Clinical and genetic characteristics of a large international cohort of individuals with rare *NR5A1*/SF-1 variants of sex development

Chrysanthi Kouri,[a,b,c,k] Grit Sommer,[a,b,d,k] Idoia Martinez de Lapiscina,[a,b,e,f,g,h] Rawda Naamneh Elzenaty,[a,b,c] Lloyd J. W. Tack,[i] Martine Cools,[i] S. Faisal Ahmed,[j] and Christa E. Flück,[a,b,*] SF1next study group[l]

[a]Pediatric Endocrinology, Diabetology and Metabolism, Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Bern 3010, Switzerland
[b]Department for BioMedical Research, University of Bern, Bern 3008, Switzerland
[c]Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern 3012, Switzerland
[d]Institute of Social and Preventive Medicine, University of Bern, Switzerland, University of Bern, Bern 3012, Switzerland
[e]Research into the Genetics and Control of Diabetes and Other Endocrine Disorders, Biobizkaia Health Research Institute, Cruces University Hospital, Barakaldo 48903, Spain
[f]CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Instituto de Salud Carlos III, Madrid 28029, Spain
[g]CIBER de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Madrid 28029, Spain
[h]Endo-ERN, Amsterdam 1081 HV, the Netherlands
[i]Department of Paediatric Endocrinology, Department of Paediatrics and Internal Medicine, Ghent University Hospital, Ghent University, Ghent 9000, Belgium
[j]Developmental Endocrinology Research Group, University of Glasgow, Royal Hospital for Sick Children, Glasgow G51 4TF, UK

eBioMedicine
2024;99: 104941
Published Online xxx
https://doi.org/10.1016/j.ebiom.2023.104941

Summary

Background **Steroidogenic factor 1 (SF-1/*NR5A1*) is essential for human sex development. Heterozygous *NR5A1*/SF-1 variants manifest with a broad range of phenotypes of differences of sex development (DSD), which remain unexplained.**

Methods **We conducted a retrospective analysis on the so far largest international cohort of individuals with *NR5A1*/SF-1 variants, identified through the I-DSD registry and a research network.**

Findings **Among 197 individuals with *NR5A1*/SF-1 variants, we confirmed diverse phenotypes. Over 70% of 46, XY individuals had a severe DSD phenotype, while 90% of 46, XX individuals had female-typical sex development. Close to 100 different novel and known *NR5A1*/SF-1 variants were identified, without specific hot spots. Additionally, likely disease-associated variants in other genes were reported in 32 individuals out of 128 tested (25%), particularly in those with severe or opposite sex DSD phenotypes. Interestingly, 48% of these variants were found in known DSD or SF-1 interacting genes, but no frequent gene-clusters were identified. Sex registration at birth varied, with <10%**

*Corresponding author. University Children's Hospital Bern, Freiburgstrasse 65 / C845, Bern 3010, Switzerland.
E-mail address: christa.flueck@unibe.ch (C.E. Flück).
[k]Shared first authorship for equal contribution.
[l]***Members of SF1next study group:*** Saygin Abali, Acibadem Mehmet Ali Aydinlar University, School of Medicine, Istanbul, Turkey; Zehra Yavas Abali, Marmara University, Department of Pediatric Endocrinology and Diabetes, Pendik, Istanbul, Turkey; Leyla Akin, Department of Pediatric Endocrinology and Diabetes, Ondokuz Mayis University, Samsun, Turkey; Maricruz Almaraz, Department of Endocrinology and Nutrition, Hospital Regional Universitario de Málaga, Instituto de Investigación Biomédica de Málaga-IBIMA, Málaga, Spain; Laura Audí, Growth and Development Group Vall d'Hebron Research Institute (VHIR), CIBERER, Barcelona, Spain; Murat Aydin, Department of Pediatric Endocrinology and Diabetes, Ondokuz Mayis University, Samsun, Turkey; Antonio Balsamo, Researcher of Alma Mater Studiorum, S. Orsola-Malpighi, University Hospital, Bologna, Italy; Federico Baronio, Department of Medical and Surgical Sciences, IRCCS S. Orsola-Malpighi, University Hospital, Bologna, Italy; Jillian Bryce, Office for Rare Conditions, University of Glasgow, Glasgow, United Kingdom; Kanetee Busiah, Paediatric Endocrinology, Diabetology and Obesity Unit, Lausanne University Hospital, University of Lausanne, Lausanne, Switzerland; Maria Caimari, Hospital Universitario Son Espases, Palma de Mallorca, Spain; Núria Camats-Tarruella, Growth and Development Group, Vall d'Hebron Research Institute (VHIR), CIBERER, Barcelona, Spain; Ariadna Campos-Martorell, Hospital Universitario Vall d'Hebron, Barcelona, Spain; Luis Castaño, Biobizkaia Health Research Institute, Cruces University Hospital, UPV-EHU, CIBERDEM, CIBERER, Endo-ERN, Barakaldo, Spain; Anna Casteràs, Department of Endocrinology, Hospital Universitario Vall d'Hebron, Barcelona, Spain; Semra Çetinkaya, University of Health Sciences Turkey, Ankara, Turkey, Clinic of Pediatric Endocrinology, Dr. Sami Ulus, Obstetrics and Pediatrics Training and Research Hospital, Ankara, Turkey; Yee-Ming Chan, Division of Endocrinology, Boston Children's Hospital, Boston, Massachusetts, USA, Department of Pediatrics Harvard Medical School, Boston, Massachusetts, USA, Eunice Kennedy Shriver National Institute of Child Health and Human Development grant R01 HD089521; Hedi L Claahsen - van der Grinten, Department of Paediatric Endocrinology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; Ines Costa, Pediatric Department, Manises Hospital, Manises,

undergoing reassignment. Gonadectomy was performed in 30% and genital surgery in 58%. Associated organ anomalies were observed in 27% of individuals with a DSD, mainly concerning the spleen. Intrafamilial phenotypes also varied considerably.

Interpretation The observed phenotypic variability in individuals and families with *NR5A1*/SF-1 variants is large and remains unpredictable. It may often not be solely explained by the monogenic pathogenicity of the *NR5A1*/SF-1 variants but is likely influenced by additional genetic variants and as-yet-unknown factors.

Funding Swiss National Science Foundation (320030-197725) and Boveri Foundation Zürich, Switzerland.

Keywords: Steroidogenic factor 1 (SF-1/*NR5A1*); Differences of sex development (DSD); Broad phenotype; Genetics of sex determination and differentiation; Intersex

Spain; Fatma Feyza Darendeliler, Istanbul University, Istanbul Faculty of Medicine, Pediatric Endocrinology Unit, Istanbul, Turkey; Justin H Davies, Southampton Children's Hospital, University Hospital Southampton, Southampton, United Kingdom; Isabel Esteva, Endocrinology Department, Gender Identity Unit, Regional University Hospital of Malaga, Málaga, Spain; Helena Fabbri-Scallet, Laboratory of Human Molecular Genetics, Center of Molecular Biology and Genetic Engineering (CBMEG)/Unicamp, Sao Paulo, Brazil; Courtney A Finlayson, Division of Endocrinology, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois, USA; Emilio Garcia, Hospital Virgen del Rocío, Sevilla, Spain; Beatriz Garcia-Cuartero, Pediatric Endocrinology Department, Ramón y Cajal University Hospital, Madrid, Spain; Alina German, Haemek Medical Center, Afula, Israel, Technion, Israel Institute of Technology; Evgenia Globa, Ukrainian Research Center of Endocrine Surgery, Endocrine Organs and Tissue Transplantation, Paediatric Endocrinology Department, Ministry of Health of Ukraine, Kyiv, Ukraine; Gil Guerra-Junior, Interdisciplinary Group for the Study of Sex Determination and Differentiation – GIEDDS and Department of Pediatrics, School of Medical Sciences, State University of Campinas, Sao Paulo, Brazil; Julio Guerrero, Hospital Infantil La Paz, Madrid, Spain; Tulay Guran, Marmara University, Department of Pediatric Endocrinology and Diabetes, Pendik, Istanbul, Turkey; Sabine E Hannema, Department of Paediatric Endocrinology, Amsterdam UMC location Vrije Universiteit, Amsterdam, the Netherlands, Erasmus Medical Center, Sophia Children's Hospital, Department of Pediatric Endocrinology, Rotterdam, the Netherlands and Leiden University Medical Center, Department of Pediatrics, Leiden, Netherlands; Olaf Hiort, Division of Pediatric Endocrinology and Diabetes, Department of Pediatrics, University of Lübeck, Lübeck, Germany; Josephine Hirsch, Division of Urology, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois, USA; Leuan Hughes, Department of Paediatrics, University of Cambridge, Cambridge, United Kingdom; Marco Janner, Pediatric Endocrinology, Diabetology and Metabolism, Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland; Zofia Kolesinska, Department of Pediatric Endocrinology and Rheumatology, Poznan University of Medical Sciences, Poznan, Poland; Katherine Lachlan, Wessex Clinical Genetics Service, University Hospital Southampton, Southampton, United Kingdom; Anna Lauber-Biason, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland; Dagmar L'Allemand, Department of Endocrinology, Children's Hospital of Eastern Switzerland, St. Gallen, Switzerland; Jana Krenek Malikova, Department of Paediatrics, Second Faculty of Medicine of Charles University and University Hospital Motol, Prague, Czech Republic; Nina Lenhnerr-Taube, Division of Pediatric Endocrinology and Diabetology and Children's Research Center, University Children's Hospital, University of Zurich, Zurich, Switzerland; Angela Lucas–Herald, Developmental Endocrinology Research Group, School of Medicine, Dentistry & Nursing, University of Glasgow, Glasgow, United Kingdom; Jamala Mammadova, Department of Pediatric Endocrinology and Diabetes, Ondokuz Mayis University, Samsun, Turkey; Kenneth McElreavey, Human Developmental Genetics, Institute Pasteur, Paris, France; Veronica Mericq, Institute of Maternal and Child Research, University of Chile, Santiago, Chile; Isabel Mönig, Division of Pediatric Endocrinology and Diabetes, Department of Pediatrics, University of Lübeck, Lübeck, Germany; Francisca Moreno, Hospital Infantil La Fe, Valencia, Spain; Julia Mührer, Division of Pediatric Endocrinology and Diabetology and Children's Research Center, University Children's Hospital, University of Zurich, Zurich, Switzerland; Marek Niedziela, Department of Pediatric Endocrinology and Rheumatology, Poznan University of Medical Sciences, Poznan, Poland; Anna Nordenstrom, Pediatric Endocrinology, Karolinska University Hospital, Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden; Burçe Orman, University of Health Sciences Turkey, Ankara; Clinic of Pediatric Endocrinology, Dr. Sami Ulus, Obstetrics and Pediatrics Training and Research Hospital, Ankara, Turkey; Sukran Poyrazoglu, Istanbul University, Istanbul Faculty of Medicine, Pediatric Endocrinology Unit, Istanbul, Turkey; Jose M Rial, Pediatric Endocrinology Department, Hospitem Rambla, Santa Cruz de Tenerife, Spain; Meilan M Rutter, Division of Endocrinology, Cincinnati Children's Hospital Medical Center, Department of Pediatrics, University of Cincinnati, Cincinnati, USA, DSD Translational Research Network, USA; Amaia Rodríguez, Biobizkaia Health Research Institute, Cruces University Hospital, UPV-EHU, CIBERDEM, CIBERER, Endo-ERN, Barakaldo, Spain; Tara Schafer-Kalkhoff, Division of Endocrinology, Cincinnati Children's Hospital, Cincinnati, USA; Kay-Sara Sauter, Pediatric Endocrinology, Diabetology and Metabolism, Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland, Department for BioMedical Research, University of Bern, Bern, Switzerland; Sumudu Nimali, Seneviratne Department of Paediatrics, University of Colombo, Colombo, Sri Lanka; Maria Sredkova-Ruskova, University Pediatrics Hospital, Medical University, Department of Clinical Genetics, Sofia, Bulgaria; Rieko Tadokoro-Cuccaro, Department of Paediatrics, University of Cambridge, Cambridge, United Kingdom; Ajay Thankamony, Department of Paediatrics, Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom; Mónica Tomé, Department of Endocrinology and Nutrition, Hospital Regional Universitario de Málaga, Instituto de Investigación Biomédica de Málaga-IBIMA, Málaga, Spain; Amaia Vela, Biobizkaia Health Research Institute, Cruces University Hospital, UPV-EHU, CIBERDEM, CIBERER, Endo-ERN, Barakaldo, Spain; Malgorzata Wasniewska, University of Messina, Department of Human Pathology of Adulthood and Childhood, Messina, Italy; David Zangen, Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel, Pediatric Endocrinology Unit, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; Nataliya Zelinska, Ukrainian Scientific and Practical Center of Endocrine Surgery, Transplantation of Endocrine Organs and Tissues of the Ministry of Health of Ukraine, Kyiv, Ukraine.

Research in context

Evidence before this study

Steroidogenic Factor 1/Nuclear Receptor Subfamily 5 Group A Member 1 (SF-1/*NR5A1*) plays a pivotal role in the development and functioning of human sex and steroid organs. *NR5A1*/SF-1 variants are associated with rare differences of sex development (DSD), giving rise to a wide spectrum of phenotypes ranging from healthy carriers to severe forms of DSD. Despite numerous studies addressing the possible mechanism underlying these diverse clinical presentations of *NR5A1*/SF-1 variants, a comprehensive explanation for the broad range phenotypes remains elusive. Recent studies indicate potential organ anomalies in *NR5A1*/SF-1 variant carriers, especially spleen-related issues. Oligogenic inheritance appears to play a role in the variable phenotypes seen in individuals with a DSD and *NR5A1*/SF-1 variants, with multiple genetic variants collectively influencing the manifestation. Advanced next-generation sequencing (NGS) and bioinformatics tools are crucial for identifying such complexities, although assembling large cohorts for study remains challenging due to the rarity of specific conditions within the broader DSD spectrum.

Added value of this study

Our large international cohort study provides comprehensive phenotype-genotype data on 197 individuals with a *NR5A1*/SF-1 variant, collected retrospectively. It informs on new insights and provides guidance for clinicians involved in counselling and care for individuals carrying a *NR5A1*/SF-1 variant. 46, XY individuals manifest most often with a severe DSD phenotype, with variable sex registration at birth, and abnormal pubertal development later in life. Most 46, XX individuals with a *NR5A1*/SF-1 variant show female-typical sex development, but some have primary ovarian insufficiency or opposite sex DSD. Spleen anomalies are frequent findings. Additional variants in other genes related to sex development are often found, suggesting a possible oligogenic disease mechanism.

Implications of all the available evidence

NR5A1/SF-1 variants are often identified in individuals manifesting with a broad spectrum of rare DSD conditions requiring specific follow-up and management depending on individual's characteristics. Spleen anomalies are a frequent finding with significant consequences calling for screening for all carriers of *NR5A1*/SF-1 variants. In contrast, other organ anomalies are seldom seen. Individuals with a *NR5A1*/SF-1 variant and a severe DSD phenotype need close follow-up at puberty and beyond. Further studies are needed to inform about long-term outcomes. Genotype-phenotype correlation remains elusive. Additional variants in other genes, mostly related to DSD, are often found, but no clusters have been identified pointing towards a complex mechanism of DSD and emphasizing the importance of considering a broader genetic context in the evaluation of *NR5A1*/SF-1 variants.

Introduction

Human sex development is complex and may be disturbed at any stage of gonadal sex determination and/or sex differentiation and maturation. A critical factor in this multistep process is Steroidogenic Factor 1/Nuclear Receptor Subfamily 5 Group A Member 1 (SF-1/*NR5A1*), which plays a crucial role in the development and function of sex organs, in steroidogenesis and beyond.[1] SF-1 was initially identified as a transcription factor that regulates gene expression of steroidogenic enzymes.[2,3] Further research in the mid-1990s showed its importance in the typical development of the adrenal glands and gonads, as the *Nr5a1* (*Ftzf1*) knock-out mouse presented with adrenal and gonadal agenesis, male-to-female sex reversal, persistent Müllerian structures, absence of the ventromedial hypothalamus, and a small spleen.[4–7] The first human disease-causing *NR5A1*/SF-1 variant was described in 1999.[8] This phenotypically female patient presented with primary adrenal failure and 46, XY complete sex reversal, similar to the *Nr5a1* knock-out mouse. Since then, numerous patients with *NR5A1*/SF-1 variants have been reported, but their phenotypic manifestation has been vast.[9] Disease-causing *NR5A1*/SF-1 variants are common among individuals with mild to severe differences of sex development (DSD).[9–11] However, *NR5A1*/SF-1 variants may also be found in healthy carriers (individuals with a *NR5A1*/SF-1 variants who do not have DSD), infertile men or in women with primary ovarian insufficiency (POI).[12–15] Variability in DSD phenotypes exist between individuals with different *NR5A1*/SF-1 variants, but also among those with the same variant, even within families.[9,10,16,17] This variability complicates genetic counselling, prognostic prediction, and preventive health care for carriers of *NR5A1*/SF-1 variants.

Even less is known beyond the DSD phenotype for those with *NR5A1*/SF-1 variants. It has been suggested that these individuals may have other associated organ anomalies that may lead to adverse (metabolic) health outcomes later in life. Spleen anomalies have, for instance, been described in several patients[18,19] and in the mouse model.[20] Whether *NR5A1*/SF-1 variants affect blood pressure and late-onset obesity remains in question.[21–23]

So far, more than 218 *NR5A1*/SF-1 variants have been reported according to the Human Gene Mutation Database (HGMD).[24] They are distributed throughout

the entire gene with no apparent hot spots, and they can manifest as missense, nonsense, frameshift, insertions, deletions, or complex variants. Heterozygous inheritance is most common, but the broad phenotype cannot be attributed to a dominant negative mechanism of action.[14] Haploinsufficiency has also been considered, but so far not confirmed.[25] Numerous studies have attempted to understand the mechanism of disease-causing *NR5A1*/SF-1 variants using various *in vitro* tools and bioinformatic approaches[14,26–30]; however, the reason for the lack of genotype–phenotype correlation remains unclear. Therefore, it remains a challenge to anticipate how different *NR5A1*/SF-1 variants influence the expression of SF-1 target genes and the levels of proteins, regulators, and modulators in the complex networks of adrenal and gonadal development, as well as steroidogenesis,[1,15] leading to the ultimate phenotype.

Recent studies have suggested that oligogenic inheritance may contribute to the variability of the phenotype observed with *NR5A1*/SF-1 variants,[16,17,27,31,32] and other forms of DSD.[33–35] Oligogenic inheritance refers to the interaction of multiple genetic variants, each of which may contribute to the phenotype.[36] This concept is particularly relevant in the context of complex genetic conditions such as DSD, where the presence of multiple genetic variants may explain the variability and severity of the phenotype, when a single gene variant does not suffice. To identify oligogenicity in complex conditions such as DSD, next-generation sequencing (NGS) technologies and advanced bioinformatic pipelines are necessary and may help solve unexplained genotype–phenotype correlations. Although demonstrating the impact of multiple gene variants will remain a challenge, investigation of a large cohort may be the first step towards identifying common genes of a core network for a complex condition.[37] However, as DSD comprises a large group of individually rare genetic conditions, such large cohorts have not existed until recently when international registries and collaborations have been established.[38–40]

To address gaps in understanding the broad variability of rare DSD associated with *NR5A1*/SF-1 variants, a worldwide collaborative project called SF1next was initiated to collect data on the largest cohort to date. By employing a standardized dataset and adhering to international recommendations for optimal clinical care of DSD individuals,[41] we aimed to phenotype and genotype individuals and families with *NR5A1*/SF-1 variants from existing data retrospectively. We particularly focused on describing the precise DSD phenotype, intrafamilial DSD, additional genetic variants related to DSD, and other associated organ anomalies. The findings of this study provide insights into the complex genotype–phenotype correlations of individuals with *NR5A1*/SF-1 variants and contribute to the development of better diagnostic and treatment strategies for individuals with DSD.

Methods

Identification of eligible individuals and study design

Individuals were eligible if they had a genetically confirmed *NR5A1*/SF-1 variant. We excluded individuals with 46, XX disorders of androgen excess, disorders of Müllerian development or cloacal exstrophy, and 46, XY DSD individuals with Leydig cell defects, persistent Müllerian duct syndrome or cloacal exstrophy as these defects are caused by other specific genetic defects. We identified individuals with *NR5A1*/SF-1 variants through the I-DSD registry (https://sdmregistries.org/), by contacting clinicians and researchers who had previously reported data of individuals with *NR5A1*/SF-1 variants, or through the DSD research community. All individuals who were identified consented to participate in international studies. We also included family members with confirmed *NR5A1*/SF-1 variants. We collaborated with clinical partners in 55 centers from 18 countries (Fig. 1a). We excluded individuals with insufficient data in mandatory variables (e.g., precise genetic description of the *NR5A1*/SF-1 variant at DNA and protein level, karyotype, phenotypic details allowing categorization of DSD severity, etc.).

Data collection and phenotype

Data collection took place between September 2019 and August 2022. We collected standardized variables at predefined age intervals based on current international recommendations for optimal clinical care of DSD individuals[41] and developed a Research Electronic Data Capture (REDCap) database for data entry (Supplementary Table S1). Each clinical partner entered anonymized data on the phenotype and on available results of genetic analyses of individuals with *NR5A1*/SF-1 variants in the REDCap database.[42,43] If available, we used existing data from the I-DSD registry on DSD diagnosis and karyotype; otherwise, we surveyed the clinicians and captured these data in the REDCap database. We described the phenotype of overall health according to the terminology of the Human Phenotype Ontology (HPO) vocabulary.[44,45] We asked for associated organ anomalies of the adrenal glands, urinary system, metabolism and homeostasis, endocrine system, blood system, immune system, spleen, abdomen, respiratory system, cardiovascular system, vasculature, integument, connective tissue, musculature, head and neck, skeletal system, central nervous system (CNS), peripheral nervous system (PNS) and psychosocial disorders. We defined abnormalities of the endocrine system as not associated with a DSD, if the individual had received growth hormone, thyroid hormone or insulin. Individuals with more than 50% of missing data with respect to information on associated organ anomalies were not included in this analysis. Collected data (basic data) of family members with *NR5A1*/SF-1 variants for

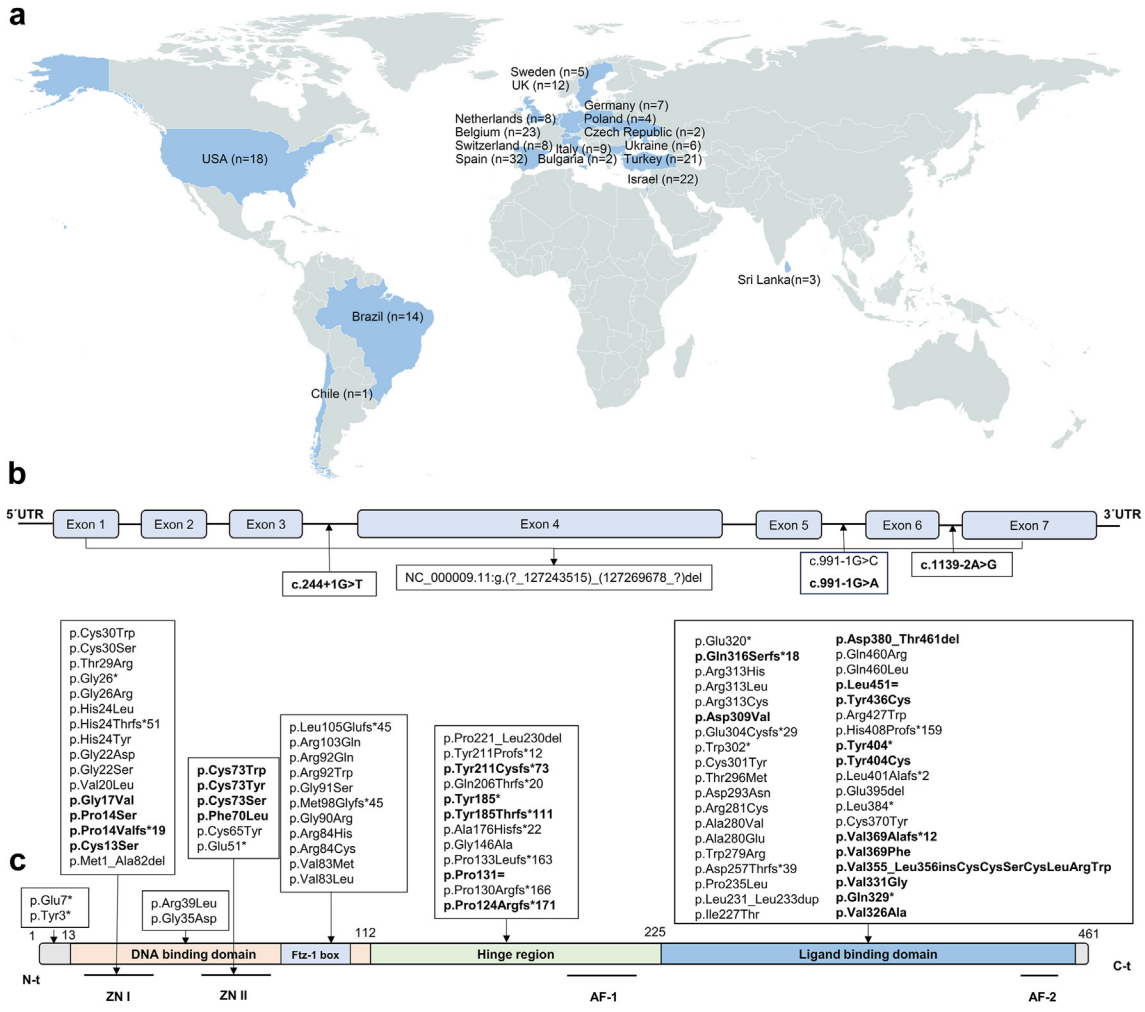

***Fig. 1:* Overview of the participants and summary of the *NR5A1*/SF-1 variants in the international SF1next study cohort.** (a) Number of individuals collected from each country comprising the SF1next study cohort are shown (n = 197). (b and c) Identified variants in the *NR5A1* gene are shown with respect to the gene and protein sequence. (b) Location of four intronic variants and one whole gene deletion of *NR5A1*/SF-1 (NC_000009.11). (c) Location of 87 *NR5A1*/SF-1 variants identified in the SF1next cohort, shown at their protein level (NM_004959.5). Novel variants are shown in bold, while previously reported variants are shown in normal font. The SF-1 protein comprises the DNA-binding domain, which contains two zinc fingers (Zn1 and Zn2), a FTZ-F1 box, the accessory hinge region, and the ligand-binding domain. It harbors two activation functional domains, AF-1 and AF-2. *NR5A1*, nuclear receptor subfamily 5 group A member 1; UTR, untranslated region.

whom we did not have informed consent included the degree of relationship to index patient, genetic details of their *NR5A1*/SF-1 variant and a brief description of overall health and DSD phenotype as routinely assessed by physicians in patient's family history during consultations with index patients.

We used a modified external genitalia score (EGS), similar to the one described previously[46] to classify the severity of the DSD. This classification was based on the karyotype and external genitalia phenotypic features at birth or before genital surgery. For the modified EGS, we assessed phenotypic features of five anatomical landmarks of the external genitalia: degree of labioscrotal fusion, length of the genital tubercle, position of the urethral meatus, and locations of the right and left gonads. We defined four categories based on the results of the modified EGS assessment: typical for karyotypic sex (typical female-type phenotype, typical male-type phenotype), mild DSD, severe DSD, and opposite sex karyotype (see Supplementary Table S2). We classified family members in whom only minimal information (basic data) was available into these four groups, based on DSD or related conditions reported by the clinicians. We categorized pubertal development into three groups: normal, abnormal, or unknown. Individuals who did not experience a spontaneous start of

puberty, received sex hormones, had a Tanner stage outside the expected range for the given age, or did not have menarche by the age of 15 were classified as having abnormal puberty. For family members, we asked clinicians to directly indicate the puberty category without asking for further details. Individuals who were too young at last follow up to draw conclusions about pubertal development (aged <13 years for 46, XX karyotype or <14 years for 46, XY karyotype) were not included in the descriptive analyses of puberty.

Genetic data collection and analysis

Genetic data were provided by collaborators. The methods used for genetic analysis included candidate gene analysis (CGA), gene panels, whole exome sequencing (WES)/whole genome sequencing (WGS) approach and array comparative genomic hybridization (aCGH). We assessed the *NR5A1*/SF-1 gene variants for each individual according to the NM_004959 reference sequence. We then reanalysed all genetic data available for their disease-causing effect using various *in silico* webtools: Polyphen-2 (Polymorphism Phenotyping v2), Panther (Protein Analysis Through Evolutionary Relationships), SNPs and GO, CADD (Combined Annotation Dependent Depletion), and the calibrated scores given by VarSome[47] for Revel (Rare Exome Variant Ensemble Learner), SIFT (Scale-invariant feature transform), Provean (Protein Variation Effect Analyzer), Mutation taster and M-CAP (Mendelian Clinically Applicable Pathogenicity). These webtools, provide insights on the impact of identified genetic variants on structure and functionality of the corresponding proteins. We assessed potential pathogenicity using ACMG (American College of Medical Genetics and Genomics) criteria[48] using VarSome and searched previously reported clinical associations in ClinVar and HGMD[49] databases. Pathogenicity of variants in other genes found in these individuals were assessed using a similar pipeline. In addition, we tested, if reported additional variants were included in our previously reported algorithm searching for oligogenic disease related to DSD with *NR5A1*/SF-1,[16] as well as previously described in the literature.[31] We also tested variants classified as VUS, LP or P found in combination with *NR5A1*/SF-1 variants for oligogenic pathogenicity using the Oligogenic Resource for Variant AnaLysis (ORVAL) online platform.[50]

Ethics

All data included in the SF1next study cohort were collected in anonymized form. We had ethical approval and informed consent from all study participants providing comprehensive data. All study participants had a family history taken, and this information included the reported genetic information of family members. If we were able to contact such family members and receive their study participation informed consent, they became study participants with comprehensive study data included. In family members that we were not able to contact and receive informed consent, we were allowed to include basic data as of our ethical approval. The University of Glasgow has ethical approval and provides guidelines to international partner centers for collecting routine data of individuals with a DSD in the I-DSD registry (UKCRN ID 12729). International clinical partners had approval from their respective ethical committees.

Statistics

Descriptive analysis included median with interquartile ranges (25th percentile P25 and 75th percentile P75) for continuous variables and frequencies and percentages for categorical variables. For anthropometric variables (birth weight at gestational age, height and BMI), SDS (standard deviation score) were calculated based on karyotype, using reference data of the general population from the INTERGROWTH-21st study[51] and the World Health Organisation (WHO).[52] Standard deviation scores are the z scores representing the number of standard deviations by which an individual's anthropometric variables differ from the population mean at a certain age. We conducted one-sample Wilcoxon signed-rank tests to assess the median of differences between the SF1next study cohort and the general population, with the assumption that the median of differences equals zero. We also tested differences of anthropometric variables (SDS) between the DSD phenotype groups using Kruskal–Wallis tests. We used Stata 16.1 statistical software for data cleaning and analyses and R software (version 4.0.3, 2020-10-10) for graphs and figures.

Role of the funders

None of the funding sources (Swiss National Science Foundation and Boveri Foundation Zürich), which supported this study, had any role in study design, data collection, analysis, interpretation, manuscript writing or decision for publication.

Results

Description of the SF1next study cohort

In the SF1next study, a total of 222 eligible individuals with *NR5A1*/SF-1 variants were identified, 74 (33%) through the I-DSD registry and 148 (67%) by directly contacting DSD researchers (Fig. 1a and Supplementary Fig. S1). After excluding 23 individuals (10%) for incomplete data, the final SF1next study cohort included 197 individuals with verified *NR5A1*/SF-1 variants, consisting of 113 (57%) index cases with comprehensive data, and 84 (43%) family members. Among the 84 family members, we had comprehensive data on 15 and basic data on 69 individuals. Of all 197 individuals, 131 (67%) had a DSD phenotype and seven (4%) women had

POI. The majority had a 46, XY karyotype (79%, 155/197), 20% (40/197) had a 46, XX karyotype, one individual had a 47, XXY and another a 47, XYY karyotype. Almost all individuals (94%, 185/197) were heterozygous for *NR5A1*/SF-1 variants, only eleven (6%) were homozygous, and one was compound heterozygous; mosaic variants were found in four (2%). In total, 93 different *NR5A1*/SF-1 variants were found, with 29 (31%) being variants that, to the best of our knowledge, were not previously reported (Fig. 1b and c and Supplementary Table S3). Most variants were located in the DNA-binding domain (DBD) (38%, 35/93) and the ligand binding domain (LBD) (41%, 38/93) of the SF-1 protein (Fig. 1c). The predicted pathogenicity of these variants varied considerably: 47% (44/93) were likely pathogenic (LP), 31% (29/93) were pathogenic (P), 17% (16/93) were variants of unknown significance (VUS) and 4% (4/93) were benign (B) or likely benign (LB) variants (Supplementary Table S3). The majority of the variants were substitutions (70%, 65/93), followed by deletions (22%, 20/93), insertions or/and duplications (3%, 3/93) and delins (2%, 2/93).

DSD phenotype and genotype classification

We classified all individuals into four groups based on the severity of the DSD phenotype as detailed in the methods section and in Supplementary Table S2. Using this classification, 41% revealed a severe DSD phenotype, 35% had a typical phenotype for their karyotype, 19% an opposite sex DSD phenotype and 5% had a mild DSD phenotype (Fig. 2a and b). For five (3%) 46, XY male individuals, complete data were unavailable, but DSD was not reported, therefore we classified them as having a typical male-type phenotype. Among the 155 individuals with a 46, XY karyotype, 49% (76/155) had a severe DSD phenotype, followed by 23% (36/155) with an opposite sex phenotype, 21% (33/155) with a typical male-type phenotype and 7% (10/155) with a mild phenotype. Among the 40 individuals with a 46, XX karyotype, 90% (36/40) had a typical female-type phenotype and 10% (4/40) a severe DSD phenotype. Both individuals with a 47, XYY or 47, XXY karyotype had an opposite sex DSD phenotype.

For all individuals, identified *NR5A1*/SF-1 variants were newly tested for predicted pathogenicity using different bioinformatic tools, incorporating the ACMG classification and data related to DSD phenotypes. Variants classified as P, LP or VUS (95%, 187/197) were found in individuals across all four DSD phenotype groups. LB and B variants were found in individuals who had severe DSD phenotype (90%, 9/10) or typical male-type phenotype (10%, 1/10) (Fig. 2c). Thus, no association between the predicted pathogenicity of the *NR5A1*/SF-1 variants and the severity of the DSD phenotypes was observed.

Additional characteristics of individuals with *NR5A1*/SF-1 variants

Table 1 and Supplementary Figs. S2 and S3 provide detailed characteristics of 128 individuals with comprehensive data. Of 121 individuals with DSD, 45% (54/121) of individuals with a 46, XY karyotype were registered female at birth, while 2% (3/121) of individuals with 46, XX karyotype were registered male. Eleven individuals with a 46, XY karyotype had sex reassignment, nine from female to male, one from male to female, and one from other to male; eight before and three after the age of two years (at 4, 10 and 19 years, respectively). At their last consultation, 30% of individuals were reported to have undergone gonadectomy at a median age of 17 years (P25 13, P75 22), and all of these had an opposite sex or severe DSD phenotype (Supplementary Fig. S2f).

Moreover, 58% were reported to have undergone genital surgery at a median age of 12 years (P25 6, P75 16), most of them with a severe phenotype (Supplementary Fig. S2g and h). Out of the 74 individuals with genital surgery, 70 had a 46, XY karyotype. Of these, 38 (54.3%) underwent masculinizing genitoplasty, 13 (18.6%) feminizing genitoplasty, 11 (15.7%) had both masculinizing genitoplasty and orchidopexy and seven (10%) had only orchidopexy. One individual had orchidopexy in combination with phalloplasty. In the four individuals with a 46, XX karyotype, three had masculinizing and one had feminizing genitoplasty.

Pubertal development was assessed in 109 individuals (54 with a DSD, 5 with POI) as described in the methods section. Seventy-one individuals were too young to assess puberty, and 17 individuals had missing data. In 62 individuals, puberty was normal, of whom 81% did not have a DSD (24 46, XX and 26 46,XY) and 19% had a 46, XY DSD (seven with a mild and five with a severe DSD phenotype, Supplementary Fig. S4). Forty-seven individuals (43%) had abnormal puberty, of whom 42 had a DSD phenotype (35 46,XY, 5 46,XX, two with 47, XYY or 47,XYY); 57% showed an opposite sex DSD, 38% a severe DSD, and 5% a typical female-type phenotype (46,XX). Thirty of these 42 individuals had gonadectomy, 12 at an early age, thus spontaneous pubertal development could not be assessed; in two individuals, data were not available. Sixteen individuals underwent gonadectomy at later age, and prior to the procedure, they experienced abnormal puberty (five with streak gonads). After gonadectomy, 28 individuals received pubertal induction and/or hormonal replacement therapy with normal pubertal development thereafter observed in three, while in 17 individuals the treatment response assessed by Tanner stage progression was insufficient. Four individuals had gonadectomy in adulthood and in four data were missing. Of eight 46, XY individuals with a severe DSD without gonadectomy, normal pubertal development was

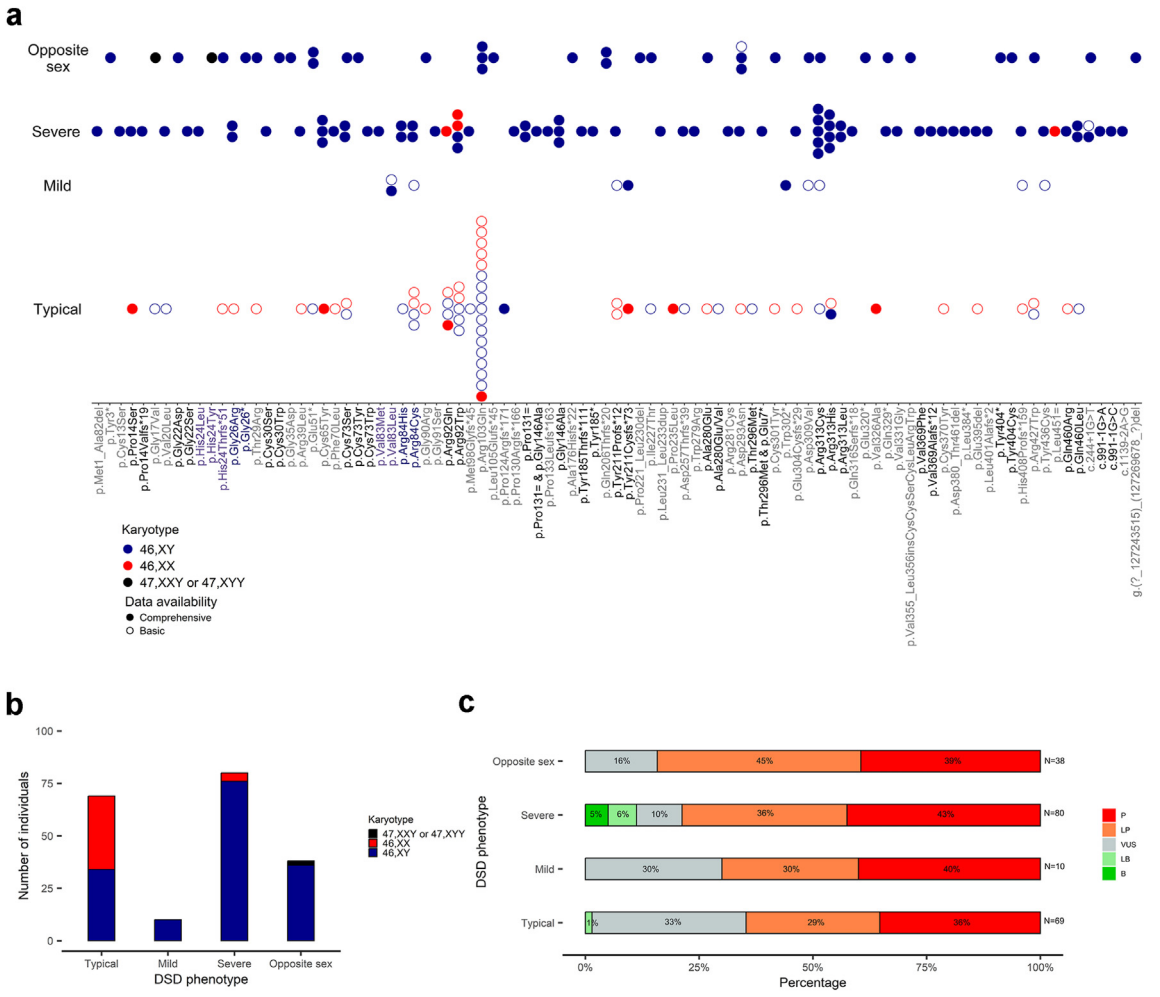

***Fig. 2:* Characterization of *NR5A1*/SF-1 variants identified in the SF1next study cohort.** (a) Genotype-phenotype correlation. Each dot represents one individual (n = 197) with the corresponding DSD phenotype of the external genitalia and karyotype, stratified by karyotype and type of data. Filled dots show individuals who required medical care while white filled dots show individuals who came to medical attention because of the genetic workup of their *NR5A1*/SF-1 positive relatives (i.e., family members with basic data who did not require medical care because of DSD). *NR5A1*/SF-1 variants affecting the same amino acid residue/intronic region are highlighted in black, purple or blue colour, while others are shown in light grey. (b) Karyotype and DSD phenotype of individuals in the cohort. (c) Summary of the pathogenicity of the 93 different *NR5A1*/SF-1 variants according to ACMG classification, stratified by DSD phenotype of the identified individuals in the cohort. P, pathogenic, LP, likely pathogenic, VUS, variant of unknown significance, LB, likely benign, B, benign.

reported in five, while three were noted to have abnormal puberty.

Germ cell tumors of the gonads were reported in two individuals at the age of three and 21 years, respectively (2/128, 1.6%).

Based on available anthropometric data (Table 1 and Supplementary Fig. S3), median birth weight SDS of newborns with *NR5A1*/SF-1 variants was - 0.7 SDS (P25–1.3, P75–0.1) ($p < 0.0001$, Wilcoxon signed-rank test). At their last consultation (median age 14 years (P25 9, P75 17)), median height SDS was - 0.2 (P25 -1, P75 0.6) ($p = 0.181$, Wilcoxon signed-rank test) and median body mass index (BMI) SDS was 0.6 (P25–0.5, P75 1.7) ($p = 0.021$, Wilcoxon signed-rank test). Adult height SDS (available in 14 individuals) was - 0.8 SDS (P25–1.8, P75 0.6) ($p = 0.064$, Wilcoxon signed-rank test). We did not find a difference in birth weight, height or BMI between the four DSD phenotype groups (birth weight $p = 0.443$, height $p = 0.715$, BMI $p = 0.519$, Kruskal–Wallis test).

Associated organ anomalies

We had information on associated organ anomalies for 173 individuals (88%); 116 had DSD and six had POI. Out of 57 individuals without DSD or with POI, eight (14%) had associated organ anomalies. Of the 116

	Total cohort		46,XY		46,XX		47, XXY or 47,XYY	
	n	%	n	%	n	%	n	%
Year of birth								
<1989	13	10	8	7	4	36	1	ND
1990–<1999	22	17	18	16	4	36	0	0
2000–<2009	43	34	41	36	1	9	1	ND
2010–<2021	50	39	48	42	2	18	0	0
Age at last follow-up (years)								
<5	28	22	27	24	0	0	1	ND
5–<10	29	23	26	22	3	27	0	0
10–<15	27	21	27	24	3	27	1	ND
15–<25	32	25	28	24	5	46	0	0
25–<55	12	9	7	6	0	0	0	0
Sex registration at birth								
Male	61	48	58	50	3	27	0	0
Female	64	50	54	47	8	73	2	ND
Other	1	1	1	1	0	0	0	0
Unknown	2	2	2	2	0	0	0	0
Sex reassignment	11	9	11	10	0	0	0	0
No reassignment	117	92	104	90	0	0	0	0
DSD phenotype								
Typical	9	7	2	2	7	64	0	0
Mild	3	2	3	3	4	36	0	0
Severe	79	62	75	65	0	0	0	0
Opposite sex	37	29	35	30	0	0	2	ND
DSD related surgery								
Biopsy of gonads								
Yes	30	23	25	22	4	36	1	ND
No	95	74	87	75	7	64	1	ND
Unknown	3	2	3	3	0	0	0	0
Gonadectomy								
Yes	39	30	37	32	1	9	1	ND
No	87	68	76	66	10	91	1	ND
Unknown	2	2	2	2	0	0	0	0
Genital surgery								
Yes	74	58	70	61	4	36	0	0
No	50	39	41	36	7	64	2	ND
Unknown	4	3	4	3	0	0	0	0

	n*	Median (P25,P75)	n*	Median (P25, P75)	n*	Median (P25, P75)	n*	Value (P25,P75)
Year of birth	128	2008 (1999–2012)	115	2008 (2001–2013)	11	1994 (1982–2009)	2	1992 (ND)
Age at last follow-up (years)	128	12 (6–18)	115	12 (5–16)	11	25 (10–35)	2	10 (ND)
Gestational age (weeks)	93	38.7 (38–40)	87	38.7 (38–40)	5	39.6 (39–40)	1	32 (ND)
Birth weight (SDS)	90	−0.7 (−1.3 to −0.1)	84	−0.7 (−1.3 to −0.2)	5	−0.8 (−0.1 to −0.3)	1	1.7 (ND)
BMI at last follow-up (SDS)	67	0.6 (−0.5 to 1.7)	60	0.6 (−0.6 to 1.7)	6	−0.02 (−0.8 to 1.5)	1	2.3 (ND)
Height at last follow-up (SDS)	69	−0.2 (−1 to 0.6)	60	−0.2 (−0.9 to 0.6)	8	−0.5 (−2 to 0.8)	1	0.2 (ND)

n*, Number available for analysis; ND, not determined, median (P25, P75), median with interquartile ranges (25th percentile P25 and 75th percentile P75).

Table 1: **Characteristics of 128 individuals with comprehensive data in the SF1next study cohort.**

individuals with DSD, 38 (33%) had associated organ anomalies (Fig. 3a): 55% had a severe DSD phenotype, 32% had an opposite sex DSD phenotype, 5% had a mild DSD phenotype, and 8% had a typical female-type phenotype. Among the 38 individuals with associated organ anomalies, one organ system was affected in 39% (15/38), two organ systems in 47% (18/38), three organ systems in 8% (3/38), and more than three organ systems in 6% (2/38) (Fig. 3b and c). The spleen and blood system were most frequently affected (45%),

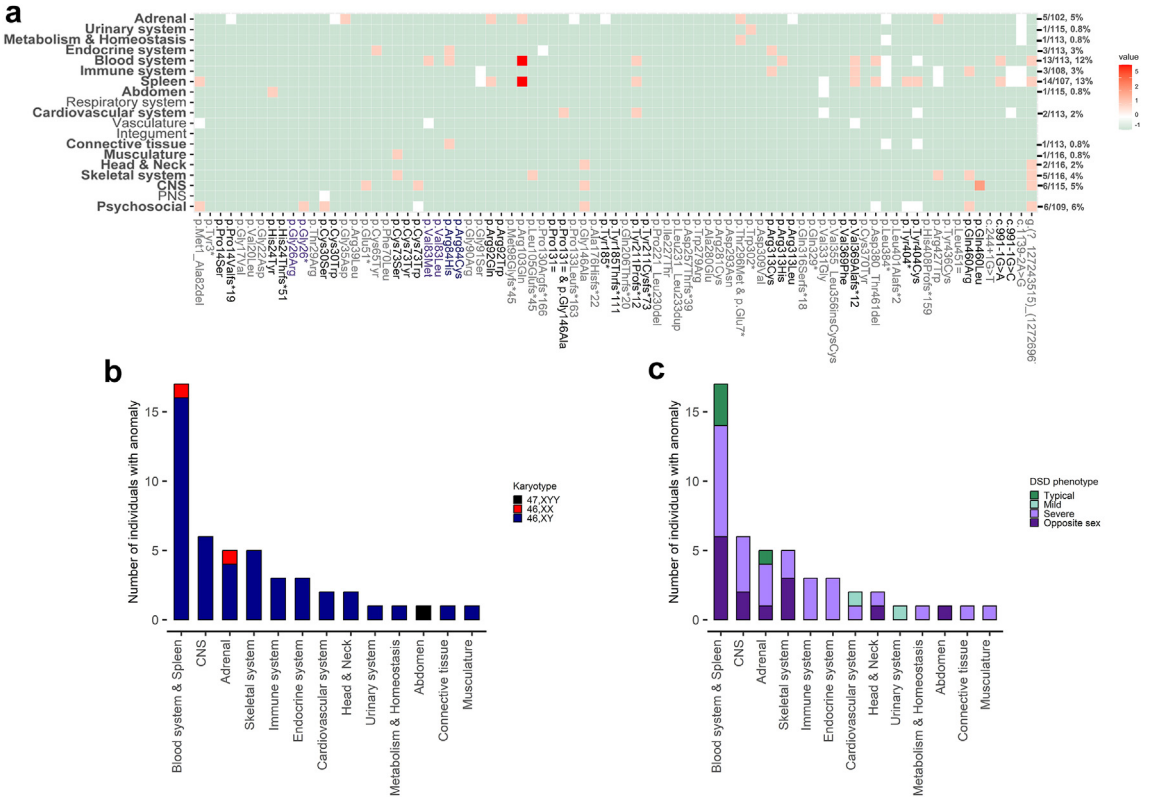

***Fig. 3*: Associated organ anomalies found in individuals with a DSD and *NR5A1*/SF-1 variants (n = 116).** (a) Heatmap depicts frequency of organ anomalies found with specific *NR5A1*/SF-1 variants. Sufficient information on organ anomalies was available in 116 out of 131 individuals with a DSD. *NR5A1*/SF-1 variants affecting the same amino acid residue/intronic region are highlighted in blue or purple colour, while others are shown in light grey. The colour of a single cell provides the information on how many individuals are affected (light red to dark red (≥1)) or unaffected (green (≤−1)) by the specific organ anomaly (see value scale). Organ systems with reported anomalies are shown in bold. Number of individuals with reported anomaly divided by the number of individuals with sufficient information per organ system is shown on the right side of the heatmap. Individuals with organ anomalies and without DSD (n = 8) are not reported in the figure; in those, anomalies were found in the blood system and spleen (3/8), metabolism and homeostasis (2/8), endocrine system (1/8), head and neck (1/8), skeletal system (1/8), musculature (1/8), CNS (1/8), cardiovascular system (1/8), connective tissue (1/8), or abdomen (1/8). For white coloured cells no data were available. (b and c) Range of anomalies reported in each organ system according to karyotype (b) or (c) DSD phenotype (n = 38). CNS, Central nervous system; PNS, Peripheral nervous system.

followed by the central nervous system (16%). Interestingly, only five individuals (13%) had adrenal anomalies, with three of them having homozygous *NR5A1*/SF-1 variants, one a compound heterozygous and another a heterozygous variant. Among the 38 individuals with reported organ anomalies, 31 different *NR5A1*/SF-1 variants were identified. Pathogenic variants were most frequent (42%), followed by LP variants (35%), VUS (13%), LB (7%) and B (3%) variants. We also searched for correlations between specific *NR5A1*/SF-1 variants and associated anomalies in these individuals. Apart from the *NR5A1*/SF-1 variant p.Arg103Gln (c.308G > A), which was identified in five individuals of four unrelated families with spleen anomalies, we did not observe any other significant correlations.

Intrafamilial DSD

In 48 families (42%), we had data of at least one relative with the same *NR5A1*/SF-1 variant as the index case. Among these 48 families, we identified 84 relatives, of whom 21 (25%) individuals from 16 families had a DSD phenotype. The intrafamilial DSD phenotype in these 16 families varied significantly, with 12 family members (57%) presenting the same phenotype, seven (33%) a less severe phenotype, and two relatives (10%) presenting a more severe phenotype than their respective index family member (Supplementary Fig. S5).

Genetic findings - additional genetic variants

Information on genetic testing used for the identification of *NR5A1*/SF-1 variants was available from 128 individuals. Of these, 58 were tested with CGA, 42 with

gene panels, 22 with WES, two with WGS and four with aCGH (Supplementary Fig. S6a).

Additional genetic variants (n = 81) were reported for 32 individuals using different genetic approaches, such as CGA (6%, 2/32), aCGH (9%, 3/32), gene panels (53%, 17/32) and WES (31%, 10/32). Of the 81 variants in 62 genes, 42 variants (52%) were identified in 34 DSD-related or SF-1-related genes (Supplementary Table S4). Each individual had between one to 16 additional variants (see Supplementary Fig. S7). We classified all the reported additional variants for their pathogenicity according to ACMG guidelines and found that most variants were VUS (29/81, 36%), followed by LB (23/81, 28%), B (20/81, 25%), P (6/81, 7%), and LP variants (3/81, 4%) (Supplementary Fig. S6b and c). Testing of potential disease-causing additional gene variants (VUS, LP, P), together with the specific combined *NR5A1*/SF-1 variants in 21 individuals using ORVAL, indicated oligogenic pathogenicity in eight individuals (38%) (Supplementary Table S5). Of the 32 individuals with additional variants, 59% (19/32) had a severe DSD phenotype, 34% (11/32) had an opposite sex DSD phenotype and only 6% (2/32) had a typical female-type phenotype (Supplementary Fig. S7). Ten of 32 individuals (31%) with additional genetic variants and DSD were found to have associated organ anomalies. No obvious correlations were found between the additional gene variants and the associated organ anomalies.

Discussion

NR5A1/SF-1 is among the three genes for which variants are most often identified in individuals with a 46, XY DSD.[53] Unlike variants in the androgen receptor (*AR/NR3C4*) and 5α-reductase type 2 (*SRD5A2*), which affect androgen synthesis and action and therefore sex differentiation exclusively,[54,55] *NR5A1*/SF-1 variants affect both early sex determination (e.g. gonad formation) and sex differentiation. Therefore, *NR5A1*/SF-1 variants can also lead to (ovo)testicular DSD in 46, XX individuals,[11,56,57] while X-linked *AR* mutations and autosomal recessive variants in *SRD5A2* cause a DSD phenotype in 46, XY individuals only. Overall, the mechanism of DSD associated with *NR5A1*/SF-1 variants is more complex and there are still large gaps in our understanding of this disorder, including the very broad phenotype, lack of genotype–phenotype correlation, as well as the mostly heterozygous mode of inheritance. This study provides new insights into DSD through a comprehensive description and analysis of the largest international cohort to date, consisting of 197 individuals carrying *NR5A1*/SF-1 variants. In these individuals, we confirmed the very broad range of possible phenotypes, ranging from severe or even opposite sex DSD in more than 70% of individuals with a 46, XY karyotype, to healthy carriers predominantly in 46, XX females (90%). Our study also highlights that the DSD phenotype varies within the families studied. We identified both novel and known, mostly heterozygous variants scattered throughout the whole *NR5A1*/SF-1 gene without hot spots. Pathogenicity testing using ACMG guidelines[48] revealed no genotype–phenotype correlation for specific *NR5A1*/SF-1 variants. Genetic testing using gene panels or WES discovered additional gene variants in 25% of 128 individuals with *NR5A1*/SF-1 variants tested, more likely in those with a severe or opposite sex DSD phenotype. Almost half of the identified additional variants were found in DSD-related and/or SF-1-related genes. The various additional genes did not point to recurrent, preferred gene partners; and some individuals had several additional variants, with 36% of variants classified as VUS. Overall, our data indicate that the observed phenotypic variability in individuals with identified *NR5A1*/SF-1 variants cannot be explained only by the *NR5A1*/SF-1 variants, and that additional genetic hits or other unknown factors may be involved.

The wide spectrum of phenotypes and possible genotype–phenotype correlation associated with *NR5A1*/SF-1 variants remain unsolved. Lack of correlation has been reported in several smaller studies[9,10,14,17] and can be confirmed by the data of this larger cohort study. Nevertheless, it is interesting that the p.Arg92 variants (p.Arg92Trp or p.Arg92Gln) manifest consistently in 15 46, XX individuals with a (ovo)testicular DSD, and in two 46, XY individuals with sex reversal, whether in heterozygous or homozygous state, although the variants have also been found in 14 unaffected individuals.[8,11,56–60] The specific mechanism of action of this variant has been studied, and it has been suggested that it downregulates the pro-ovarian Wnt4/β-catenin pathway.[11,56,58,59]

By contrast, many hypotheses have been formulated to explain the variability and complexity observed with *NR5A1*/SF/1 variants, including, a) haploinsufficiency and variable expressivity, e.g., by skewed allelic expression b) tissue-specific somatic reversion mechanisms, c) mosaicism, d) additional genetic hits (e.g., genetic modifiers), e) epigenetic regulation and other non-Mendelian genetic contributions, and f) gene–environment interactions. The genetic network comprising *NR5A1*/SF-1 is large and includes transcription factors, co-modulators, posttranslational modulators and signaling molecules.[1,61–63] *NR5A1*/SF-1 seems to be regulated by epigenetic modification, such as methylation,[64] and post-transcriptional mechanisms involving phosphorylation.[65] Thus, several mechanisms may contribute to the complexity of DSD associated with *NR5A1*/SF-1 variants. However, more recently, NGS approaches for genetic workup of individuals with DSD have revealed additional variants in several other genes, in combination with known and novel *NR5A1*/SF-1 variants, supporting the hypothesis that phenotypic variability might be due to oligogenic

inheritance.[17,26,31,32,37,66–68] In addition, a recent study suggested that non-coding *NR5A1/SF-1* variants may contribute to the DSD phenotype.[69]

Multilocus variant burden has been shown to impact the occurrence of rare diseases, both within and between families.[70] This has been reported for other rare endocrine disorders such as hypogonadotropic hypogonadism.[34,35] Our study points towards possible oligogenic causation of DSD associated with *NR5A1*/SF-1 variants, at least in some individuals. Additional variants in candidate genes possibly contributing to the disease phenotype were reported in 32 of the individuals with a DSD in our SF1next cohort. This number may even increase in the future with rising awareness of possible mode of oligogenic inheritance and the use of NGS approaches for genetic analysis,[31,37,71] although many of these identified variants may not be disease-causing and could be detected by chance as part of the genetic background. Confirmation of an oligogenic mode of inheritance is challenging, and calculating the occurrence of variants by chance is not feasible for small cohorts and non-standardized genetic methods. In our study, we assessed the reported variants using established bioinformatic tools following current guidelines, which are mostly designed for evaluating monogenic disorders.[16,72,73] So far, only very few bioinformatic tools are available to check for oligogenic pathogenicity, e.g., ORVAL including VarCoPP (Variant Combination Pathogenicity Predictor).[50,74] Using these tools, we found potential oligogenic pathogenicity in a third of tested individuals with a DSD. But, of course, final proof whether and how these variants in combination contributed to the DSD phenotype remains to be demonstrated. From a genetics perspective, trio family analysis and finding additional individuals with the same spectrum of gene variants can help. Experimental approaches using cell models or animal models to investigate pathogenicity are also challenging, as there are limitations to how many genes can be manipulated simultaneously, and simplified modelling might produce false negative results. In addition, classic cell experiments that investigated the promoter activation or nuclear translocation of different *NR5A1*/SF-1 variants have shown uncertain results.[8,14,21,26,28,75–77] Even mouse models do not consistently reflect the human phenotype, as only *Sf1* knock-out mice showed adrenal insufficiency and sex reversal, while heterozygous mice did not recapitulate the broad phenotype seen in humans.[78–80] However, these specific findings may also point towards an oligogenic mode of disease mechanism where a monogenic *NR5A1*/SF-1 variant is insufficient to explain the observed complex and individual phenotype.[34,35,81] In the future, experimental models of patient-derived biomaterials, which carry the individual's full genetic background and can be reprogrammed into tissue-specific cell lines, may help in evaluating the concerted impact of specific gene variants on the DSD phenotype and enhance our understanding of oligogenic disease mechanisms.

Our study aimed to investigate the SF1next cohort beyond genetics and the DSD phenotype. We found anomalies in other organ systems in 27% of individuals, which were more frequent in those with a DSD phenotype (33%), than in those without (14%). Moreover, our data indicated that individuals with a more severe DSD phenotype are more likely to have additional anomalies. We also found that 45% of individuals with a DSD had associated organ anomalies in more than one organ system. Most common organ anomalies observed were in the spleen, followed by the central nervous system. Previous studies in individuals with DSD[82] or in infants with atypical genitalia[83] have reported similar rates of associated anomalies (27% and 23%, respectively). The most common anomalies reported in these studies were small birth size, cardiac anomalies, CNS disorders, renal malformations, and extremity malformations, respectively.[82,83] By contrast, additional organ anomalies are not reported in cohorts of 46, XY DSD caused by *AR* or *SRD5A2* mutations.[84,85] With respect to comorbidities, little is known for specific DSD. Due to the retrospective study design and the young age of our study participants, we were not able to assess long-term health. This is certainly a field of interest to follow up on in future studies.

Spleen anomalies were seen in several individuals of the SF1next cohort. SF-1 plays a critical role in the development and function of the spleen,[1] and *NR5A1*/SF-1 variants have been shown to cause abnormal spleen development (e.g., asplenia).[18,19] As spleen anomalies might only be diagnosed when specifically searched for by imaging studies, we speculate that these anomalies are severely underreported. Given the potential serious consequences splenic dysfunction may have with increased susceptibility to serious infections (e.g., sepsis, meningitis), we strongly advise screening carriers of *NR5A1*/SF-1 variants for spleen anomalies. Early detection and management may prevent life-threatening complications.

In our large cohort, only five individuals had adrenal insufficiency. While the first reported patient with a heterozygous *NR5A1*/SF-1 variant had sex-reversal DSD and adrenal insufficiency,[8] it appears that adrenal insufficiency is rare in individuals with *NR5A1*/SF-1 variants.[9,75,76,86] It may occur with monoallelic or biallelic *NR5A1*/SF-1 variants. However, adrenal insufficiency might only develop over time and detected by ACTH (adrenocorticotropic hormone) testing. More data are needed to advise whether screening or long-term follow up is necessary.

Moreover, our study found that individuals with *NR5A1*/SF-1 variants had lower birth weights compared to the normal population, but later in life these individuals gained in terms of growth with normal height and BMI. However, these results have to be interpreted

with caution as our cohort is still young. Conditional *Nr5a1* mutants in the mouse model have shown that Sf-1 deficiency affects the regulation of reproduction and energy balance.[22] Selective deletion of *Nr5a1* in the ventromedial hypothalamus in mice prenatally resulted in late-onset obesity, and postnatally to deregulated thermogenesis.[87] Thus, long-term studies including more individuals are needed to conclude whether *NR5A1*/SF-1 plays a role in growth and metabolism. Birth weight and size, as well as catch-up growth and final height seem to vary in patients with different types of DSD,[88,89] but little data are available.

Many 46, XY individuals with a DSD had a severe phenotype with female sex registration at birth, but only few were sex reassigned later in life. Notably, surgical procedures such as genitoplasty and gonadectomy were performed in a considerable number of individuals with *NR5A1*/SF-1 variants. Germ cell tumors were reported in only two individuals with a DSD in our cohort. Although this small number may suggest that tumor risk with *NR5A1/SF-1* variants is low, we caution to draw this conclusion prematurely because many individuals had gonadectomy at an early age with histological investigations of the gonads not performed uniformly; in addition, many study individuals are still young. Clearly, there are case reports in the literature of individuals with *NR5A1*/SF-1 variants and germ cell tumors.[26,90] Long-term studies are therefore needed to assess tumor risk, while meanwhile surveillance is recommended.

Normal pubertal development was rather seldom observed in individuals with a DSD and *NR5A1*/SF-1 variants, even with hormonal replacement therapy. Mostly, an opposite sex 46, XY DSD phenotype related negatively to normal puberty, while even with a severe 46, XY DSD phenotype both normal and abnormal pubertal development was possible. This finding is in line with other studies on pubertal development of patients with a DSD and *NR5A1*/SF-1 variants, which describe a wide spectrum of pubertal courses with considerable virilization at puberty in 46, XY individuals, irrespective of the degree of virilization of the external genitalia at birth.[91–93] Thus, the severity of the DSD phenotype based on the assessment of the external genitalia at birth may not be a reliable predictor of later pubertal development in individuals with *NR5A1*/SF-1 variants. Similarly, the severity of external genital undervirilization at birth does not necessarily reflect the potential of virilization in patients with a loss of 5α-reductase activity or partial androgen resistance syndromes.[94–96] A recent review on pubertal development in patients with a different DSD suggested that puberty may be absent, incomplete or atypical, and even timing and progression of puberty may be modulated by the specific type of DSD.[97] These facts should be considered when counselling parents of children with a DSD in decisions such as sex registration at birth, hormonal treatments, genital surgeries or even gonadectomy. This certainly appears to be true for children with a DSD and *NR5A1*/SF-1 variants and indicates the need for prospective standardized assessment of these individuals through puberty and beyond.

Our study has several limitations. Due to the retrospective study design and inherent issues with registry data, we had to rely on pre-existing data, which were not originally collected for the specific purpose of this study and sometimes were partially incomplete or inaccurate. For instance, information regarding specific phenotypes associated with *NR5A1*/SF-1 variants, such as spleen anomalies, may have been missing. The SF1next cohort only includes cases from centers that specialize in DSD, resulting in sampling bias and underreporting of individuals with less severe phenotypes. In addition, carrier status was tested in only a subset of family members, who were also phenotypically incompletely characterized. Our cohort predominantly consists of individuals with a 46, XY karyotype, which reflects the incidence of DSD reported in the general population,[98] but which limits the applicability of our findings to less common DSD individuals with a 46, XX karyotype and *NR5A1*/SF-1 variants. In particular, POI arises over time and may not have developed at the time of data collection in some index cases or family members. The young age of our cohort does not allow the assessment of long-term outcomes, and there is limited phenotypic data on family members without DSD. Finally, the analysis and interpretation of *NR5A1*/SF-1 and other gene variants identified in our study cohort through different genetic approaches in different genetic labs over a long time period, was a big issue, although we tried our best by using established bioinformatic tools and genetic guidelines. In addition, some reported (likely) disease causing *NR5A1*/SF-1 variants included in our study were identified by our bioinformatic reanalysis as (likely) benign (4/93) raising doubts whether they should have been included in the study. Finally, the descriptive nature of our study limits its ability to establish causal relationships or determine underlying disease mechanisms. Future research employing analytical techniques, such as functional studies, molecular investigations, and in-depth genetic profiling, will be necessary to close further gaps. Also, it is important to realize that sometimes genetic data generated with less advanced techniques need to be repeated with newer methods. Despite these limitations, our study has several strengths. It provides insight into the largest international cohort of individuals with *NR5A1*/SF-1 variants to date. It offers a comprehensive overview of various outcomes including precise phenotyping, genotyping, growth assessment, and identification of additional associated anomalies. It includes data of family members, which are often overlooked, but are important for understanding the complex phenotype and genotype of DSD. Ultimately, our study was an international effort, which contributed to increase the

diversity of the cohort, improved generalizability of findings, and provided further insights into the broad phenotypic variability of DSD for individuals carrying *NR5A1*/SF-1 variants. Importantly, it informs clinicians on considerations of caring for these people on an individual basis, specifically regarding sex registration and pubertal development, spleen anomalies and surveillance of adrenal insufficiency.

In conclusion, the SF1next cohort study has significantly enhanced our knowledge on genotypic and phenotypic characteristics of individuals with *NR5A1*/SF-1 variants. It strengthens the hypothesis that the broad variability of the disease might be due to oligogenic or other non-Mendelian pathogenicities, therefore encouraging advanced genetic testing in individuals with a DSD and *NR5A1*/SF-1 variants, and further mechanistic research. Furthermore, it emphasizes the need for special surveillance and management of associated clinical features to ensure optimal patient care and outcome.

Contributors

All authors read and approved the final version of the manuscript.

C.K. Setup and organization of data collection. Data collection and assistance to clinical collaborators. Access and verification of data. Data analysis, interpretation, and presentation. Creation of tables and figures. Manuscript writing. Email: chrysanthi.kouri@students.unibe.ch. ORCID 0000-0002-7225-593X.

G.S. Study Co-PI. Overall study design, organization. Access and verification of data. Data analysis, statistics and interpretation. Creation of tables and figures. Manuscript writing. Email: grit.sommer@unibe.ch. ORCID 0000-0002-4205-7932.

I.M.dLP. Genetic data analysis and interpretation. Creation of tables and figures. Manuscript writing and reviewing. Email: idoia.martinezdelapiscina@unibe.ch. ORCID 0000-0001-8943-1429.

R.N.E. Discussion of data interpretation and presentation. Manuscript reviewing and proofreading. Email: rawda.naamneh@students.unibe.ch. ORCID 0000-0003-2669-4170.

L.J.W.T. Setup and providing of clinical and genetic data of patients. Genetic data analysis. Data interpretation and presentation. Manuscript reviewing. Email: lloydtack@gmail.com. ORCID 0000-0001-9352-5444.

M.C. Discussion of study design and data interpretation and presentation. Manuscript reviewing. Email: martine.cools@uzgent.be. ORCID 0000-0002-9552-4899.

S.F.A. Study design and registry use. Data interpretation. Manuscript reviewing. Email: faisal.ahmed@glasgow.ac.uk. ORCID 0000-0003-0689-5549.

SF1next study group. Collaborators providing clinical and genetic data of patients included in the SF1next cohort. Manuscript reading and final content approval.

C.E.F. Study PI. Grant holder. Overall idea and design, organization of study. Access and verification of data. Data analysis, interpretation and presentation. Creation of tables and figures. Manuscript writing and content responsibility and responsible for the decision to submit the manuscript. Fully employed in the academic field at University of Bern, Switzerland. Corresponding author. christa.flueck@unibe.ch. ORCID 0000-0002-4568-5504.

Data sharing statement

Access to basic data is possible through the international I-DSD registry; general rules apply (https://sdmregistries.org/about/). Additional data were collected in a project specific REDCap database governed by the Clinical Trials Unit (CTU) at University of Bern, Switzerland. These data can also be accessed upon reasonable request.

Declaration of interests

A postdoctoral fellowship from the Education Department of Basque Government (Spain) was granted to Idoia Martinez de Lapiscina. The SF1next study group was formed from the I-DSD research community (https://sdmregistries.org/), and related networks caring for rare patients with a DSD associated with *NR5A1*/SF-1 variants.

Acknowledgments

We thank all individuals with a DSD and their families for providing their medical data and trust towards this research study. Additionally, we extend our thanks to our funders: the Swiss National Science Foundation and Boveri Foundation Zürich, Switzerland.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104941.

References

1 Schimmer BP, White PC. Minireview: steroidogenic factor 1: its roles in differentiation, development, and disease. *Mol Endocrinol*. 2010;24(7):1322–1337.
2 Ikeda Y, Lala DS, Luo X, Kim E, Moisan MP, Parker KL. Characterization of the mouse FTZ-F1 gene, which encodes a key regulator of steroid hydroxylase gene expression. *Mol Endocrinol*. 1993;7(7):852–860.
3 Morohashi K, Honda S, Inomata Y, Handa H, Omura T. A common trans-acting factor, Ad4-binding protein, to the promoters of steroidogenic P-450s. *J Biol Chem*. 1992;267(25):17913–17919.
4 Luo X, Ikeda Y, Parker KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell*. 1994;77(4):481–490.
5 Shinoda K, Lei H, Yoshii H, et al. Developmental defects of the ventromedial hypothalamic nucleus and pituitary gonadotroph in the Ftz-F1 disrupted mice. *Dev Dynam*. 1995;204(1):22–29.
6 Sadovsky Y, Crawford PA, Woodson KG, et al. Mice deficient in the orphan receptor steroidogenic factor 1 lack adrenal glands and gonads but express P450 side-chain-cleavage enzyme in the placenta and have normal embryonic serum levels of corticosteroids. *Proc Natl Acad Sci U S A*. 1995;92(24):10939–10943.
7 Morohashi K. Gonadal and extragonadal functions of Ad4BP/SF-1: developmental aspects. *Trends Endocrinol Metabol*. 1999;10(5):169–173.
8 Achermann JC, Ito M, Ito M, Hindmarsh PC, Jameson JL. A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. *Nat Genet*. 1999;22(2):125–126.
9 Fabbri-Scallet H, de Sousa LM, Maciel-Guerra AT, Guerra-Junior G, de Mello MP. Mutation update for the NR5A1 gene involved in DSD and infertility. *Hum Mutat*. 2020;41(1):58–68.
10 Domenice S, Machado AZ, Ferreira FM, et al. Wide spectrum of NR5A1-related phenotypes in 46,XY and 46,XX individuals. *Birth Defects Res C Embryo Today*. 2016;108(4):309–320.
11 Baetens D, Stoop H, Peelman F, et al. NR5A1 is a novel disease gene for 46,XX testicular and ovotesticular disorders of sex development. *Genet Med*. 2017;19(4):367–376.
12 Lourenço D, Brauner R, Lin L, et al. Mutations in NR5A1 associated with ovarian insufficiency. *N Engl J Med*. 2009;360(12):1200–1210.
13 Bashamboo A, Ferraz-de-Souza B, Lourenço D, et al. Human male infertility associated with mutations in NR5A1 encoding steroidogenic factor 1. *Am J Hum Genet*. 2010;87(4):505–512.
14 Camats N, Pandey AV, Fernández-Cancio M, et al. Ten novel mutations in the NR5A1 gene cause disordered sex development in 46,XY and ovarian insufficiency in 46,XX individuals. *J Clin Endocrinol Metab*. 2012;97(7):E1294–E1306.
15 Suntharalingham JP, Buonocore F, Duncan AJ, Achermann JC. DAX-1 (NR0B1) and steroidogenic factor-1 (SF-1, NR5A1) in human disease. *Best Pract Res Clin Endocrinol Metabol*. 2015;29(4):607–619.
16 Camats N, Fernández-Cancio M, Audí L, Schaller A, Flück CE. Broad phenotypes in heterozygous NR5A1 46,XY patients with a disorder of sex development: an oligogenic origin? *Eur J Hum Genet*. 2018;26(9):1329–1338.

17 Werner R, Mönig I, Lünstedt R, et al. New NR5A1 mutations and phenotypic variations of gonadal dysgenesis. *PLoS One*. 2017;12(5):e0176720.
18 Colson C, Aubry E, Cartigny M, et al. SF1 and spleen development: new heterozygous mutation, literature review and consequences for NR5A1-mutated patient's management. *Clin Genet*. 2017;92(1):99–103.
19 Zangen D, Kaufman Y, Banne E, et al. Testicular differentiation factor SF-1 is required for human spleen development. *J Clin Invest*. 2014;124(5):2071–2075.
20 Morohashi K, Tsuboi-Asai H, Matsushita S, et al. Structural and functional abnormalities in the spleen of an mFtz-F1 gene-disrupted mouse. *Blood*. 1999;93(5):1586–1594.
21 Correa RV, Domenice S, Bingham NC, et al. A microdeletion in the ligand binding domain of human steroidogenic factor 1 causes XY sex reversal without adrenal insufficiency. *J Clin Endocrinol Metab*. 2004;89(4):1767–1772.
22 Majdic G, Young M, Gomez-Sanchez E, et al. Knockout mice lacking steroidogenic factor 1 are a novel genetic model of hypothalamic obesity. *Endocrinology*. 2002;143(2):607–614.
23 Malikova J, Camats N, Fernández-Cancio M, et al. Human NR5A1/SF-1 mutations show decreased activity on BDNF (brain-derived neurotrophic factor), an important regulator of energy balance: testing impact of novel SF-1 mutations beyond steroidogenesis. *PLoS One*. 2014;9(8):e104838.
24 Stenson PD, Ball EV, Mort M, et al. Human gene mutation database (HGMD): 2003 update. *Hum Mutat*. 2003;21(6):577–581.
25 Ferraz-de-Souza B, Lin L, Achermann JC. Steroidogenic factor-1 (SF-1, NR5A1) and human disease. *Mol Cell Endocrinol*. 2011;336(1–2):198–205.
26 Robevska G, van den Bergen JA, Ohnesorg T, et al. Functional characterization of novel NR5A1 variants reveals multiple complex roles in disorders of sex development. *Hum Mutat*. 2018;39(1):124–139.
27 Martínez de LaPiscina I, Mahmoud RA, Sauter KS, et al. Variants of STAR, AMH and ZFPM2/FOG2 may contribute towards the broad phenotype observed in 46,XY DSD patients with heterozygous variants of NR5A1. *Int J Mol Sci*. 2020;21(22).
28 Biason-Lauber A, Schoenle EJ. Apparently normal ovarian differentiation in a prepubertal girl with transcriptionally inactive steroidogenic factor 1 (NR5A1/SF-1) and adrenocortical insufficiency. *Am J Hum Genet*. 2000;67(6):1563–1568.
29 11th international meeting of paediatric endocrinology (IMPE) - abstracts. *Horm Res Paediatr*. 2023;Suppl 2:1–197.
30 Sreenivasan R, Ludbrook L, Fisher B, et al. Mutant NR5A1/SF-1 in patients with disorders of sex development shows defective activation of the SOX9 TESCO enhancer. *Hum Mutat*. 2018;39(12):1861–1874.
31 Kouri C, Sommer G, Flück CE. Oligogenic causes of human differences of sex development: facing the challenge of genetic complexity. *Horm Res Paediatr*. 2021;96(2):169–179.
32 Zidoune H, Ladjouze A, Chellat-Rezgoune D, et al. Novel genomic variants, atypical phenotypes and evidence of a digenic/oligogenic contribution to disorders/differences of sex development in a large north African cohort. *Front Genet*. 2022;13:900574.
33 Flück CE, Audí L, Fernández-Cancio M, et al. Broad phenotypes of disorders/differences of sex development in MAMLD1 patients through oligogenic disease. *Front Genet*. 2019;10:746.
34 Sykiotis GP, Plummer L, Hughes VA, et al. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. *Proc Natl Acad Sci U S A*. 2010;107(34):15140–15144.
35 Boehm U, Bouloux PM, Dattani MT, et al. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism–pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol*. 2015;11(9):547–564.
36 Badano JL, Katsanis N. Beyond Mendel: an evolving view of human genetic disease transmission. *Nat Rev Genet*. 2002;3(10):779–789.
37 Camats N, Flück CE, Audí L. Oligogenic origin of differences of sex development in humans. *Int J Mol Sci*. 2020;21(5):1809.
38 Tack LJW, van der Straaten S, Riedl S, et al. Growth, puberty and testicular function in boys born small for gestational age with a nonspecific disorder of sex development. *Clin Endocrinol*. 2022;96(2):165–174.
39 Bacila I, Freeman N, Daniel E, et al. International practice of corticosteroid replacement therapy in congenital adrenal hyperplasia: data from the I-CAH registry. *Eur J Endocrinol*. 2021;184(4):553–563.
40 Hebenstreit D, Ahmed SF, Krone N, et al. Surgical practice in girls with congenital adrenal hyperplasia: an international registry study. *Sex Dev*. 2021;15(4):229–235.
41 Flück C, Nordenström A, Ahmed SF, et al. Standardised data collection for clinical follow-up and assessment of outcomes in differences of sex development (DSD): recommendations from the COST action DSDnet. *Eur J Endocrinol*. 2019;181(5):545–564.
42 Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: building an international community of software platform partners. *J Biomed Inform*. 2019;95:103208.
43 Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)–a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377–381.
44 Köhler S, Gargano M, Matentzoglu N, et al. The human phenotype Ontology in 2021. *Nucleic Acids Res*. 2020;49(D1):D1207–D1217.
45 Köhler S, Schulz MH, Krawitz P, et al. Clinical diagnostics in human genetics with semantic similarity searches in ontologies. *Am J Hum Genet*. 2009;85(4):457–464.
46 van der Straaten S, Springer A, Zecic A, et al. The external genitalia score (EGS): a European multicenter validation study. *J Clin Endocrinol Metab*. 2019;105(3):e222–e230.
47 Kopanos C, Tsiolkas V, Kouris A, et al. VarSome: the human genomic variant search engine. *Bioinformatics*. 2019;35(11):1978–1980.
48 Richards S, Aziz N, Bale S, 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. *Genet Med*. 2015;17(5):405–424.
49 Stenson PD, Mort M, Ball EV, Shaw K, Phillips A, Cooper DN. The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. *Hum Genet*. 2014;133(1):1–9.
50 Renaux A, Papadimitriou S, Versbraegen N, et al. ORVAL: a novel platform for the prediction and exploration of disease-causing oligogenic variant combinations. *Nucleic Acids Res*. 2019;47(W1):W93–W98.
51 Villar J, Cheikh Ismail L, Victora CG, et al. International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. *Lancet*. 2014;384(9946):857–868.
52 de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ*. 2007;85(9):660–667.
53 Eggers S, Sadedin S, van den Bergen JA, et al. Disorders of sex development: insights from targeted gene sequencing of a large international patient cohort. *Genome Biol*. 2016;17(1):243.
54 Tyutyusheva N, Mancini I, Baroncelli GI, et al. Complete androgen insensitivity syndrome: from bench to bed. *Int J Mol Sci*. 2021;22(3):1264.
55 Mendonca BB, Batista RL, Domenice S, et al. Steroid 5α-reductase 2 deficiency. *J Steroid Biochem Mol Biol*. 2016;163:206–211.
56 Bashamboo A, Donohoue PA, Vilain E, et al. A recurrent p.Arg92Trp variant in steroidogenic factor-1 (NR5A1) can act as a molecular switch in human sex development. *Hum Mol Genet*. 2016;25(16):3446–3453.
57 Askari M, Rastari M, Seresht-Ahmadi M, et al. A missense mutation in NR5A1 causing female to male sex reversal: a case report. *Andrologia*. 2020;52(6):e13585.
58 Igarashi M, Takasawa K, Hakoda A, et al. Identical NR5A1 missense mutations in two unrelated 46,XX individuals with testicular tissues. *Hum Mutat*. 2017;38(1):39–42.
59 Knarston IM, Robevska G, van den Bergen JA, et al. NR5A1 gene variants repress the ovarian-specific WNT signaling pathway in 46, XX disorders of sex development patients. *Hum Mutat*. 2019;40(2):207–216.
60 Guran T, Buonocore F, Saka N, et al. Rare causes of primary adrenal insufficiency: genetic and clinical characterization of a large nationwide cohort. *J Clin Endocrinol Metab*. 2016;101(1):284–292.
61 Hoivik EA, Lewis AE, Aumo L, Bakke M. Molecular aspects of steroidogenic factor 1 (SF-1). *Mol Cell Endocrinol*. 2010;315(1–2):27–39.
62 Ferraz-de-Souza B, Lin L, Shah S, et al. ChIP-on-chip analysis reveals angiopoietin 2 (Ang2, ANGPT2) as a novel target of

steroidogenic factor-1 (SF-1, NR5A1) in the human adrenal gland. *FASEB J*. 2011;25(4):1166–1175.
63 Meinsohn M-C, Smith OE, Bertolin K, Murphy BD. The orphan nuclear receptors steroidogenic factor-1 and liver receptor homolog-1: structure, regulation, and essential roles in mammalian reproduction. *Physiol Rev*. 2019;99(2):1249–1279.
64 Xue Q, Lin Z, Yin P, et al. Transcriptional activation of steroidogenic factor-1 by hypomethylation of the 5' CpG island in endometriosis. *J Clin Endocrinol Metab*. 2007;92(8):3261–3267.
65 Hammer GD, Krylova I, Zhang Y, et al. Phosphorylation of the nuclear receptor SF-1 modulates cofactor recruitment: integration of hormone signaling in reproduction and stress. *Mol Cell*. 1999;3(4):521–526.
66 Mazen I, Abdel-Hamid M, Mekkawy M, et al. Identification of NR5A1 mutations and possible digenic inheritance in 46,XY gonadal dysgenesis. *Sex Dev*. 2016;10(3):147–151.
67 Eggers S, Smith KR, Bahlo M, et al. Whole exome sequencing combined with linkage analysis identifies a novel 3 bp deletion in NR5A1. *Eur J Hum Genet*. 2015;23(4):486–493.
68 Wang H, Zhang L, Wang N, et al. Next-generation sequencing reveals genetic landscape in 46, XY disorders of sexual development patients with variable phenotypes. *Hum Genet*. 2018;137(3):265–277.
69 Fabbri-Scallet H, Werner R, Guaragna MS, et al. Can non-coding NR5A1 gene variants explain phenotypes of disorders of sex development? *Sex Dev*. 2022;16(4):252–260.
70 Posey JE. Genome sequencing and implications for rare disorders. *Orphanet J Rare Dis*. 2019;14(1):153.
71 Audi L, Ahmed SF, Krone N, et al. Genetics in endocrinology: approaches to molecular genetic diagnosis in the management of differences/disorders of sex development (DSD): position paper of EU COST Action BM 1303 'DSDnet'. *Eur J Endocrinol*. 2018;179(4):R197–r206.
72 Baxter RM, Arboleda VA, Lee H, et al. Exome sequencing for the diagnosis of 46,XY disorders of sex development. *J Clin Endocrinol Metab*. 2015;100(2):E333–E344.
73 Lee H, Deignan JL, Dorrani N, et al. Clinical exome sequencing for genetic identification of rare Mendelian disorders. *JAMA*. 2014;312(18):1880–1887.
74 Papadimitriou S, Gazzo A, Versbraegen N, et al. Predicting disease-causing variant combinations. *Proc Natl Acad Sci U S A*. 2019;116(24):11878–11887.
75 Lin L, Philibert P, Ferraz-de-Souza B, et al. Heterozygous missense mutations in steroidogenic factor 1 (SF1/Ad4BP, NR5A1) are associated with 46,XY disorders of sex development with normal adrenal function. *J Clin Endocrinol Metab*. 2007;92(3):991–999.
76 Philibert P, Zenaty D, Lin L, et al. Mutational analysis of steroidogenic factor 1 (NR5a1) in 24 boys with bilateral anorchia: a French collaborative study. *Hum Reprod*. 2007;22(12):3255–3261.
77 Bassett MH, Zhang Y, Clyne C, White PC, Rainey WE. Differential regulation of aldosterone synthase and 11beta-hydroxylase transcription by steroidogenic factor-1. *J Mol Endocrinol*. 2002;28(2):125–135.
78 Bland ML, Jamieson CA, Akana SF, et al. Haploinsufficiency of steroidogenic factor-1 in mice disrupts adrenal development leading to an impaired stress response. *Proc Natl Acad Sci U S A*. 2000;97(26):14488–14493.
79 Bland ML, Fowkes RC, Ingraham HA. Differential requirement for steroidogenic factor-1 gene dosage in adrenal development versus endocrine function. *Mol Endocrinol*. 2004;18(4):941–952.
80 Beuschlein F, Mutch C, Bavers DL, et al. Steroidogenic factor-1 is essential for compensatory adrenal growth following unilateral adrenalectomy. *Endocrinology*. 2002;143(8):3122–3135.
81 Délot EC, Papp JC, Workgroup D-TG, Sandberg DE, Vilain E. Genetics of disorders of sex development: the DSD-TRN experience. *Endocrinol Metab Clin N Am*. 2017;46(2):519–537.
82 Cox K, Bryce J, Jiang J, et al. Novel associations in disorders of sex development: findings from the I-DSD Registry. *J Clin Endocrinol Metab*. 2014;99(2):E348–E355.
83 Thyen U, Lanz K, Holterhus PM, Hiort O. Epidemiology and initial management of ambiguous genitalia at birth in Germany. *Horm Res Paediatr*. 2006;66(4):195–203.
84 Akcan N, Uyguner O, Baş F, et al. Mutations in AR or SRD5A2 genes: clinical findings, endocrine pitfalls, and genetic features of children with 46,XY DSD. *J Clin Res Pediatr Endocrinol*. 2022;14(2):153–171.
85 Lucas-Herald A, Bertelloni S, Juul A, et al. The long-term outcome of boys with partial androgen insensitivity syndrome and a mutation in the androgen receptor gene. *J Clin Endocrinol Metab*. 2016;101(11):3959–3967.
86 Coutant RG, Mallet D, Lahlou N, et al. Heterozygous mutation of steroidogenic factor-1 in 46,XY subjects may mimic partial androgen insensitivity syndrome. *J Clin Endocrinol Metab*. 2007;92(8):2868–2873.
87 Kim KW, Zhao L, Donato J Jr, et al. Steroidogenic factor 1 directs programs regulating diet-induced thermogenesis and leptin action in the ventral medial hypothalamic nucleus. *Proc Natl Acad Sci U S A*. 2011;108(26):10673–10678.
88 Poyrazoglu S, Darendeliler F, Ahmed SF, et al. Birth weight in different etiologies of disorders of sex development. *J Clin Endocrinol Metab*. 2017;102(3):1044–1050.
89 Wu D, Chen H, Gong C. Physical assessment and reference growth curves for children with 46, XY disorders of sex development. *Pediatr Investig*. 2017;1(1):13–19.
90 Barbaro M, Cools M, Looijenga LH, Drop SL, Wedell A. Partial deletion of the NR5A1 (SF1) gene detected by synthetic probe MLPA in a patient with XY gonadal disorder of sex development. *Sex Dev*. 2011;5(4):181–187.
91 Mönig I, Schneidewind J, Johannsen TH, et al. Pubertal development in 46,XY patients with NR5A1 mutations. *Endocrine*. 2022;75(2):601–613.
92 Warman DM, Costanzo M, Marino R, et al. Three new SF-1 (NR5A1) gene mutations in two unrelated families with multiple affected members: within-family variability in 46,XY subjects and low ovarian reserve in fertile 46,XX subjects. *Horm Res Paediatr*. 2011;75(1):70–77.
93 Gabriel Ribeiro de Andrade J, Marques-de-Faria AP, Fabbri HC, de Mello MP, Guerra-Júnior G, Maciel-Guerra AT. Long-term follow-up of patients with 46,XY partial gonadal dysgenesis reared as males. *Int J Endocrinol*. 2014;2014:480724.
94 Kumar G, Barboza-Meca JJ. 5-Alpha-Reductase deficiency, 2022 Oct 17. In: *StatPearls [Internet]*. Treasure Island (FL: StatPearls Publishing; 2023.
95 Batista RL, Mendonca BB. Integrative and analytical review of the 5-alpha-reductase type 2 deficiency worldwide. *Appl Clin Genet*. 2020;13:83–96.
96 Cheon CK. Practical approach to steroid 5alpha-reductase type 2 deficiency. *Eur J Pediatr*. 2011;170(1):1–8.
97 Nordenström A. Puberty in individuals with a disorder of sex development. *Curr Opin Endocr Metab Res*. 2020;14:42–51.
98 Lee PA, Nordenström A, Houk CP, et al. Global disorders of sex development update since 2006: perceptions, approach and care. *Horm Res Paediatr*. 2016;85(3):158–180.