Addressing the Gaps in the Care of People with Conditions Affecting Sex Development and Maturation

Recommendations for Future Research by COST Actions DSDnet and GnRHnetwork, and the European Reference Network for Rare Endocrine Conditions (Endo-ERN)

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Abstract

Development of sex and gender and the elucidation of variant physiology belong to the most complex topics, both in biology and in medicine. Differences or Disorders of Sex Development (DSD) are defined as conditions with divergence between chromosomal, gonadal, and phenotypic sex. Often, the pubertal development is also atypical. 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 cases. To expedite progress in the care of people affected by DSD and CHH, scientific networks have been funded by the European Union. Not only did two Actions of the Cooperation of Science and Technology (COST) program, DSDnet (BM1303) and GnRHnetwork (BM1105), provide the framework for ground-breaking research but they also allowed the development of position papers on diagnostic procedures and special laboratory analyses, as well as clinical management. Both Actions developed educational programs increasing expertise and promoting interest in this area of science and medicine. The clinical arms of the COST Actions formed the basis for the thematic group of conditions affecting sex development and maturation in the newly constituted European Reference Network for Rare Endocrine Conditions. This allowed evaluation of the progress made, but also definition of the several gaps that need to be addressed to improve the care of people with conditions affecting sex development and maturation. In addition, such lasting networks of experts and affected individuals are crucial to make progress in controversial areas of care that are debated today.

Introduction

The Cooperation of Science and Technology (COST) program aims to increase the networking possibilities for scientists and funds activities through the European scientific funding program Horizon 2020. COST Actions provide the opportunity to hold workshops and meetings, and additionally offer support for early stage researchers through short-term scientific missions and training schools. COST Actions are joined by countries within Europe, but also include near neighboring countries as well as international partners. Interestingly, COST Actions are not closed projects, but open to all opinion leaders from the participating countries.

COST Actions provide excellent tools for scientists involved in research around rare diseases in medicine, because here, expertise is often scattered, and effective networking is necessary for increasing knowledge and achieving appropriate attention. Recently, COST funded two European concerted Actions for the systematic elucidation of Differences of Sex Development (DSD) (www.dsdnet.eu) as well as for congenital hypogonadotropic hypogonadism (CHH) resulting from GnRH deficiency and frequently associated with anosmia (Kallmann syndrome, KS) and variable clinical features (www.gnrhnetwork.eu). Both Actions were similar in their structured approach regarding clinical care, genetic and laboratory, biology and basic research, as well as education and training.

The main objective was to promote research regarding 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 wanted to aid understanding of the underlying clinical heterogeneity as well as reveal the pathophysiological commonalities between different conditions at the molecular level. Additionally, they wanted to benefit the scientific investigation of rare diseases in the international community and also 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 and included the ESPE DSD Working Group, EuroDSD (funded by FP7), and the International DSD (www.i-dsd.org) and CAH registries (www.i-cah.org) , and sought collaboration with the parallel EU FP7 funded project DSDLife (www.dsd-life.eu). The GnRHnetwork began earlier, in April 2012, and 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 recent years due to cutting-edge research on mammalian development and elucidation of underlying genetic mechanisms. In parallel, descriptive clinical outcome studies have been conducted, which provided some insight into the long-term outcome of affected people; however, the results of these studies are mostly inconclusive due to the small sample sizes and broad heterogeneity of participants and due to 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 over 8,000 rare conditions known today. The ERN for Rare Endocrine Conditions (Endo-ERN) is divided into eight Main Thematic Groups (MTG), which cover all rare endocrine disorders over the life-span. It is the aim of Endo-ERN, 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.

Achievements of the COST Actions

Clinical assessment

The clinical findings of conditions affecting sex development and maturation may be highly variable and sometimes clinically undetectable. This holds true for conditions such as complete gonadal dysgenesis, where the external phenotype is female, even if the karyotype is 46,XY; as well as in 46,XX with CHH, who are unequivocally female at birth. All conditions may have associated features with complex syndromes affecting almost all organ systems, but mainly the kidneys, the heart, the peripheral and central nervous systems[1]. Therefore, any clinical investigation needs to take further developmental anomalies into account.

The clinical assessment needs to be age-dependent and includes an extensive whole-body and genital examination. In the young child, the inspection of the genitalia might reveal micropenis 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 may be a hallmark. Both COST Actions published recommendations about standardized clinical

assessment[2-4]. For DSD, the external masculinization score[5] 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 resulting tool will become available to be incorporated into online platforms such as the I-DSD registry (www.i-dsd.org) for collection of standardized phenotypic data.

A template for longitudinal follow-up was created in conjunction with the I-DSD registry. Within such an evaluation, overall health, endocrine status, genital anatomy and function, psychosocial items and gender congruence have to be considered equally important if we want to improve quality of life of patients having a DSD condition at all ages. Suboptimal outcomes in one or more of these domains should then lead to referral for in-depth expert evaluation and management. The evidence on which the selection of age intervals and data to be collected were based has been reviewed in Cools et al. 2018[4].

GnRHnetwork achieved multicenter trials replacing gonadotropins for the induction of fertility in patients with CHH[6, 7], and besides evaluated the psychosexual development in men with CHH[8], also highlighting the importance of a structured and life-long clinical assessment.

Genetics

Both Actions stratified a diagnostic approach to elucidate the underlying genetic anomaly and published position and consensus papers on this topic[2, 9]. In people with DSD, the determination of a karyotype is still seen as an initial mandatory step, since numerical or structural chromosomal abnormalities account for a considerable subset of DSD conditions. However, many patients with DSD or hypogonadotropic hypogonadism may have an unaffected chromosomal complement and detailed studies for molecular genetic conditions are needed.

Both Actions favored collaboration between basic scientists and clinicians to understand the molecular etiology 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[10-14]. The collaborative efforts in DSDnet led to the identification of variants in a new gene associated with 46,XY DSD[15] and mutations in a nuclear factor, *NR2F2*, which cause a novel syndromic form of 46,XX DSD[16]. 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 a consensus document from 2015, a first European Consensus Statement on CHH was published, covering aspects related to the pathogenesis, diagnosis and treatment of this rare condition[2]. 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 (eg., CHH+hearing loss particularly frequent among cases with *SOX10*, *IL17RD*, *CHD7* gene defects), and provided recommendations for improved treatment of CHH examining early, but also later, outcomes including future fertility.

Despite these important advances, the majority of patients particularly with 46,XY conditions affecting sex development and maturation, excluding primary errors of the endocrine system, do not have a molecular diagnosis. The accurate clinical interpretation of high-throughput sequencing datasets is challenging. This is in part due to emerging evidence that these conditions may be caused by variants in many different genes and the prevalence of variants in a single gene may be very low. To build robust evidence to support causality, there is a need to both share genomic data between research groups and develop informative animal and cellular models. DSDnet established a secure platform for sharing data between research groups, called “SDgeneMatch”. 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 matchmaking systems do exist, SDgeneMatch is currently specific for DSD and the DSD research community will be actively encouraged to submit data into the system. This can accelerate gene/variant discovery in the field and encourage collaboration with groups working on animal/cellular model systems in order to provide evidence of causality as well as explore the molecular pathways that are involved, and it will lead to a more accurate molecular diagnosis for DSD.

Endocrine Assessment

Rare endocrine conditions are often genetically determined and feature hormonal imbalances as the result of divergent endocrine pathways. However, in childhood and puberty, endocrine abnormalities may not be easily detected and tend to be highly variable depending on age and developmental stage of the affected person.

The COST Actions therefore aimed to identify appropriate laboratory determinations useful in the complex differential diagnostics of DSD and CHH; and second, to develop guidelines for the usefulness of specific laboratory analyses and testing conditions. These guidelines aimed at the implementation of clinical standards for diagnosis and appropriate treatment of DSD and CHH to achieve the best outcome for patients, no matter where patients are investigated or managed[2, 17-19].

In a position paper, all forms of DSD were summarized and, for each condition, the required hormonal work-up and suitable analytical techniques were described. The main recommendations were: 1) to support the appropriate use of both immunoassay- and mass spectrometry-based methods for the diagnosis and monitoring of DSD; 2) that use of both serum and urine is established and appropriate matrices used for analysis of steroids; and 3) that laboratories should aim to participate in activities of peer comparison[17].

A next step in harmonization of laboratory assessments affected the important plasma/serum analyte, 17-hydroxyprogesterone, a key marker in the diagnosis of congenital adrenal hyperplasia. Hereto, in collaboration with colleagues in China, Singapore and Australia, a worldwide survey on mass spectrometric determinations of this analyte was conducted. This resulted in another publication[20] which 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/serum 17OHP by mass spectrometry[20].

Education and Training

Both COST Actions employed the specific tools of the COST program to provide training and education to early stage researchers. Specifically, they developed the Action websites www.dsdnet.eu and www.chuv.ch/en/hhn/hhn-home/, 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 www.dsdnet.eu/dissemination.html and www.chuv.ch/en/hhn/hhn-home/neuroendocrine-control-of-reproduction/publications), meetings (6 WG meetings/workshops for DSDnet, the last one in combination with GNRHnetwork) and position papers[2, 4, 9, 17, 21].

The Actions used part of their funds to provide training for eligible young scientists and organized three Training Schools (TSs) each and with the primary objective of providing multidisciplinary training to young professionals (trainees) and encouraging ongoing activity in the field of DSD and CHH. These interactive meetings, each including approximately 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 TS was to encourage the trainees to be involved in and to improve the national and international networking capabilities. Within DSDnet, for example, through participation in the I-DSD registry (www.i-dsd.org), through grant and fellowship applications in the field of DSD and by becoming active members of the forming European Reference Network for Rare Endocrine Conditions (Endo-ERN https://endo-ern.eu/). A report that analyzed, through specific surveys, the success and subsequent outcome of the TSs for the trainees was recently published[22]. Briefly, the high rate of positive responses from trainees demonstrates the success of the TS model that DSDnet adopted and shows that the great majority of the participating trainees are still active in the DSD field. This positive result justifies the continuation of this form of postgraduate multidisciplinary training.

Another educational tool is the organization of the Short-Term Scientific Missions (STSM). They were aimed at supporting individual mobility and at strengthening the existing networks 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/years 2015-2017; 3.33/year) and COST Action GnRHnetwork (n.23/years 2013-2016; 5.75/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 higher participation of basic scientists in GnRHnetwork, willing and able to perform an STSM in another laboratory site. In contrast, the participation of clinical scientists in such exchanges may be hampered by their clinical duties.

Improving patient care

A major task is the promotion and optimization of patient management and care for the complex conditions involving 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[23, 24].

The survey aimed at paediatric endocrinologists revealed that 40% of the DSD centres had a multidisciplinary team (MDT) available at initial presentation. Half of the centres reported sharing their data in a multicentre registry. Local access to specialist biochemical and genetic tests influenced the diagnostic process, and detailed molecular genetic testing was increasingly 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. A number of research priorities were suggested, highlighting amongst others the need for more studies that aim at understanding and improving the quality of life (Table 1). In another survey access to and modalities of psychosocial care, organisation and practice, was investigated. It was learned that psychosocial care is mainly provided to parents and focuses on coping and acceptance of the diagnosis and the atypical genital, decisions on genital surgery, disclosure and education. Adults have less access to counselling and given that this age group may particularly require support coping with a range of DSD-related health and psychosexual issues, this is an area which deserves attention in the future. Among providers of psychosocial care there is a large variety in training background. This and the absence of an overarching professional body hinder professional development[24].

The assessment of patient needs was particularly emphasized by both Actions. A DSDnet face to face workshop that was held for 33 patients, their relatives and professionals included one or more patient(s) and a healthcare professional from the same centre from eight different European countries[25]. In addition, there were five professionals who ran the breakout sessions and there were also 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 young people experiences, 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.

GnRHnetwork included patients' perspectives into their annual meetings[26]. The patients' point of view formed a significant component of these meetings, thus providing a novel opportunity to

identify particular gaps in patient care. A specific component within the Action's web-page (www.gnrhnetwork.eu) was created and currently represents a friendly and functional tool for EU researchers/physicians 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. Additionally, educational materials co-created by expert clinicians and patients and translated in different languages were developed and were included in the web page. This will help patients and their families to understand all forms of GnRH deficiency, find centers of excellence to optimize their treatment, learn how to prepare for a visit with their doctor, discover research centers where they can participate in studies, browse online resources and support groups, and finally find answers to their questions.

Future Research Needs

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

Needs that can be addressed by the proposed systematic and standardized data collection include the development of treatment protocols that improve clinical outcomes and quality of life[27]. Furthermore, the collection and analysis of long-term post-surgery data, the impact of living with atypical genitalia, gender well-being, and overall health in individuals who have a DSD or CHH through the transition phase and at older ages need to be investigated. A major challenge is that all these data, 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 is the improvement of clinical care and long-term outcomes.

As larger numbers of patients with rare and presumably genetically determined complex conditions are subject to genomic sequencing, there is an increasing concern about the ability to robustly establish causality for novel candidates. Thus, basic research is equally 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 may not be possible, despite data sharing platforms. One important additional tool 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: i) 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; ii) Specific sequence variants (point variants etc.) can also be introduced by genome editing, and this facility is important if the human variant does not represent a loss-of-function allele; iii) Gain-of-function may also be modelled by over-expression transgenesis. The power of the mouse model lies in the ability to perform examination of gene function in a whole organismal context, in a mammalian model that shares many fundamental genetic pathways with humans. It also permits study of the role of genetic background, which can have profound effects on penetrance and expressivity.

The drawback of mouse models, however, consists of them not being 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/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 the patient’s response to treatment, the influence of the patient’s genome on long-term outcomes and the potential development of comorbidities are very poorly understood. In part, this reflects our lack of understanding of the genetic aetiology of variant sex development and maturation in general and the virtual absence of follow-up studies combined with deep phenotyping and harmonization of laboratory data. Thus, collection of detailed and standardized phenotypic data over the longer term should be performed in parallel to improve our understanding of the wider health implications of existing and novel genetic variants in the field. This will be approached through the development of a European Registry for Rare

Endocrine Conditions, and through increasing the awareness and participation in existing registries[27].

The diagnostic pathway in patients with variant sex development and maturation requires close interlinking between the clinical, biochemical and genetic diagnostic work-up. However, currently, healthcare systems across Europe differ greatly in structure and funding. This results 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. Consequently, diagnosis and management depend heavily on local or national pathways. Regarding reasonable hormonal determinations, highly qualified and specialized laboratories across European countries are required. This task is currently promoted by the European Reference Networks on Rare Conditions (ERN https://ec.europa.eu/health/ern_en), to be established in EU countries.

Conclusion

The development of two COST Actions led to key advancements in the field of sex development and maturation thanks to the involvement of experts from different disciplines and affected individuals. However, there is also a clear need to continue the momentum in this rapidly evolving field, with studies following on from this large body of work to solve 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.

This might be possible through the development of Endo-ERN, which is a structure without time limits, harboring 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, because pure research institutions are not part of the ERNs at this time. Therefore, further possibilities allowing both clinical as well as 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 Horizon 2020, to promote research, education and training in rare diseases. Furthermore, connection to clinicians, translational and basic scientists outside of Europe has to be sought and respective co-funding programs for international collaborative research need to be developed.

Box: **Optimization of diagnosis**

- Differential diagnosis of DSD conditions, especially of 46,XY DSD with a stratified approach using both biomarkers and next generation sequencing methodology
- Differential diagnosis of CHH vs constitutional delay of growth and puberty (CDGP)
- Discovery of novel biomarkers for diagnosis and assessment of the utility of refining the phenotype to enhance genetic testing
- Role of environmental factors (eg disruptors, nutrition, exercise) on sex development and delayed puberty or early onset central hypogonadism and later outcomes
- Personalized counselling of patients using results obtained by next-generation sequencing technologies
- Genetic counselling (monogenic versus oligogenic forms; estimation of variations of undefined significance (VUS); extremely variable penetrance and expressivity of heterozygous variants)

Box: **Optimization of Patient Management**

- Increasing patient participation in an international registry; investigate and overcome the hurdles preventing such participation.
- Continuous assessment of outcome, patient satisfaction and quality of care.
    - Impact of delayed diagnosis and/or inappropriate treatment on psychological outcome
    - Targeted psychosocial interventions and enhanced peer-to-peer support
- Development of clinical benchmarks based on outcomes reported by clinicians, patients and parents
- Greater involvement of patients and parents in setting research priorities
- Identification of specialized care centers
    - Assessing the quality of care delivered by the specialist centre
    - Need for professional education and development of the Multi-Disciplinary Care within the specialist centre
- Greater awareness of the availability of diagnostic tests in accredited labs
- 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: **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 DSD and in CHH
- Need for multicenter RCTs in patients:
    - without minipuberty and micropenis at birth
    - with delayed puberty (start of treatment at 14-18 years)
    - Gonadotropins vs 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 males and females

Table 1: Research priorities that are considered to be important by clinicians (Survey by DSDnet)

AREAS OF RESEACH RANKED IN ORDER OF IMPORTANCE					
	Area of Research	Sum	Average	Least Important	Most Important
1	Quality of Life	1005	13.05	0	24
2	Gender Development	912	11.84	0	10
3	Sexual Function	855	11.1	1	1
4	Genetic Aetiology	815	10.58	0	5
5	Fertility	812	10.55	1	4
6	Communication & Understanding	798	10.36	2	9
7	Hormone Replacement	768	9.97	1	1
8	Biochemical Investigation	730	9.48	3	6
9	Basic Mechanisms	710	9.22	1	13
10	Cancer	601	7.81	2	2
11	Neurocognitive Development	531	6.9	6	1
12	Epidemiology	514	6.68	10	1
13	Cardiovascular Health	409	5.31	8	0
14	Bone Health	406	5.27	9	0
15	Model organism research (e.g.mouse, zebrafish)	309	4.01	17	0
16	In-vitro stem cell research	297	3.86	16	0
			Yes		No
Fundamental research is priority in DSD research			86%		14%
Molecular diagnosis as main goal for fundamental research			78%		22%

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