

RESEARCH

The genetic diagnosis of rare endocrine disorders of sex development and maturation: a survey among Endo-ERN centres

Luca Persani^{1,2}, Martine Cools³, Stamatina Ioakim¹, S Faisal Ahmed⁴, Silvia Andonova⁵, Magdalena Avbelj-Stefanija⁶, Federico Baronio⁷, Jerome Bouligand⁸, Hennie T Bruggenwirth⁹, Justin H Davies¹⁰, Elfride De Baere³, Iveta Dzivite-Krisane¹¹, Paula Fernandez-Alvarez¹², Alexander Gheldof¹³, Claudia Giavoli^{14,15}, Claus H Gravholt¹⁶, Olaf Hiort¹⁷, Paul-Martin Holterhus¹⁸, Anders Juul¹⁹, Csilla Krausz²⁰, Kristina Lagerstedt-Robinson²¹, Ruth McGowan^{4,22}, Uta Neumann²³, Antonio Novelli²⁴, Xavier Peyrassol²⁵, Leonidas A Phylactou²⁶, Julia Rohayem²⁷, Philippe Touraine²⁸, Dineke Westra²⁹, Valeria Vezzoli² and Raffaella Rossetti²

¹Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy

²Department of Endocrinology and Metabolic Diseases, IRCCS Istituto Auxologico Italiano, Milan, Italy

³Departments of Internal Medicine and Paediatrics and of Paediatric Endocrinology, Ghent University Hospital, Ghent, Belgium

⁴Developmental Endocrinology Research Group, School of Medicine, Dentistry & Nursing, University of Glasgow, Glasgow, United Kingdom

⁵National Genetic Laboratory, UHOG "Maichin dom", Medical University, Sofia, Bulgaria

⁶Department for Pediatric Endocrinology, Diabetes and Metabolic Diseases, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

⁷Pediatric Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

⁸Université Paris-Saclay, Inserm UMR51185 & Service de Génétique Moléculaire, Pharmacogénétique et Hormonologie, Hôpital Bicêtre, France

⁹Department of Clinical Genetics, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

¹⁰Faculty of Medicine, University of Southampton, Southampton, United Kingdom

¹¹Children's University Hospital, Riga, Latvia

¹²Department of Clinical and Molecular Genetics, Vall d'Hebron University Hospital, Barcelona, Spain

¹³Center for Medical Genetics, Vrije Universiteit Brussel, Universitair Ziekenhuis Brussel, Brussels, Belgium

¹⁴Unit of Endocrinology, Fondazione IRCCS Ospedale Maggiore Policlinico, Milano, Italy

¹⁵Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy

¹⁶Departments of Endocrinology, of Clinical Medicine and of Molecular Medicine, Aarhus University, Aarhus, Denmark

¹⁷University Hospital Schleswig-Holstein, Campus Lübeck, and University of Lübeck, Lübeck, Germany

¹⁸University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany

¹⁹Departments of Growth and Reproduction and of Clinical Medicine, Copenhagen University Hospital – Rigshospitalet, Copenhagen, Denmark

²⁰Endocrinology and Andrology Units, University Hospital of Careggi and Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy

²¹Department of Clinical Genetics, Karolinska University Laboratory, Karolinska University Hospital, Stockholm, Sweden

²²West of Scotland Centre for Genomic Medicine, Queen Elizabeth University Hospital, Glasgow, United Kingdom

²³Charité Medicine University, Berlin, Germany

²⁴Translational Cytogenomics Research Unit, Bambino Gesù Children's Hospital IRCCS, Rome, Italy

²⁵Université Libre de Bruxelles, Brussels, Belgium

²⁶Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

²⁷University Hospital Münster, Munster, Germany

²⁸Center for Rare Endocrine and Gynecological Disorders, Department of endocrinology and reproductive Medicine, Hospital Pitié Salpêtrière, Paris, France

²⁹Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands

Correspondence should be addressed to Luca Persani luca.persani@unimi.it

This paper forms part of a special series collated by European Reference Network on Rare Endocrine Conditions celebrating its fifth year. The guest editors for this section are Violeta Iotova, Jérôme Berherat, and George Mastorakos.

Abstract

Differences of sex development and maturation (SDM) represent a heterogeneous puzzle of rare conditions with a large genetic component whose management and treatment could be improved by an accurate classification of underlying molecular conditions, and next-generation sequencing (NGS) should represent the most appropriate approach. Therefore, we conducted a survey dedicated to the use and potential outcomes of NGS for SDM disorders diagnosis among the 53 health care providers (HCP) of the European Reference Network for rare endocrine conditions. The response rate was 49% with a total of 26 HCPs from 13 countries. All HCPs, except 1, performed NGS investigations for SDM disorders on 6720 patients, 3764 (56%) with differences of sex development (DSD), including 811 unexplained primary ovarian insufficiency, and 2956 (44%) with congenital hypogonadotropic hypogonadism (CHH). The approaches varied from targeted analysis of custom gene panels (range: 11–490 genes) in 81.5% of cases or whole exome sequencing with the extraction of a virtual panel in the remaining cases. These analyses were performed for diagnostic purposes in 21 HCPs, supported by the National Health Systems in 16 cases. The likelihood of finding a variant ranged between 7 and 60%, mainly depending upon the number of analysed genes or criteria used for reporting, most HCPs also reporting variants of uncertain significance. These data illustrate the status of genetic diagnosis of DSD and CHH across Europe. In most countries, these analyses are performed for diagnostic purposes, yielding highly variable results, thus suggesting the need for harmonization and general improvements of NGS approaches.

Key Words

- ▶ next-generation sequencing
- ▶ rare diseases or syndromes
- ▶ disorders of sex development
- ▶ congenital hypogonadotropic hypogonadism
- ▶ primary ovarian insufficiency

Endocrine Connections
(2022) **11**, e220367

Introduction

The technological advancements in genetics have had a profound impact on the diagnosis of non-communicable diseases. A next-generation sequencing (NGS) approach may lead to the identification of genetic variants with an unprecedented critical impact on the management of the affected patient and their families. Thanks to the progressive diminution of costs, the diffusion of these approaches has been occurring in a short period of time but with high variability among different European countries and the respective National Health Systems (NHS) (1, 2), thus representing an example of significant disparity among citizens within the European Union.

The disorders or differences of sex development and maturation (SDM) include the differences of sex development (DSD), that are generally associated with phenotypical manifestations incongruent with chromosomal sex (46,XY DSD and 46,XX DSD), and those associated with absent/delayed puberty due to congenital hypogonadotropic hypogonadism (CHH) or unexplained 46,XX primary ovarian insufficiency (POI). These conditions are rare, with variable and complex aetiology, and their differential diagnosis using only clinical and biochemical parameters may be difficult (3). Therefore, NGS retains great potential for an accurate

diagnosis and personalized management of affected individuals and families (4).

Here, we report the results of a survey on the application of NGS within the health care providers (HCPs) of the Main Thematic Group 7 (MTG7) dedicated to rare conditions of SDM within the European Reference Network on rare endocrine conditions (Endo-ERN; www.endo-ern.eu) (3).

Methods

An international survey was circulated among the HCPs of MTG7 within Endo-ERN in spring 2020 and a second round, asking some more details on the NGS protocols, was run in December 2021. Responses from new Endo-ERN HCPs were collected up to April 2022. Contact details of clinicians were retrieved through the Endo-ERN coordinating office. Endo-ERN steering committee officially approved the performance of this survey as part of its clinical research activities under the European Union grant agreement (#739572). The survey did not contain personal data of patients (Supplemental Materials, see section on [supplementary materials](#) given

at the end of this article) and included questions on (i) the number of analysed patients, (ii) the composition of the gene panels (total and specific numbers of candidate genes for DSD, 46,XX POI or CHH), (iii) the percentage of patients with positive NGS reports (i.e. cases with at least one rare non-synonymous variant in the candidate genes), (iv) quality criteria used for reporting, including bioinformatic support (i.e. reports including class IV-V variants indicating a clear genetic diagnosis or including also class III variants of unknown significance (VUS) indicating a likely genetic diagnosis) (5, 6), (v) the reasons justifying the analyses (i.e. clinical or research purposes), (vi) the origin of economical support for genetic analyses and (vii) impact on clinical management. Patient and/or parental consent was obtained in each centre prior to genetic analyses as part of routine clinical care.

Results

The response rate from the 53 expert HCPs was 49% with a total of 26 HCPs from 14 countries (see Fig. 1). Several countries were represented by more than one HCP (three

from Belgium, two from Denmark, two from France, four from Germany, five from Italy, two from The Netherlands and two from United Kingdom).

These 26 HCPs performed investigations for SDM disorders on at least 6720 patients, 3764 (56%) with DSD (either 46,XX or 46,XY), including 811 for unexplained POI, and 2956 (44%) with CHH (Fig. 1). At the time of the survey, 10 HCPs had a large experience with NGS (≥200 individuals with SDM conditions), whereas 7 HCPs had analysed <50 individuals referred for SDM conditions and the remaining 9 HCPs had an intermediate experience (55–156 individuals with SDM conditions). The Bulgarian National Genetic Lab analysed a panel of 10 candidate genes in 50 patients with 46,XY DSD by Sanger method, but the large majority (81.5%) of the patients (n = 5477) underwent a targeted NGS analysis using custom gene panels. The number of analysed genes was highly variable across the various HCPs, ranging from a panel of 11 genes in the Kiehl HCP (Germany) to custom panels of >150 candidate genes for disorders of SDM across Sweden, Slovenia, Italy, Denmark and Belgium and up to whole exome sequencing (WES) analyses (Figs 2 and 3). This latter approach is prioritized

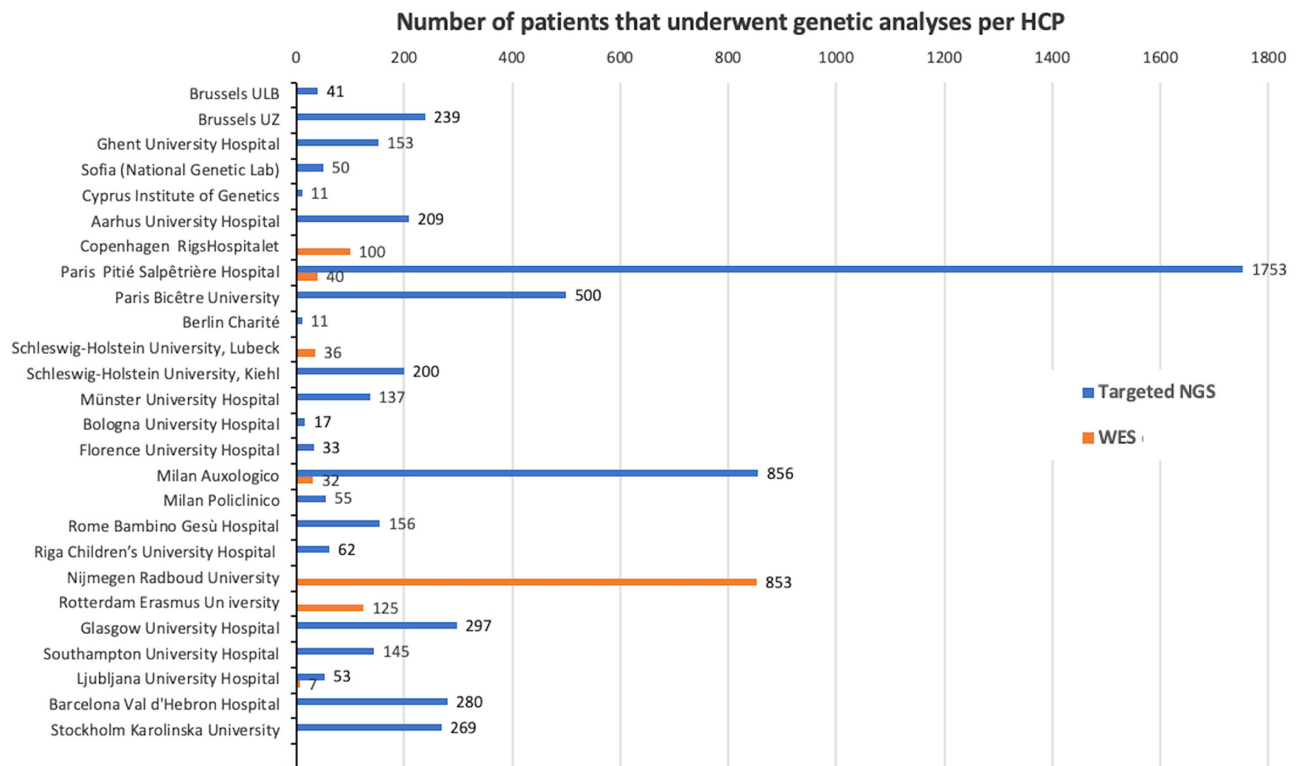


Figure 1

Graph illustrating the number of patients referred for genetic diagnosis by NGS in the 26 health care providers of Endo-ERN. As first-line investigation, the vast majority of patients were analysed by targeted NGS with custom panels of candidate genes. The Sofia HCP analysed 10 candidate genes by Sanger sequencing in 50 patients with 46,XY DSD.

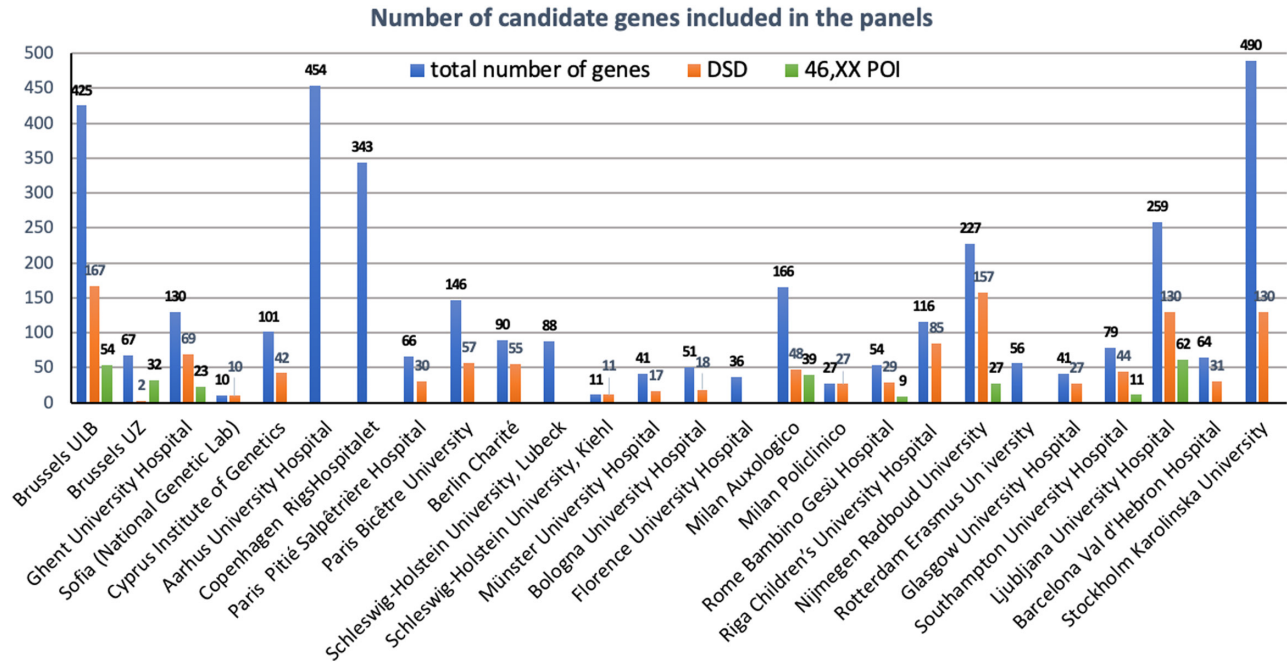


Figure 2 Graph illustrating the variable panel of candidate genes analysed in the 26 health care providers for the genetic diagnosis of DSD or POI. Some HCPs did not give details on the list of candidate genes included in the custom or virtual panel. Note the highly variable number of genes included in the custom panels.

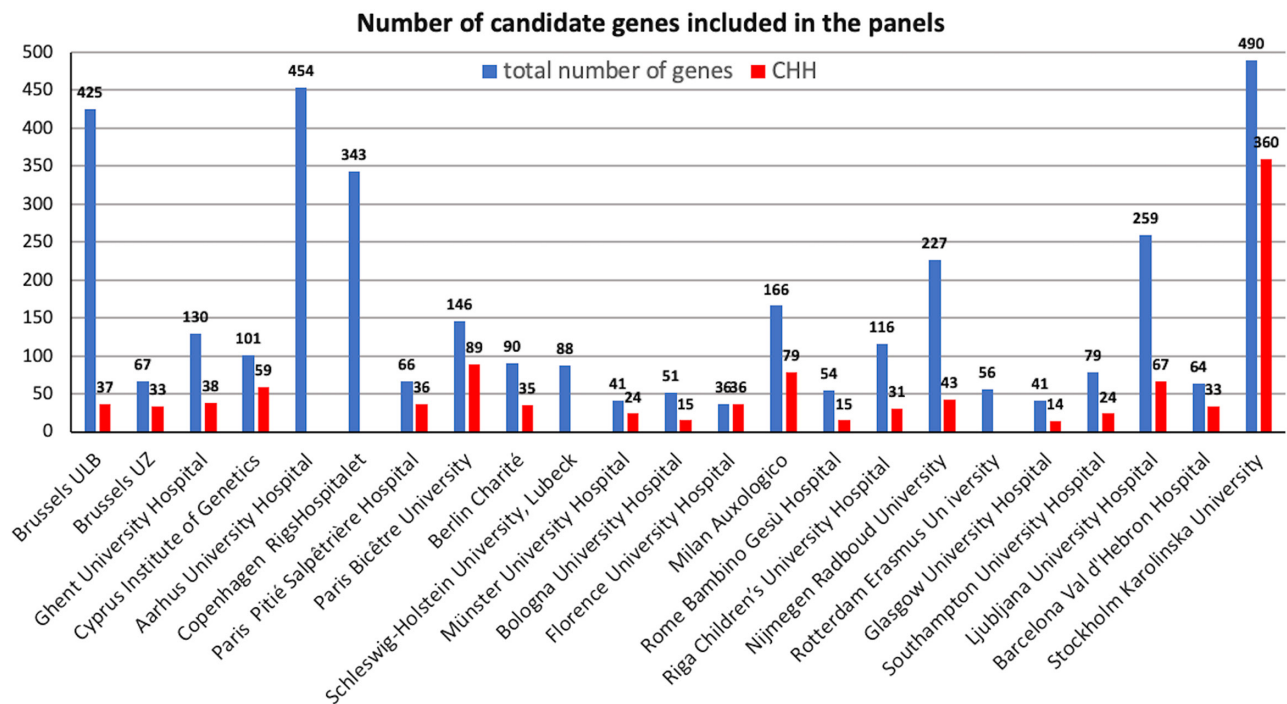


Figure 3 Graph illustrating the variable panel of candidate genes analysed in 23 health care providers for the genetic diagnosis of CHH. Some HCPs did not give details on the list of candidate genes included in the custom or virtual panel. Note the highly variable number of genes included in the custom panels.

in some HCPs (Copenhagen, Lubeck, Nijmegen and Rotterdam) performing a WES enrichment with an initial analysis of variants in a virtual gene panel that is applied by bioinformatic restriction of variant call format (VCF) files (Fig. 1). In case no causative mutation is found in the chosen candidate genes and if the informed consent to proceed was previously given by the patient or their parents, these expert centres proceed with an open exome analysis. Two centres (Paris Pitié Salpêtrière and Milan Auxologico) performed subsequent WES analyses in SDM cases selected by negative results at the targeted NGS (Fig. 1).

Twenty-one HCPs declared to perform these analyses for diagnostic purposes. The diagnostic analyses are supported by the National Health Care Systems in 15 HCPs from 9 different countries (Belgium, Bulgaria, Denmark, France, Germany, Italy, Latvia, Slovenia and The Netherlands). Instead, the remaining five HCPs performed the NGS analyses for research purposes. NGS analyses were performed on institutional diagnostic platforms, except in one case (Riga HCP).

Most of the HCPs analyse candidate genes for most SDM conditions, whereas some HCPs are specialized on specific conditions, like 46,XY DSD in Sofia and Milan Policlinico HCPs.

All HCPs declare a coverage >95% by their custom panels and run an *in silico* bioinformatic analysis using several professional algorithms. Also, all of them declare to follow the standards and guidelines for the interpretation of sequence variants published by Richards *et al.* (5) and/or Matthijs *et al.* (6).

The outcome of NGS analyses is highly variable across the 26 HCPs: the centres with >150 candidate genes in their custom or virtual panel report are more likely to give a positive outcome (40–60% of the cases) whereas positive results fall <15% in some HCPs. Moreover, the diagnostic yield ranges 6.3–39.0% (mean: 9.0%) among the 9 centres that gave details of the pathogenic or likely pathogenic variants at bioinformatic analyses (ACMG Class IV or V variants) and 21.5–60% (mean: 46%) when rare non-synonymous VUS (minor allele frequency < 0.01%) are also considered. However, more than 90% of the HCPs include not only ACMG Class IV or V variants but also rare non-synonymous VUS in the clinical reports for genetic counselling of affected families.

Results are confirmed by Sanger sequencing except in 1 out of 19 HCPs. Four HCPs declared to perform complimentary multiplex ligation-dependent probe amplification (MLPA) or arrayCGH for the detection of exon or allelic deletions on specific cases. In all affected

cases who had a molecular genetic diagnosis, the expert centres responded that NGS analyses revealed to be useful for a more precise management of the patients or their families.

Discussion

We are reporting a detailed illustration on the NGS approach for the diagnosis of rare conditions affecting SDM across several European countries based on the results of a survey conducted among the HCPs of Endo-ERN. Contributions were received from 26 centres distributed across 14 different countries. These HCPs analysed DNA samples from >6000 patients with DSD, 46,XX POI or CHH; in some centres, the numbers are low either because the afferent population is limited (e.g. Cyprus HCP) or because the clinical NGS was recently introduced for the SDM disorders (e.g. Berlin HCP) (Fig. 1).

The results of the survey reveal that technical approaches are highly variable across these expert HCPs, with several reference centres offering highly informative panels containing >150 candidate genes at the time the survey was conducted. Other centres provided gene panels focusing on specific conditions (congenital adrenal hyperplasia (CAH), CHH or 46,XY DSD) as the result of specific clinical expertise on these rare conditions.

Interestingly, all HCPs except one use their institutional NGS platforms for these genetic diagnoses, but few centres equipped with adequate high-throughput NGS platforms offer a first-line WES analysis with a clinical report on virtual candidate gene panel. The custom NGS panel of candidate genes can be run on less expensive instrumentations and has high accuracy in detecting variants within the genomic regions of interest, but its development is time-consuming, labour-intensive and has limited future flexibility for the addition of new candidate genes. The virtual panel approach has the advantage of the flexible adaptation of the virtual panel with the rapid inclusion of novel candidates and the possibility to revise previous reports according to new discoveries in the field, but the sequencing depth of WES (or whole genome sequencing, WGS) may be insufficient to detect low-frequency variants due to the higher sequencing error rates or poor coverage (1, 4). Nevertheless, Sanger sequencing and MLPA still retain a particular relevance in the detection or confirmation of the most frequent form of CAH, 21-hydroxylase defects due to variations in *CYP21A2* because of the high homology in *CYP21A2* and its pseudogene *CYP21A1P* frequently leading to false positive or negative results.

The survey revealed the definition of a molecular genetic diagnosis of otherwise unexplained DSD, 46,XX POI or CHH in 10–60% of the cases across the 26 HCPs. Such variable yields of the NGS approach in ‘real life’ can depend upon (i) variable pre-analytical selection of patients (diagnostic yield can be very low when all cases with isolated hypospadias or cryptorchidism are investigated by NGS); (ii) variable criteria of withheld variants (the inclusion of rare non-synonymous VUS makes a significant difference in diagnostic yield from 9 to 46%, but the reclassification of these VUS is possible and should in principle be done whenever possible according to further studies of phenotypical co-segregation in affected families) and (iii) poorly comprehensive and sporadically updated NGS panels. Indeed, further efforts should be done for a more pervasive availability of comprehensive and informative genetic analyses, with frequent technical updates in all centres or the centralization of the NGS analyses in specialized centres of the same area with more comprehensive and informative approaches, as already done by the Latvian HCP. Since candidate genes for SDM disorders are numerous and the positive yields are still below 50%, the WES/WGS approach should become the method of choice for these expert centres. The choice of focusing on specific disorders sometimes depends also on the availability of more comprehensive approaches in close HCPs of the same city/region to avoid overlaps and centralize specific analyses (e.g. the two HCPs in Milan). Furthermore, these data also indicate the need for a more stringent approach and uniform reporting method of NGS analyses across the various HCPs which should include a clear and referenced indication of the pathogenic potential of the identified variants. Efforts should also be made within Endo-ERN to provide information on reference labs performing functional analyses for the correct reclassification of VUS when other approaches (e.g. genotype–phenotype co-segregation studies) are not informative or impossible to be performed.

These updates are particularly relevant as NGS analyses were supported by the NHS at least in nine European countries and the clinical management and outcome of affected patients and families was generally reported to be improved by the positive results of NGS analyses, in accordance with recent recommendations (2, 3, 4).

An inherent limitation of a study performed in such a rapidly evolving scientific field is that the required time interval between data collection (2020 for some centres, 2021–2022 for other centres) and publication may lead to the reporting of outdated data (e.g. UK has introduced NHS supported targeted panels since 2021, and UK HCPs

contributed the survey only in 2020 when they were still part of Endo-ERN before the Brexit). Also, we have not kept track of the reason why HCPs chose not to participate in the study. Therefore, we were unable to discriminate between HCPs where NGS was not performed at the time of data collection and centres that relied on NGS technology but chose not to participate for other reasons.

In conclusion, these data illustrate how clinical genetic diagnostics in the field of rare diseases, more specifically unexplained disorders of SDM, are currently performed across Europe. The number of SDM patients who had detailed molecular genetic analyses constitute a practical demonstration of the great clinical and research potential of the European Reference Networks for the diagnosis and care of rare diseases. This potential can be further enhanced when these genetic data will be linked to the clinical information available in disease registries (7, 8).

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/EC-22-0367>.

Conflict of Interest

The authors have nothing to disclose related to the content of this manuscript

Funding

This publication has been supported by Endo-ERN, which is co-funded by the European Union's 3rd Health Programme (CHAFAEA Framework Partnership Agreement No 739527).

Acknowledgements

This work is generated within the European Reference Network for Rare Endocrine Conditions (Endo-ERN). The authors wish to thank all those people that contributed the results of the survey.

References

- Persani L, de Filippis T, Colombo C & Gentilini D. Genetics in endocrinology: genetic diagnosis of endocrine diseases by NGS: novel scenarios and unpredictable results and risks. *European Journal of Endocrinology* 2018 **179** R111–R123. (<https://doi.org/10.1530/EJE-18-0379>)
- Eggermann T, Elbracht M, Kurth I, Juul A, Johannsen TH, Netchine I, Mastorakos G, Johannsson G, Musholt TJ, Zenker M, *et al.* Genetic testing in inherited endocrine disorders: joint position paper of the European reference network on rare endocrine conditions (Endo-ERN). *Orphanet Journal of Rare Diseases* 2020 **15** 144. (<https://doi.org/10.1186/s13023-020-01420-w>)
- Hiort O, Cools M, Springer A, McElreavey K, Greenfield A, Wudy SA, Kulle A, Ahmed SF, Dessens A, Balsamo A, *et al.* Addressing gaps

- in care of people with conditions affecting sex development and maturation. *Nature Reviews. Endocrinology* 2019 **15** 615–622. (<https://doi.org/10.1038/s41574-019-0238-y>)
- 4 Ahmed SF, Alimusina M, Batista RL, Domenice S, Lisboa Gomes N, McGowan R, Patjamontri S & Mendonca BB. The use of genetics for reaching a diagnosis in XY DSD. *Sexual Development* 2022 1–18. (<https://doi.org/10.1159/000524881>)
- 5 Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine* 2015 **17** 405–424. (<https://doi.org/10.1038/gim.2015.30>)
- 6 Matthijs G, Souche E, Alders M, Corveleyn A, Eck S, Feenstra I, Race V, Sistermans E, Sturm M, Weiss M, *et al.* Guidelines for diagnostic next-generation sequencing. *European Journal of Human Genetics* 2016 **24** 2–5. (<https://doi.org/10.1038/ejhg.2015.226>)
- 7 Ali SR, Bryce J, Tan LE, Hiort O, Pereira AM, van den Akker ELT, Appelman-Dijkstra NM, Bertherat J, Cools M, Dekkers OM, *et al.* The EuRRECa project as a model for data access and governance policies for rare disease registries that collect clinical outcomes. *International Journal of Environmental Research and Public Health* 2020 **17** 8743. (<https://doi.org/10.3390/ijerph17238743>)
- 8 Lucas-Herald AK, Rashid Ali S, McMillan C, Rodie ME, McMillan M, Bryce J & Ahmed SF. 1-DSD – the first 10 years. *Hormone Research in Paediatrics* 2022 [epub]. (<https://doi.org/10.1159/000524516>)

Received in final form 6 October 2022

Accepted 13 October 2022

Accepted Manuscript published online 13 October 2022