OPINION

Addressing gaps in care of people with conditions affecting sex development and maturation

Olaf Hiort, Martine Cools, Alexander Springer, Ken McElreavey, Andy Greenfield, Stefan A. Wudy, Alexandra Kulle, S. Faisal Ahmed, Arianne Dessens, Antonio Balsamo, Mohamad Maghnie, Marco Bonomi, Mehul Dattani, Luca Persani and Laura Audi on behalf of COST Actions DSDnet and GnRHnetwork as well as the European Reference Network for Rare Endocrine Conditions (Endo–ERN)

Abstract

Differences of sex development (DSD) are defined as conditions with discrepancies between chromosomal, gonadal and phenotypic sex. In congenital hypogonadotropic hypogonadism (CHH), a lack of gonadotropin activity results primarily in the absence of pubertal development with prenatal sex development being (almost) unaffected in most patients. To expedite progress in the care of people affected by DSD and CHH, the European Union has funded a number of scientific networks. Two Actions of the Cooperation of Science and Technology (COST) programmes — DSDnet (BM1303) and GnRHnetwork (BM1105) — provided the framework for ground-breaking research and allowed the development of position papers on diagnostic procedures and special laboratory analyses, as well as clinical management. Both Actions developed educational programmes to increase expertise and promote interest in this area of science and medicine. In this Perspectives, we discuss the success of the COST Actions DSDnet and GnRHnetwork, and the European Reference Network for Rare Endocrine Conditions (Endo–ERN) and provide recommendations for future research.

Introduction

The Cooperation of Science and Technology (COST) programme aims to increase the networking possibilities for scientists and to fund activities through the European scientific funding programme Horizon 2020. COST Actions, which are networks dedicated to scientific collaboration and complement national research funds, provide the opportunity to hold workshops and meetings and offer support for early stage researchers (PhD students or post-doctoral fellows within 8 years of their PhD) through short-term scientific missions and training schools. COST Actions are joined by countries within Europe, but also include near neighbouring countries and international partners. Interestingly, COST Actions are not closed projects, but are open to all opinion leaders from the participating countries.

COST Actions provide excellent tools for scientists involved in research on rare diseases in medicine, because here, expertise is often scattered, and effective networking is necessary for increasing knowledge and achieving appropriate attention. From 2012 to 2017, COST funded two European concerted Actions for the systematic elucidation of differences of sex development (DSD) and for congenital hypogonadotropic hypogonadism (CHH) resulting from gonadotropin-releasing hormone (GnRH) deficiency. CHH is frequently associated with anosmia (Kallmann syndrome) and has variable clinical features. Both Actions had similar structured approaches concerning clinical care, genetic testing and scientific research, as well as education and training.

The development of sex and gender, and the elucidation of variant physiology, are some of the most complex topics in biology and medicine, as well as in society. The main objective of the COST Actions was to promote research into sex development and maturation, spanning the whole patient journey from diagnostic molecular studies to treatment, in order to improve the structured care and health of people with DSD or CHH. The Actions were designed to aid understanding of the underlying clinical heterogeneity of DSD or CHH, as well as reveal the pathophysiological commonalities between the different conditions at the molecular level. Additionally, we wanted the Actions to benefit the scientific investigation of rare diseases in the international community and to promote the formation of a European Reference Network for better visibility of patient care.

DSDnet was built on the framework of pre-existing collaborations that took shape following the Chicago consensus in 2005[1] and included the DSD Working Group of the European Society for Paediatric Endocrinology, EuroDSD (funded by 7th Framework Programme of the European Union),

and the international I-DSD and I-CAH registries. DSDnet was designed to promote collaboration with the project DSDLife, which was also funded within the 7th Framework Programme of the European Union. The GnRHnetwork began earlier, in April 2012, lasted until April 2016 and was characterized by interdisciplinary interaction of physicians and more basic scientists.

Our knowledge on the prenatal sex development and pubertal maturation pathways has improved considerably in the past 20 years due to cutting-edge research on mammalian development and elucidation of underlying genetic mechanisms[29,30]. In parallel, descriptive clinical outcome studies have provided some insight into the long-term outcome of affected people[31,32,33]. However, the results of these studies are mostly inconclusive due to the small sample sizes and broad heterogeneity of participants as well as differences in applied methodology and measures.

To provide better care for its citizens with rare and complex health issues, the EU has established the European Reference Networks (ERNs) for rare conditions. In 2017, 24 ERNs were founded, encompassing the majority of the >8,000 rare conditions known today. The ERN for Rare Endocrine Conditions (Endo–ERN) is divided into eight Main Thematic Groups (MTGs), which cover all rare endocrine disorders over the lifespan. The aim of Endo–ERN is to diagnose these conditions promptly and effectively, whilst minimizing the inequities that exist for the care of affected people across the EU. Within Endo–ERN, the MTG 'Sex Development and Maturation' was established, which stems from previous participants of the COST Actions DSDnet and GnRHnetwork and whose members will build its future aims for patient care on the achieved results.

In this Perspectives article, we describe the achievements of these two related COST Actions and highlight the gaps in research that were identified. Future investigations should take these considerations into account

[H1] Achievements of the COST Actions

[H2] Clinical assessment

The clinical findings of conditions that effect sex development and maturation can be highly variable and sometimes clinically undetectable[34]. This clinical observation holds true for conditions such as complete gonadal dysgenesis (where the external phenotype is female, even if the karyotype is 46,XY) and in patients who are 46,XX and have CHH (who are unequivocally female at

birth). All DSD can have associated features, with complex syndromes affecting almost all organ systems, but mainly the kidneys, the heart, the peripheral and central nervous systems[2]. Therefore, any clinical investigation needs to take further developmental anomalies into account.

The clinical assessment of a patient needs to be age-dependent and include an extensive whole-body examination, including the genital status. In the young child, the inspection of the genitalia might reveal micropenis (<2 SD of normative value) and cryptorchidism. Only in DSD conditions, hypospadias or further genital ambiguity will be found. At the time of puberty, in both DSD and CHH delay of pubertal development might be a hallmark. Both of the COST Actions published recommendations on standardized clinical assessment[3-5]. For DSD, the external masculinization score[6], which was originally designed to describe genitalia in undervirilized male infants, was modified into a more widely applicable 'external genitalia score' (EGS) to cover the whole spectrum of genital appearance in both male and female infants. The tool that resulted following the modifications will become available to be incorporated into online platforms such as the I-DSD for collection of standardized phenotypic data by the end of 2019.

A template for longitudinal follow-up has been created in conjunction with the I-DSD registry. Within this longitudinal follow-up tool some questions on overall health, endocrine status, genital anatomy and function, need for psychosocial support and gender congruence have to be considered equally important if we want to improve the quality of life of all patients with a DSD condition. A simple screen such as this might be sufficient for use in the routine clinical setting and could be followed up with more detailed assessment if indicated.

For clinical and research purposes, we considered it crucial to standardize a minimal set of time points, corresponding to important developmental milestones, at which relevant clinical data should be collected, although most patients will need medical evaluations more often than these minimums. Consensus was reached on the following time points: assessment at diagnosis and at ages 4 years and 8 years allows for documentation of psychological developmental milestones and gender development, presence of associated symptoms and growth patterns, and ensures the provision of timely and adequate information to the child and their parents. At the start and at the termination of puberty, and at the transition to adult care, documenting the outcome of pubertal development and the physical and mental status was considered paramount. Various age intervals were identified in adulthood and each has specific requirements. At ages 18-25 years independence is gained and new intimate relationships are formed. At ages 25-40 years issues around fertility and forming a family might dominate discussions with patients. By contrast, at age intervals 40-60 years and 60-80 years,

long-term effects of treatment or (lack of) hormonal treatment and co-morbidities might become apparent (unpublished data and REF [5])

Several experts of the GnRHnetwork COST Action conducted or reviewed multicentre trials that highlight the need for improved management of affected patients and the importance of a structured and life-long clinical assessment. In particular, Andrew Dwyer and colleagues conducted the first and only randomized clinical trial on the induction of puberty, testicular growth and fertility in treatment naive young adults with GnRH deficiency[7]. They evaluated the fertility outcomes by sperm count and surrogate markers during a 24-month pulsatile GnRH administration versus 4-month of recombinant human FSH (rhFSH) pretreatment followed by 24 months of GnRH therapy. They demonstrated the superiority of rhFSH pre-treatment on both surrogate markers (including testicular volume) and sperm count. A subsequent review of the GnRH-network experts confirmed the superiority of rhFSH pretreatment before the combined gonadotropin or pulsatile GnRH administration for the induction of pubertal development and testicular growth in patients with severe GnRH deficiency[8]. In addition, the GnRH COST action evaluated the psychosexual development of 101 men with CHH[9]. The study revealed that male patients with CHH frequently experience psychosexual problems that hamper intimate relationships and the initiation of sexual activity. These persistent effects cause considerable distress and are not ameliorated by long-term treatment. Therefore, psychosexual assessment followed by appropriate psychological support and treatment is warranted in these patients.

[H2] Genetics

Both Actions, DSDnet and GnRHnetwork, stratified a diagnostic approach to elucidate the underlying genetic anomaly and published position and consensus papers on this topic[3,10]. In people with DSD, the determination of a karyotype is still seen as an initial mandatory step, as numerical or structural chromosomal abnormalities account for a considerable subset of DSD conditions. However, many patients with DSD or CHH might have an unaffected chromosomal complement, and so detailed studies for molecular genetic conditions are needed.

Both Actions favoured collaboration between basic scientists and clinicians to understand the molecular aetiology and develop collaborative bioinformatics tools to rapidly aid the identification of novel genetic causes. Collaborations within GnRHnetwork led to the discovery of several novel candidate genes underlying CHH[11-15]. The collaborative efforts in DSDnet led to the identification

of variants in a new gene associated with 46,XY DSD[16] and mutations in a nuclear factor, *NR2F2*, which cause a novel syndromic form of 46,XX DSD[17]. We predict that it is highly likely that other new genes causing DSD will be identified through the DSDnet and Endo–ERN collaborative network in the near future.

In 2015, the first European Consensus Statement on CHH was published. The consensus document covers aspects related to the pathogenesis, diagnosis and treatment of this rare condition[3]. This document is the result of the interaction between the members of the clinical and genetic working groups of the GnRHnetwork. In particular, the experts documented the existence of particular phenotype–genotype correlations (for example, CHH and hearing loss are particularly frequent among patients with *SOX10*, *IL17RD* or *CHD7* gene defects) and provided recommendations for improved treatment of patients with CHH examining early, but also later, outcomes including future fertility.

Despite these important advances, the majority of patients, particularly those with 46,XY conditions that effect sex development and maturation (excluding primary errors of the endocrine system) do not have a molecular diagnosis. The accurate interpretation of high-throughput sequencing datasets is challenging in the clinical setting. The challenges arise in part due to emerging evidence that these conditions might be caused by variants in many different genes, and the prevalence of variants in a single gene could be very low[35]. To build robust evidence to support causality, sharing genomic data between research groups and developing informative animal and cellular models are required.

To improve the way in which we share genomic data, DSDnet established a secure platform for sharing data between research groups, called 'SDgeneMatch'[36]. A 'match' occurs when two users of the system are found with a variant in the same gene. Matches are reported to the two researchers that supplied the relevant gene identifier. Reporting of matches will be done behind the password-protected environment of SDgeneMatch, ensuring only the users that originally uploaded the match will be able to learn the gene name of the match. Although other gene matching systems do exist, SDgeneMatch is currently specific for DSD, and the DSD research community will be actively encouraged to submit data into the system. Matching data in this way can accelerate gene and/or variant discovery in the field and encourage collaboration with groups working on animal and/or cellular model systems in order to provide evidence of causality, as well

as explore the molecular pathways that are involved. In addition, data matching will lead to a more accurate molecular diagnosis for DSD.

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[H2] Endocrine assessment

Rare endocrine conditions, such as DSD and CHH, are often genetically determined and feature hormonal imbalances as the result of divergent endocrine pathways. However, in childhood and puberty, endocrine abnormalities can be difficult to detect and tend to be highly variable depending on age and developmental stage of the affected person[34].

Therefore, the COST Actions first aimed to identify appropriate laboratory determinations that are useful in the complex differential diagnostics of DSD and CHH. Second, the COST Actions aimed to develop guidelines for the usefulness of specific laboratory analyses and testing conditions. These guidelines were directed toward the implementation of clinical standards for diagnosis and appropriate treatment of DSD and CHH to achieve the best outcomes for patients, no matter where patients are investigated or managed[3,18-20].

In a position paper by Alexandra Kulle and colleagues, all forms of DSD were summarized and, for each condition, the required hormonal work-up and suitable analytical techniques were described [18]. The main recommendations were: support the appropriate use of both immunoassay-based and mass spectrometry-based methods for the diagnosis and monitoring of DSD; use of both serum and urine is established and appropriate matrices used for analysis of steroids; and laboratories should aim to participate in activities of peer comparison[18].

The next step for the harmonization of laboratory assessments relates to the important plasma and/or serum analyte 17-hydroxyprogesterone, which is a key marker in the diagnosis of congenital adrenal hyperplasia[21]. Hereto, we conducted, in collaboration with colleagues in China, Singapore and Australia, a worldwide survey on mass spectrometric determinations of this analyte. This collaboration resulted in a publication[21] that pointed out that although mass spectrometry-based methods are similar in many facets, they are highly disparate, leading to heterogeneous reference values over the whole lifespan. Consecutively, five recommendations have been developed to support the continued improvement of analysis of plasma or serum 17-hydroxyprogesterone by mass spectrometry[21].

[H2] Education and training

Both COST Actions used the specific tools of the COST programme to provide training and education to early stage researchers. Specifically, they developed the Action websites DSDNet and CHUV and coordinated and integrated the activities of the other Working Groups. Some of the scientific efforts were translated into public dissemination through a series of articles (full list at DSDNet and CHUV meetings (6 working group meetings and/or workshops for DSDnet, the last one in combination with GNRHnetwork) and position papers[3,5,10,18,22].

The COST Actions used part of their funds to provide training for eligible young scientists and organized three training schools. The three training schools were set up to provide multidisciplinary training to young professionals (trainees) and encourage ongoing activity in the field of DSD and CHH. These interactive meetings, each of which included ~30 trainees and 10 trainers, have been designed to cover important topics relevant to science and clinical work. The key long-term aim of each training school was to encourage the trainees to be involved in and to improve the national and international networking capabilities of interdisciplinary research for breakthrough science. To achieve the key long-term aim within DSDnet, for example, trainees were encouraged to participate in the I-DSD registry, apply for grant and fellowship applications in the field of DSD and become active members of the European Reference Network for Rare Endocrine Conditions (Endo–ERN).

A report that analyzed, through specific surveys, the success and subsequent outcome of the training schools for the trainees was published in 2019[23]. Briefly, the high rate of positive responses from trainees demonstrates the success of the training school model that the DSDnet has adopted, and shows that the majority of the participating trainees are still active in the DSD field. These positive results justify the continuation of this form of postgraduate multidisciplinary training.

Another educational tool is the organization of the short-term scientific missions (STSM). The STSMs were aimed at supporting individual mobility in order to share knowledge and technology that might not be available in the home institution, strengthening the existing networks in the field and fostering collaborations by allowing scientists to visit an institution or laboratory in another participating COST country. There were three grant periods throughout the duration of the COST Actions. The overall comparison between the number of STSMs during COST Action DSDnet (*n* = 10 between 2015 and 2017; 3.33 per year) and COST Action GnRHnetwork (*n* = 23

between 2013 and 2016; 5.75 per year) of our partners in the European Reference Network for Rare Endocrine Conditions (Endo–ERN) demonstrates some differences in missions between the two actions. One possible explanation could be the increased participation of basic scientists in GnRHnetwork, who are willing and able to perform an STSM in another laboratory site. By contrast, the participation of clinical scientists in such exchanges could be hampered by their clinical duties.

[H2] Improving patient care

We need to promote and optimize patient management and care for the complex conditions involved in sex development and maturation. DSDnet intended to understand the current practice and research priorities amongst professionals and patients. These objectives were primarily achieved through a series of web-based surveys that targeted paediatric endocrinologists, providers of psychosocial support and paediatric surgeons and urologists[24,25].

The survey that was aimed at paediatric endocrinologists revealed that 40% of the DSD centres had a multidisciplinary team available at initial presentation[24]. Half of the centres reported that they share their data in a multicentre registry. In addition, the survey revealed that local access to specialist biochemical and genetic tests influenced the diagnostic process, and that detailed molecular genetic testing was becoming routine. Approximately one third of regions surveyed had seen the development of clinical networks. Expert centres were increasingly disseminating knowledge to non-expert centres through arranging local clinical meetings, case discussion and regional DSD clinics. Evidence of conferences and training days and e-learning tools was also reported. Survey participants suggested a number of research priorities, which highlights, among other requirements, the need for more studies that aim to understand and improve the quality of life.

In this survey, paediatric endocrinologists were asked to rate their research priorities in order of importance[24], see table 1 for a summary. Most frequently indicated topics were development of DSD-specific HRQoL measures and interventions that will improve quality of life, gender development and fundamental research on basic mechanisms underlying DSD and DSD-related disease [24]. The survey among providers of psychosocial care investigated access to and modalities of psychosocial care, organization and practice[25]. The survey revealed that psychosocial care is predominantly provided to parents and focuses on coping and acceptance of the diagnosis and

the atypical genitalia, decisions on genital surgery, disclosure and education. Adult patients have less access to counselling than parents, children and adolescents[25]. Given that this age group in particular require support for coping with a range of DSD-related health and psychosexual issues[37,38,39,40] , improving the accessibility of psychosocial counselling for adults should be prioritized. Sexuological counselling for men and women with DSD hardly received attention in the survey; however, there is a compelling need for development of sex therapies focused on sexual problems experienced by men and women with DSD. Providers of psychosocial care have a wide range of training backgrounds. This variety and the absence of an overarching professional body hinder professional development[25].

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The assessment of patient needs was emphasized by both Actions, DSDnet and GnRHnetwork. A DSDnet face-to-face workshop that was held for 33 patients, their relatives and professionals included one or more patients and a health-care professional from the same centre from eight different European countries[26]. The focus of this workshop was to understand the needs and perceptions of those patients and parents who had recent experience of health care within the centres represented within DSDnet. In addition, there were five professionals who ran the breakout sessions and ten people who represented support groups. The background of the professionals included endocrinology, psychology, nursing, sociology and urology. Topics discussed included experiences around diagnosis, childhood and experiences of young people, experiences of transition to adult services, the I-DSD registry, future research areas, obtaining consent in practice and patient education and information resources. All attendees acknowledged the constructive nature of the workshop. The collaborative model of the workshop offered valuable ideas to improve clinical services, perform patient oriented research and optimize the development and use of registries[25]. The model that was used for these workshops can be extended in the future to engage in a separate setting with human rights and intersex advocacy organizations.

GnRHnetwork included patients' perspectives in their annual meetings[27]. The patients' point of view formed an important component of these meetings, thus providing a novel opportunity for health-care professionals to identify particular gaps in patient care. In particular, the GnRH Cost Action conducted the first survey evaluating the unmet needs in 105 adult males with CHH[27]. The study was publicized online via a closed (private) CHH social media group (Facebook), CHH forum (chat room), a clinical trials registry and the COST Action website. The survey revealed that male

patients with CHH often have long gaps in care and still struggle with considerable psychosocial sequelae that are often unrecognized by the health-care community. A specific component within the GnRHnetwork's web-page was created and currently represents a friendly and functional tool for researchers and physicians in the EU and patients involved in the area of CHH. Researchers and physicians are able to connect to other members of the consortium through this web page, in order to share their data and establish productive collaborations. In addition, educational materials co-created by expert clinicians and patients, which have been translated into different languages, were developed and included in the web page[41]. The addition of the educational material will help patients and their families to understand all forms of GnRH deficiency, find centres of excellence to optimize their treatment, learn how to prepare for a visit with their doctor and discover research centres where they can participate in studies. This additional resource also means that patients and their families can browse online resources and support groups and find answers to their questions. This process might serve as a roadmap for creating patient education materials for other rare diseases.

< BOX 3 >

[H1] Future research needs

Future research priorities can be identified through the achievements of the international networks as they incorporated a broad variety of professionals with clinical and basic science backgrounds. In addition, the international networks also involved patients and patient advocates as important opinion leaders.

Needs that can be addressed by the systematic and standardized data collection strategies proposed by the COST Actions include the development of treatment protocols that improve clinical outcomes and quality of life[28]. Furthermore, the collection and analysis of long-term post-surgery data, the effect of living with atypical genitalia, gender well-being and overall health of individuals who have a DSD or CHH through the transition phase and at older ages need to be investigated. A major challenge that needs addressing is that all these data, which are often the result of medical management that took place several decades ago, need to be interpreted in the societal and medical context of today. Research priorities will have to be set in discussion with clinicians and patient advocates, who are currently involved as European Patient Advocacy Groups (EPAGs) in the Endo–ERN, acting as a partnership between patients, researchers and health-care

providers. The common objective of research into this area is the improvement of clinical care and long-term outcomes.

As increasing numbers of patients with rare, and presumably genetically determined complex conditions, undergo genomic sequencing[42], there is increasing concern about the ability to robustly establish causality for novel therapeutic candidates. Thus, basic research is equally as important and complements clinical science, as it minimizes misidentification of condition-related genes and an erroneous interpretation that will have severe consequences for the patients and their families. Some forms of DSD and CHH are very rare and providing statistical evidence in favour of causality might not be possible, despite data sharing platforms.

An important additional tool for the identification of novel therapeutic candidates consists of the study of mouse models. Variants in a human gene associated with sex development and maturation can be modelled in the mouse in a number of ways. First, a null (complete loss of function) allele can be generated rapidly using CRISPR–Cas9 genome editing technology — such a mouse variant immediately answers the question of whether the gene is required for normal sex development in a related mammal and conditional gene deletion approaches can also be performed[43, 44]. Secondly, specific sequence variants (point variants, for example) can also be introduced by genome editing[45]. This facility is important if the human variant does not represent a loss-of-function allele. Thirdly, gain-of-function might also be modelled by over-expression transgenesis[46]. The power of mouse models lies in the ability of researchers to perform examination of gene function in a whole organismal context, in a mammalian model that shares many fundamental genetic pathways with humans. Mouse models also permit the study of the role of genetic background, which can have profound effects on penetrance and expressivity.

The drawback to mouse models, however, is that they are not human. Differences between mouse and human reproductive biology are widespread. Another complementary tool for the analysis of novel gene variants consists of the use of cellular reprogramming technology. Here, candidate variants can be introduced into cell lines and/or induced pluripotent stem cells (iPSCs) and their effect on the reprogramming or derivation of somatic cells of either the testis or ovary can be assessed. Such approaches can be combined with genome editing techniques to permit the generation of cell lines in which rare variants are ‘corrected’ such that a common allele is now present, thereby acting as a powerful control.

The biology and genetics of a patient's response to treatment, the influence of a patient's genome on long-term outcomes and the potential development of comorbidities are poorly understood. In part, this lack of understanding reflects the need for us to further investigate the genetic aetiology of variant sex development and maturation in general, in addition to the absence of follow-up studies combined with deep phenotyping and harmonization of laboratory data. Thus, the collection of detailed and standardized phenotypic data over the longer term should be performed in parallel to the compilation of genetic data to improve our understanding of the wider health implications of existing and novel genetic variants in the field. Such studies will be made possible following the development of a European Registry for Rare Endocrine Conditions and by increasing the awareness and participation in existing registries[28].

The diagnostic pathway in patients with variant sex development and maturation requires close interlinking between the clinical, biochemical and genetic diagnostic work-up. The relevance of genetic testing for the differential diagnosis between CHH and constitutional delays of growth and puberty, a frequent para-physiological condition confounding the early recognition of patients with GnRH deficiency, was recently reported by a group of experts of the GnRH COST action[47]. However, currently, health-care systems across Europe differ greatly in structure and funding (see European Reference Networks on Rare Conditions (ERN) for examples). These disparities result in heterogeneous clinical and laboratory resources in the respective countries. In addition, and although highly desirable in such a complex field, interaction with research laboratories is often not possible, due to lack of access and funding, as well as ethical implications of data security measures. Consequently, diagnosis and management depend heavily on local or national pathways. With regards to reasonable hormonal determinations, highly qualified and specialized laboratories across European countries are required. This task is currently promoted by the ERN, which is due to be established in EU countries. Close interactions between patients and their families and health-care providers, as well as valid research, can improve medical care for people with conditions affecting sex development and maturation, and diminish the discomfort in society and the medical community towards gender variances[21].

< BOX 4 >

[H1] Conclusion

The development of two COST Actions — DSDnet and GnRHnetwork — led to key advances in the field of sex development and maturation, thanks to the involvement of experts from different disciplines and affected individuals. However, there is a clear need to continue the momentum in this rapidly evolving field with studies following on from this large body of work. Such studies would fill the gaps that still hamper basic understanding of the physiological and pathophysiological events and their inclusion into optimal patient management. Furthermore, ongoing progress must be made in science to aid other public and political stakeholders in their decision-making regarding various aspects of dealing with diversity of sex in society and culture.

These advancements might be possible through the development of Endo–ERN, which is a structure without time limits that harbors the clinical participants and stakeholders from the affected community within the political EU. Endo–ERN will make expert patient care visible and allow for clinical management progress. However, there are several issues that need to be addressed. First, the ERNs currently do not allow non-EU participants, because the ERNs have to adhere to the EU legal frameworks of health care. Second, one of the main purposes of the COST Actions, namely the networking of basic and clinical scientists, is restricted in ERNs because pure research institutions are not part of the ERNs at this time. Therefore, further possibilities that allow both clinical and translational science to be part of networking activities need to be sought. One possibility is the inclusion of ERNs and researchers into the European Joint Program Cofund, which was just positively reviewed and granted as a collaborative project in the European funding programme Horizon 2020, to promote research, education and training in rare diseases. Furthermore, connection to clinicians, translational and basic scientists outside of the European Union has to be sought and respective co-funding programmes for international collaborative research need to be developed.

Olaf Hiort[1*], Martine Cools[2], Alexander Springer[3], Ken McElreavey[4], Andy Greenfield[5], Stefan A. Wudy[6], Alexandra Kulle[7], S. Faisal Ahmed[8], Arianne Dessens[9], Antonio Balsamo[10], Mohamad Maghnie[11], Marco Bonomi[12,13], Mehul Dattani[14], Luca Persani[12,13] and Laura Audi[15] on behalf of COST Actions DSDnet and GnRHnetwork as well as the European Reference Network for Rare Endocrine Conditions (Endo–ERN)

1. Division of Paediatric Endocrinology and Diabetes, Department of Paediatrics and Adolescent Medicine, University of Lübeck and University Hospital of Schleswig-Holstein, Campus Lübeck, Lübeck, Germany

2. Department of Paediatric Endocrinology, Ghent University Hospital and Department of Internal Medicine and Paediatrics, Ghent University, Ghent, Belgium

3. Department of Paediatric Surgery, Interdisciplinary Center for Pediatric Urology, Medical University of Vienna, Vienna, Austria

4. Human Developmental Genetics, Department of Developmental and Stem Cell Biology, Institut Pasteur, Paris, France

5. Mammalian Genetics Unit, MRC Harwell Institute, Oxfordshire OX11 0RD, UK

6. Center for Child and Adolescent Medicine, Justus Liebig University, Giessen, Germany

7. Division of Paediatric Endocrinology and Diabetes, Christian-Albrechts-University Kiel & University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany

8. Developmental Endocrinology Research Group, School of Medicine, Dentistry & Nursing, University of Glasgow, Glasgow, UK

9. Department of Child- and Adolescent Psychiatry and Psychology, Erasmus Medical Center – Sophia, Rotterdam, The Netherlands

10. Paediatric Endocrinology Unit, External consultant of the Reference Center for Rare Endocrine Conditions, S.Orsola-Malpighi University Hospital, Bologna, Italy.

11. Departments of Paediatrics, IRCCS, Istituto Giannina Gaslini, University of Genova, Genova; Italy

12. Department of Clinical Sciences and Community Health, University of Milan, 20122 Milan, Italy

13. IRCCS Istituto Auxologico Italiano, Division of Endocrine and Metabolic Diseases, Milan, Italy

14. Molecular Basis of Rare Disease Section, Genetics and Genomic Medicine Programme, University College London, Great Ormond Street Institute of Child Health, London, UK

15. Growth and Development Research Unit, Vall d'Hebron Research Institute (VHIR), Center for Biomedical Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Barcelona, Spain

*e-mail: olaf.hiort@uksh.de

Author contributions

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Related links

COST Action DSDnet: www.dsdnet.eu

GHRH Network: www.gnrhnetwork.eu

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I-CAH Registry: www.i-cah.org

DSD-Life: https://www.dsd-life.eu/

DSDNet: www.dsdnet.eu

CHUV: www.chuv.ch/en/hhn/hhn-home/

Endo-ERN: https://endo-ern.eu/

European Commission European Reference Networks: https://ec.europa.eu/health/ern_en

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Box 1: Steps to **optimize diagnosis of DSD**

We need to be able to differentiate the diagnosis of differences of sex development (DSD) conditions, especially of 46,XY DSD, with a stratified approach using both biomarkers and next-generation sequencing methodology

- We need to be able to differentiate congenital hypogonadotropic hypogonadism versus constitutional delay of growth and puberty
- We need to identify novel biomarkers for diagnosis and assessment of the utility of refining the phenotype to enhance genetic testing
- We need to further understand the role of environmental factors (such as endocrine disruptors, nutrition and exercise) on sex development and delayed puberty or early onset central hypogonadism and later outcomes
- We need to develop personalized counselling of patients using results obtained by next-generation sequencing technologies

- We need to improve genetic counselling (monogenic versus oligogenic forms; estimation of variations of undefined significance; extremely variable penetrance and expressivity of heterozygous variants)

Box 2: Steps to **optimize patient management**

- We need to increase patient participation in an international registry; investigate and overcome the hurdles preventing such participation.
- The continuous assessment of outcome, patient satisfaction and quality of care is needed.
    - We need to understand the effect of delayed diagnosis and/or inappropriate treatment on psychological outcome
    - Patients need targeted psychosocial interventions and enhanced peer-to-peer support
- The development of clinical benchmarks based on outcomes reported by clinicians, patients and parents is required
- Greater involvement of patients and parents in setting research priorities will aide patient management
- We need to identify specialized care centers
    - Assessment of the quality of care delivered by the specialist centre
    - There is a need for professional education and development of the multi-disciplinary care team within the specialist centre
- A greater awareness of the availability of diagnostic tests in accredited labs is required
- Adherence to treatment and transition to adult services
    - Interventions to promote adherence and transitional care from pediatric to adult services
- Development of patients' associations in several countries

Box 3: **Need for randomized clinical trials (RCT)**

- RCTs for the induction of puberty: need to identify the best treatment and timing of initiating treatment both in differences of sex development (DSD) and in congenital hypogonadotropic hypogonadism (CHH)
- Need for multicentre RCTs in patients:
    - without minipuberty and micropenis at birth
    - with delayed puberty (start of treatment at 14–18 years)
    - Gonadotropins versus testosterone on fertility outcome in CHH
- Long-term studies evaluating: neonatal gonadotropin treatment to optimize fertility; gonadotropin treatment during adolescence versus adulthood for fertility optimization; and role of prior androgen treatments on fertility outcomes
- Optimal approach to the induction of fertility in male and female patients

Box 4: **Needs for acceptance of variant sex development**

- Integration of basic science into DSD-related research to explain the broad range of variabilities in sex development and maturation
- Development of defined prospective outcome studies of medical interventions and their omission for better understanding of patient needs in the medical context
- Guarantee psychosexual assessment and psychological support for patients with DSD and CHH
- Focusing on gender well-being rather than gender incongruence
- Defining personalized sex development as a human rights issue and reconciliation of opposing views on appropriate management
- Interactive multidisciplinary and interdisciplinary approaches of natural sciences and humanities for better understanding of the socio-cultural context
- Integration of a broad range of stakeholders into public information and education of both professionals as well as the lay public

Table 1: Research priorities that are considered to be important by clinicians (Survey by DSDnet Ref. [24])

Area of research	Sum	Average	Least important	Most important
Quality of life	1,005	13.05	0	24
Gender development	912	11.84	0	10
Sexual function	855	11.1	1	1
Genetic aetiology	815	10.58	0	5
Fertility	812	10.55	1	4
Communication and understanding	798	10.36	2	9
Hormone replacement	768	9.97	1	1
Biochemical investigation	730	9.48	3	6
Basic mechanisms	710	9.22	1	13
Cancer	601	7.81	2	2
Neurocognitive development	531	6.9	6	1
Epidemiology	514	6.68	10	1
Cardiovascular health	409	5.31	8	0
Bone health	406	5.27	9	0
Model organism research (such as mouse and zebrafish)	309	4.01	17	0
In vitro stem cell research	297	3.86	16	0

Survey based on 78 respondents (63% response rate). When asked whether “Fundamental research is priority in DSD research”, 86% of responders said yes, while 14% said no. When asked if “Molecular diagnosis as main goal for fundamental research”, 78% yes, while 22% said no.