patients were evaluated in RCMGT between January 2015-November 2018, 567 males and 471 females, 74.3% from 4 surrounding counties and 25.6% from other counties. Most patients were between 1-7 years (32%), with median age 5.58 years (range 0–78). Most frequent disease suspicions were chromosomal anomalies, including micro-deletion/duplication syndromes (203 patients), followed by conditions with intellectual disability (195), and multiple developmental anomalies or syndromes (179). Diagnostic yield calculated for 820 patients addressing RCMGT was: karyotype-26.4%, SNP array (molecular karyotype)-20.5%, Next Generation Sequencing: Cardio panel-45% and"Clinical exome"-47%. 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. Conclusions. Rare diseases have become a health priority worldwide and in each community, aligning policies to improve strategies in the coordination of care, diagnosis and access to treatment in patients.

Keywords: Rare diseases, genetic testing, diagnosis, SNP array, NGS panel

Introduction

The state of the art in rare diseases around the world

A rare disease (RD), also named orphan disease, is a health condition with low prevalence affecting a small number of people in general population compared to common disorders, however raising specific issues in relation to their rarity [1,2]. Rare diseases term is relatively new, the first use of this term was introduced in relation to "orphan drugs" in the United States of America in the mid-1970s [3], and adopted by Europe and France between 1987-2000 [4,5,6]. The threshold that defines RD varies across countries worldwide, European Union accepting a prevalence inferior to 5 cases for every ten thousand inhabitants, USA 7.5 per 10.000 persons, Japan 1 per 2.500 and Russia and Australia 1 per 10.000 people [7, 8].

To date, there are approximately 6.000 to 8.000 described RD, with an estimation of 5 new entities every week, but 80% of patients are affected by approximately 350 RD [9]. Each affecting less than 0, 1% of the population, but collectively they affect a considerable proportion of the population in any country (between 6% and 10%), and about 350-400 million people around the world [11]. Data has shown that 80% of rare diseases have a genetic etiology, and the rest may occur as a result of viral or bacterial infections, allergies and other environmental causes [10]. Their clinical expression is different, persons having the same condition may express different signs and symptoms, or there may be subtypes of the same disease. Most of them appear at an early age and associate multiple disabilities (physical, sensorial or intellectual disability) being responsible for 35% of child mortality in children with less than one year of age, while others have a benign evolution and do not affect intellectual development, if diagnosed and treated at an early stage [11,12,13,14,15]. Some RD have onset in adulthood. This variety, along with their number, challenge healthcare practitioners and scientists alike in terms of being able to acquire experience of a given condition for the most appropriate and timely diagnosis and management [15].

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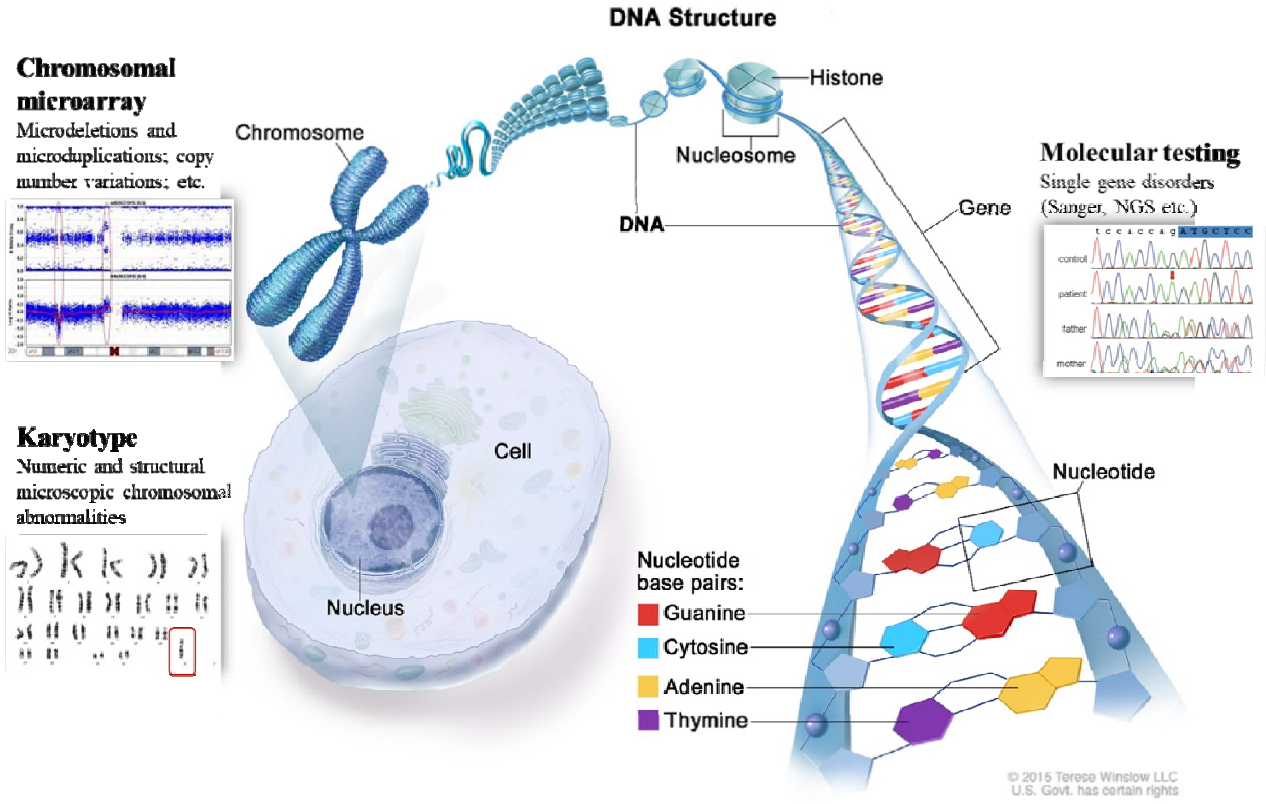

Fig. 1. Recommendations of different types of genetic testing based on clinical data of patients (adapted from: © 2015 Terese Winslow LLC, U.S. Govt. has certain rights)

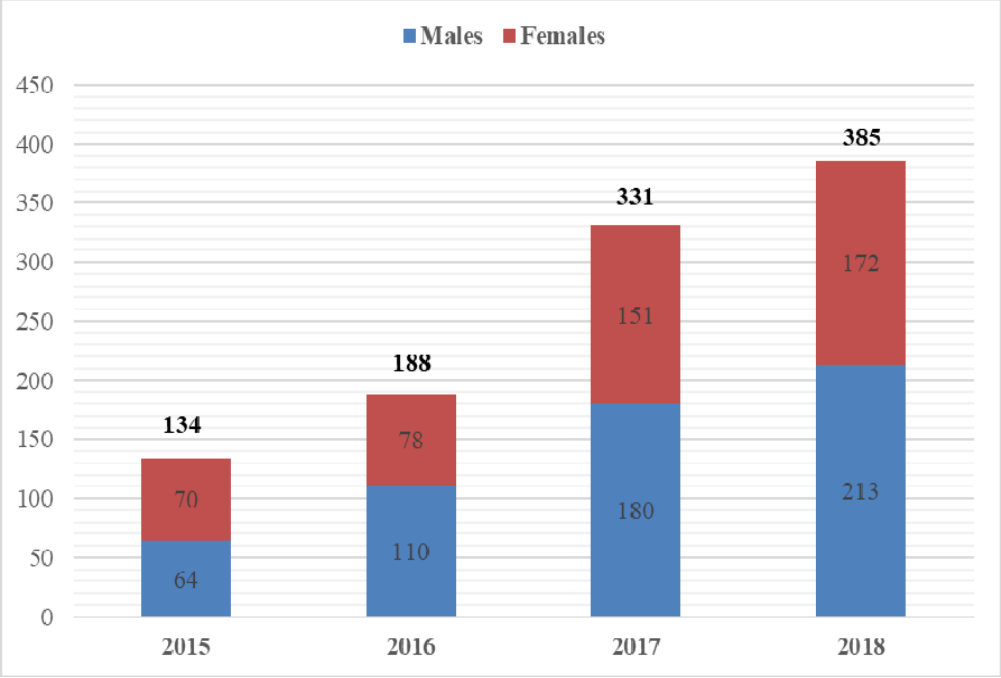

Fig. 2. Number of unique patients evaluated in RCMGT, distributed by the year of first presentation

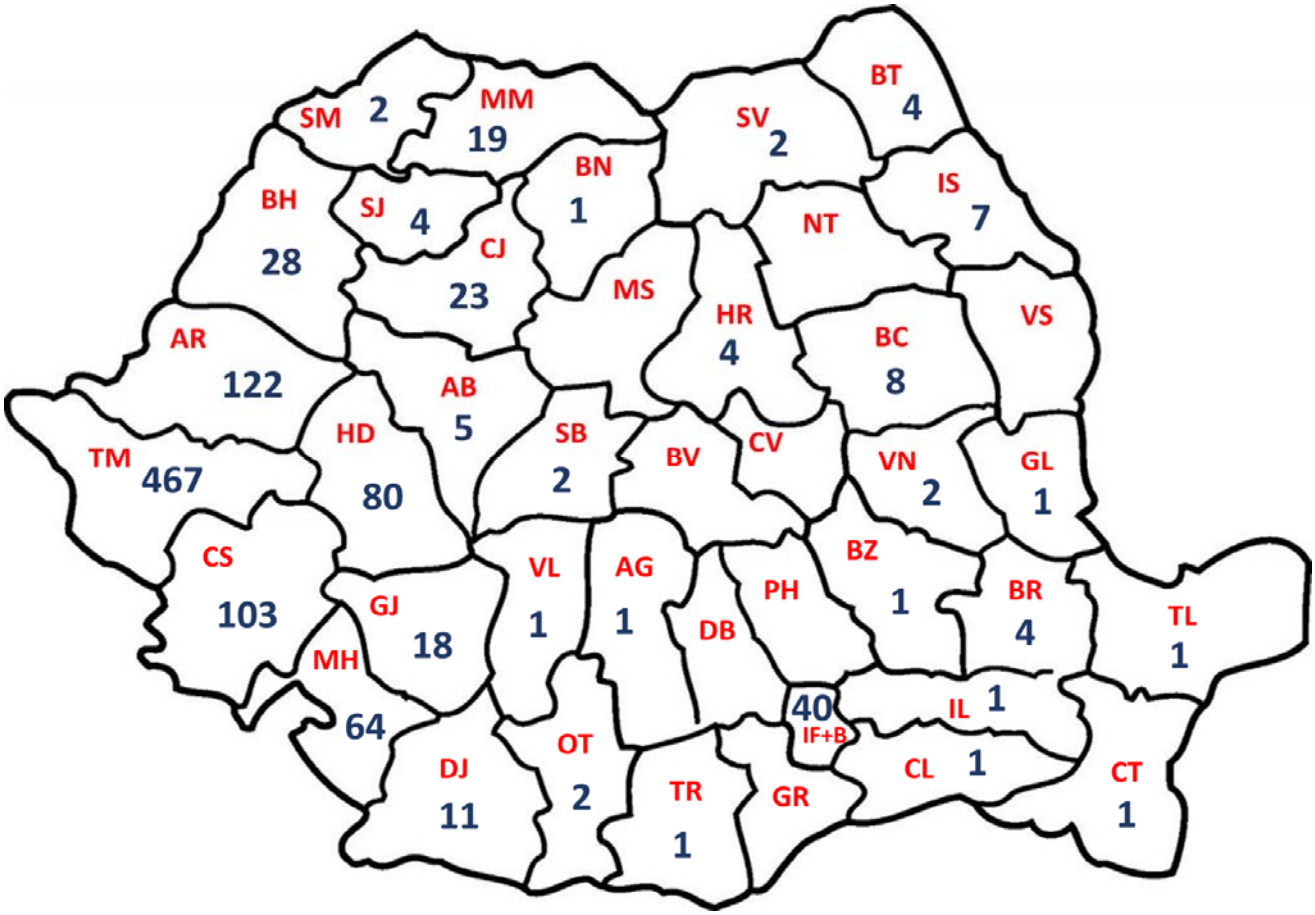

Fig. 3. Geographical repartition of our cohort of patients

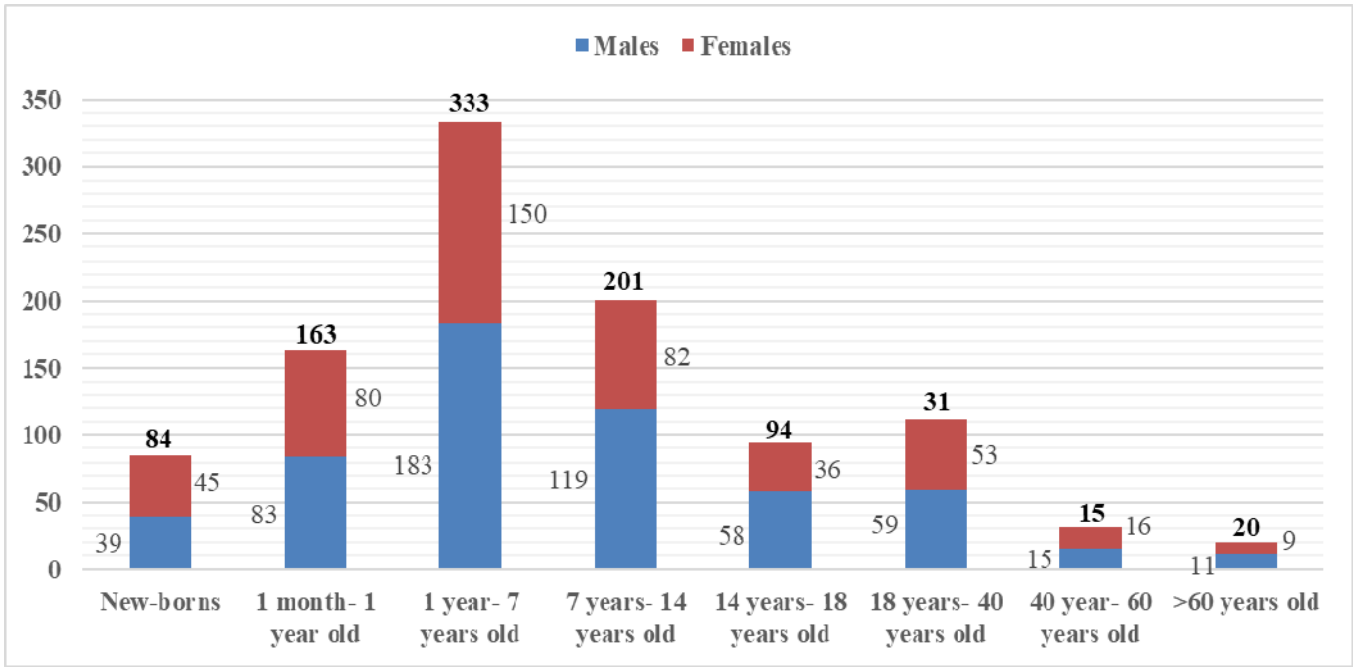

Fig. 4. Age repartition for both sexes in our patients

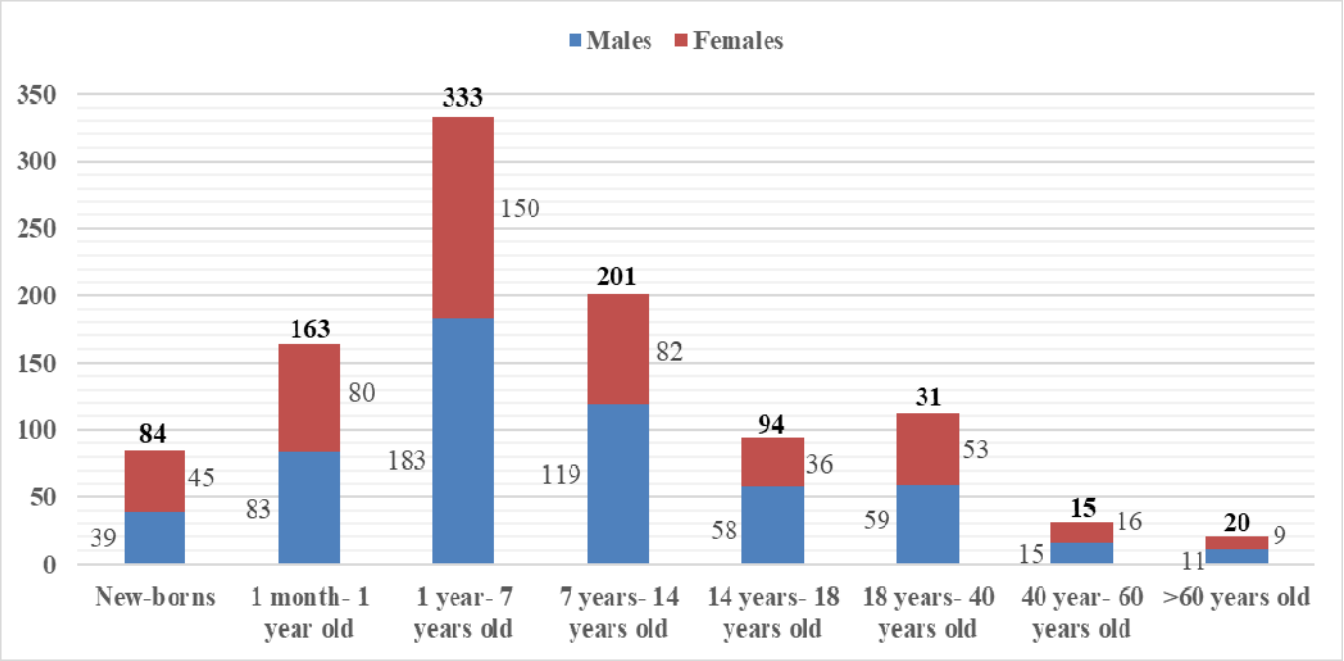

Fig. 4. Age repartition for both sexes in our patients

Genetic testing methods	Total number of tested patients/ test	Number of patients with pathogenic/ probably pathogenic variants/ findings	Diagnostic yield
Karyotype	242	64	26.4 %
SNP array	180	37	20.5 %
TruSightCardio Next Generation Sequencing panel	100	45	45 %
TruSightOne Next Generation Sequencing panel	92	51	47 %

Table 1. Diagnostic yields for each test

A patient's journey to diagnosis, also called "diagnostic odyssey", has a mean time of 7.6 years in the United States and 5.6 years in the United Kingdom and, during this period, patients receive several misdiagnoses after presenting to 8 physicians in average, inappropriate treatments or miss treatment opportunities, all these associating with increased morbidity and mortality [16, 17, 18]. Even more, rare diseases are further characterized by limited or non-existing treatment options (95% have no treatment option), lack of resources, significant disease burden and chronic course even when treatment is available. Healthcare systems and contributions of patient advocacy groups in moving forward implementation and inclusion of rare disease programs have diminished gaps across the policy landscape for different countries and are fighting the global burden of rare diseases by improving coordination of care, data registries, diagnostic resources, access to treatments, patient awareness and support, and promoting innovative research [19, 20, 21, 22, 23]. Therefore, the «EU Public Health Programme» identified Rare Diseases as a main priority for action. EURORDIS, the European Organization for Rare Diseases, in cooperation with national alliances rare disease patient organizations, and with previously described stakeholders, Member States developed National Commissions and put the basis of European Reference Networks [1, 7, 24].

Romanian landscape of rare diseases

With a total area of 238,397 square kilometers, Romania is the 12th largest country and also the 7th most populous member state of the European Union, having 20.121.641 inhabitants (2011) [25]. In Romania, the accepted definition of a rare is a health condition with less than 5 in 10,000 persons within the general population, 6-10% afflicted inhabitants [26]. These data allow to appreciate a number between 1.207.298 and 2.012.164 people in Romanian population presenting a RD.

The first national initiative taken in relation to rare diseases was advocated by a patient association, the Romanian Prader Willi Association- President Dorica Dan [27], who established the fundaments of the Romanian National Alliance for Rare Diseases- ANBRaRo or RONARD [28] in August 2007, together with members of Romanian Society of Medical Genetics [29], other specialists and patient organizations. The first National Plan for Rare Diseases was adopted in 2013. These sustained efforts gained a place for RD in the National Public Health Strategy for 2014-2020 ("Prosperity of Health"), approved by Government Decision no. 1028/2014. The strategy established a regulatory and political framework which generated a system to integrate health and social services. A dedicated budget did not exist for the National Plan in Romania. The Government approved, by Decision, the national programmes for rare diseases to be carried out and funded in 2015 and 2016: Ministry of Health (dietary treatment of RD) and National Health Insurance House (curative treatment for rare diseases). Between 2014 and 2017, all the Romanian stakeholders (patients, health professionals, policymakers and academia) worked together and made notable progress towards improving the quality of life for RD patients. As a result of continuous activity and effort, the Ministry of Health acknowledged the six Regional Centers for Medical Genetics (Timis, Bihor, Dolj, Iasi, Bucuresti, Cluj) (1358/13.11.2014). Timis Regional Center of Medical Genetics along with other Regional centers were included in a European Reference Network – ITHACA on congenital malformations and rare intellectual disability [26, 28].

Herein, we present the experience and strategy for diagnosis of RD in the Timis Regional Centre of Medical Genetics (RCMGT), in collaboration with the Centre for Genomic Medicine in the University of Medicine and Pharmacy "Victor Babes" Timisoara [30].

Material and Method

Clinical assessment and investigations

Timis Regional Centre of Medical Genetics, affiliated to "Louis Turcanu" Emergency Hospital for Children offers medical services through inpatient and outpatient care. The cohort of patients includes a variety of people referred by different specialties, RCMGT having 4 assigned counties: Timis, Arad, Caraş-Severin, and Hunedoara, however available to evaluate patients from all the country.

Comprehensive clinical assessment is crucial for planning investigations in diagnosing a rare disease. A data set was collected for each patient, as requested in the medical genetics consultation chart of RCMGT including: 1) patient demographics and general information, 2) family history of diseases, 3) data about the antenatal and perinatal period, 4) personal physiological history, 5) symptoms and pathological medical history, 6) clinical findings in physical examination, 7) documentation of relevant investigation results, 8) medication, 9) 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. Genetic counseling is offered for every situation.

Genetic testing

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, [26]. Genetic testing was performed at the Centre for Genomic Medicine in UMF Timisoara, a partner research laboratory [30]. Test that were not available, 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. Most test were performed for children (and affected siblings if necessary) as first tier of investigation and further analysis of parent was recommended if needed.

The following tests are available in RCMGT: 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).

The classic karyotype used the indirect method of studying human chromosomes, performed on a 72h cell culture and GTG banding technique (450 bands). Twenty metaphases are usually analyzed. Turnaround time varied between 10 days and 4 weeks.

The FISH (Fluorescent in situ hybridization) used the indirect method of analyzing targeted sequences stained with fluorescent dye. Visualization was performed by fluorescence microscopy and the hybridization signal was observed both in metaphase spreads and in interphase nuclei [31]. Turnaround time varied between 5 days and 2 months. Ten specific regions for the diagnosis of Prader Willi Syndrome, Angelman Syndrome, DiGeorge Syndrome, Williams Syndrome, Smith –Magenis Syndrome, Miller – Dieker Syndrome, Saethre-Chotzen Syndrome, Rubinstein – Taybi Syndrome, Neurofibromatosis, SRY deletion were available.

Genotyping analysis using RealTime-PCR for some frequent pathogenic variants in relation to Noonan Syndrome, 21-Hydroxylase deficiency, Galactosemia, Cystic fibrosis, Rett Syndrome, Bardet-Biedl Syndrome were performed. Turnaround time varied between 4 weeks and 6 months. Fragile X Syndrome was screened using the cut-off over 44 CGG repeats in FMR1 gene. Turnaround time varied between 4 weeks and 6 months.

SNP array was performed using specific kits with either 750k or 300k SNPs. Scanning was done with IScan Illumina and GenomeStudio v2.0 software was used for primary scanning of data generated. In data analysis the following databases: UCSC Genome Browser, DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources), OMIM (Online Mendelian Inheritance in Man), ISCA (International Standards for Cytogenomic Arrays), DGV (Database of Genomic Variants) were used. No genomic imbalances below the 100 kb limit, polymorphic variants CNV (copy number variation) were reported [32]. Turnaround time varied between 2 weeks and 6 months.

Next generation sequencing using panels of genes was performed with the MiSeq Illumina platform. For patients with cardiovascular diseases, a panel including 174 genes was used (Illumina TruSight Cardio). For patients with other type of disorders the next-generation sequencing was performed using the Illumina TruSight One Sequencing Panel kit, a broad panel including 4813 genes. End-to-end bioinformatics algorithms have been implemented and data analysis was conducted in concordance to current knowledge using several databases: UCSC Genome Browser, OMIM (Online Mendelian Inheritance in Man), DGV (Database of Genomic Variants). In interpretation of variants, all variants that can cause illness reported in HGMD®, ClinVar (class 1), and all minor allele frequency (MAF) variants less than 1%-2% in the ExAc database were considered. The evaluation was focused on exons. Only variants related to the phenotype for which the patient was sent were reported. Variants are interpreted according to the ACMG Guide [33]. Classification of Mendelian variants with ACMG: Class 1 - Pathogen/ Class 2 - Probably pathogenic/ Class 3 - Uncertainty of significance (VUS)/ Class 4 - Probably benign/ Class 5 - Benign. Turnaround time varied between 6 weeks and 1 year. An overview of main genetic testing performed at the Centre for Genomic Medicine in UMF Timisoara, are presented in Figure 1.

If a diagnosis is 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 genetic counselling.

Data analysis

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. The age for each patient was calculated as date of presentation minus date of birth. The following age subgroups were used: new-born, infancy: 1 month- 1 year, early childhood: 1–7 years, middle childhood: 7-14 years, adolescence: 14-18 years, young adult: 18-40 years, middle-aged adult: 40-60 years, old adult: > 60 years. Geographical location was categorized as either urban (metropolitan) or rural and was determined by a person's last known postal code of residence. Diagnostics yields (positive predictive value for different genetic tests) were calculated as the proportion of positive findings in each test for all referred patients for that specific test.

Results

A total of new, unique, 1038 patients were examined in Timis Regional Centre of Medical Genetics between January 2015 and November 2018 and referred for testing to our diagnostic laboratory and partner national or international laboratories. For statistical analysis 6 patients were excluded because of missing data. 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.

The majority of evaluated patients were male, 567 (54.6%) vs. 471 females (45.4%). Increasing number of patients were evaluated in RCMGT in the 4 years of activity 2015-2018, as seen in Figure 2. In 2018, the number of new unique patients receiving a genetic consultation per month was in average 35.

Patients from whole Romania presented for genetic consultation, as seen in Figure 3, 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. 60% of patients are established in urban areas, while 40% in rural Romanian areas. 260 males and 207 females were examined from Timis county, 58% coming from urban areas and 42% from villages and communes.

Age repartition of patient's cohort examined in RCMGT is described in Figure 4. The median age was 5.58 years (range 0–78), average at 10.51, and the majority of patients from the 1 to 7 years old subgroup (32%). Majority of patients addressed for a genetic.

All patients having a genetic disease suspicion addressed to RCMGT are presented in Figure 5, classified in accordance to the global standard for diagnostic health information, International Classification of Diseases 11th Revision (ICD 11) [34], including developmental anomalies but also other categories of genetic diseases. 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).

Different genetic tests were performed for each patient according to suspected disorder. Diagnostic yields for each test were calculated for the first 820 patients addressed to RCMGT, from January 2015 to July 2018, as detailed in table 1.

Discussions

Rare diseases have become a worldwide health priority mostly in the last 10 years, aligning policies to improve coordination of care in patients by facilitating the provision of timely, equitable, and evidence-informed services. Dharssi et al. published in March 2017 a review of 11 national policies for rare diseases in the context of key needs of the RD community, and found five common dimensions: coordination of care, diagnosis, access to treatments, patient awareness and support, and research. This study also showed important differences in the status of RD across economic, political, healthcare national factors and examined the role of patient organizations in shaping national policy and programs, including rare disease legislation, national rare disease plans, and coordinated comprehensive services directed to rare diseases [5]. Our present work illustrates the intersection of these aspects in Romania, more specific, the activity in Timis Regional Centre of Medical Genetics (RCMGT), a part of the European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability (ERN ITHACA) in collaboration with the Centre for Genomic Medicine in the University of Medicine and Pharmacy "Victor Babes" Timisoara.

Concerning coordination of care, on a national level, Romania has gained the recognition of rare diseases as a priority for health care since the end of 2013 by adopting the Romanian National Plan for Rare Diseases [26], expanded the official Political Decision that supported the designation of Centers of Expertise using the EUCERD Recommendations on Quality Criteria, and ensured Centers of Expertise have adapted to the national status, and to ERN membership [25, 27]. Given that most rare diseases are genetic in origin, and beyond awareness, an important promoted strategy was to offer access for education in health care practitioners. Thereby, if in 2013 there were only 17 consultant geneticists, today Romania has around 60 doctors specialized in medical genetics (1 geneticist for 330.000 Romanians, and for 33.000 possible RD patients), increasing number of geneticists in training, but still no genetic counsellor profession recognition [35].

Patient registries, recognized as crucial tools for rare diseases national management, prevalence establishment, diagnosis and research [36], are implemented in our daily routine, but in need for standardization, coordination, and further development. 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. Although RCMGT serves inhabitants from 4 counties, 25.6% of the addressed patients are from the rest of the country. This fact may be due to specific genetic testing performed in the Centre for Genomic Medicine in the University of Medicine and Pharmacy "Victor Babes" Timisoara (SNP array and NGS panels), but also to patient needs. 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 [16, 17].

Diagnostic strategy for each case 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 [37]. After this pathway towards a detailed clinical phenotyping, patients evaluated in RCMGT were mostly categorized as presenting unspecified developmental anomalies, followed by chromosomal anomalies, including micro-deletion/duplication syndromes, conditions with disorders of intellectual development as a relevant clinical feature, multiple developmental anomalies or syndromes and neuromuscular disorders. As such report publications are yet very rare, it was not possible to rely on comparisons between Romanian or international data. However, we found an extended population-based cohort study in Western Australia using ICD9 [38] describing highest rates in rare developmental defects during embryogenesis (19.1%), followed by rare neurological diseases (12.4%) and also a higher proportion of male population. The Italian National Rare Diseases Registry also reported disease-distribution in accordance to ICD9 and diseases of the central nervous system and sense organs were the most frequent (26%), followed by congenital anomalies (19.7%) [39].

In Romania, genetic testing is voluntary and by law written informed consent must be obtained prior to any form of genetic testing. Only medical geneticists are allowed to provide genetic testing, counselling and therapy for genetic diseases. Medical Laboratories may offer different types of genetic tests: new-born screening, diagnostic testing, and carrier testing, prenatal testing (RCMGT also has a Prenatal Diagnostic Department, data not included in this study), predictive and presymptomatic testing, forensic testing.

There are conventional stepwise strategies in choosing the right genetic testing for a given phenotype. This comes especially from each individual's unicity and the complex

interaction between their genetic background and environmental factors (phenotypic heterogeneity/ locus heterogeneity). Often, genetically heterogeneous phenotypes request costly and time-consuming investigations, a combination of chromosomal microarray analysis to detect copy number variation and targeted next-generation sequencing gene panels to detect single-nucleotide variants and small insertions and deletions [40, 41]. 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% [42]). As for NGS panels, molecular diagnostic yields were high for both Cardio and extended "Clinical exome" panels compared to literature (11.3% [43], 26% [44]), 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.

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

Conclusions

Despite outstanding advances in policies, technology and bioinformatics, 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.

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.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Authors' contributions

All authors have contributed to either the assessment of subjects, phenotyping, data analysis, statistic interpretation, writing or final critical appraisal of the manuscript. JSIE, PM and CEA conceived the idea for the study and supervised the data collection, the research and the statistical analyses, taking responsibility for the integrity of the data and the accuracy of the data analysis. AN, MiA and TP performed genetic testing. ZC analyzed data from NGS and SNP array. CEA, AN, MiA, TP and DAI interpreted genetic testing data. JSIE drafted the manuscript and undertook data analysis. CEA and PAM researched data and contributed to the discussion and review and editing of the manuscript. PM, CEA and AN contributed

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