Manuscript submitted to: Volume 2, Issue 2, 127-147.

AIMS Genetics DOI: 10.3934/genet.2015.2.127

Received date 30 November 2014, Accepted date 23 March 2015, Published date 26 March 2015

Research article

Sex attribution, gender identity and quality of life in disorders of sex development due to 45,X/46,XY mosaicism: methods for clinical and psychosocial assessment

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Abstract: The choice of sex in newborns with genital ambiguity is challenging. Information concerning the satisfaction of subjects with disorders of sex development from childhood to adulthood is required in order to address sex attribution policies. This study focuses on the methods that enable clinicians to investigate the alignment of phenotypes with gender identity and quality of life in people with disorders of this kind. These methods are presented as tools for studying a cohort of ten subjects with 45,X/46,XY mosaicism examined between 1985 and 2014 in the Department of Pediatric Endocrinology, Regina Margherita Children's Hospital, Turin: five children and five young adults, four reared as females and six as males. Clinical outcome was assessed by means of a clinical scoring system considering height, genital appearance, gonads and pubertal development. The Gender Identity Questionnaire for Children and the World Health Organization Quality of Life assessment were adopted. The four male children strongly identified with their assigned sex: male attribution was satisfactory until pubertal age. In young adults the clinical scores ranged between 55–65% for both genders. In the young male, the reduced sexual activity and the poor body image perception strongly affected his quality of life. The clinical scores of the two young female adults (60% for both) were not balanced with their quality of life scores (87.5% and 68.75% respectively): individual traits and social-familial context should be investigated in order to explain these differences. Clinical and psychosocial assessment in people with disorders of sex development is mandatory in order to plan

care procedures; a detailed analysis requires adequate tools. Clinical scoring system, Gender Identity Questionnaire for Children and World Health Organization Quality of Life assessment can be used to investigate the alignment of physical phenotype with gender identity and quality of life.

Keywords: 45,X/46,XY mosaicism; disorders of sex development; sex attribution; gender identity development; quality of life assessment

Abbreviations:

DSD = Disorders of Sex Development
FSH = Follicle Stimulating Hormone
GIQC = Gender Identity Questionnaire for Children
hGH = human Growth Hormone
LH = Luteinizing Hormone
SHOX = Short Stature Homeobox
WHOQOL-100 = World Health Organization Quality of Life assessment

1. Introduction

Disorders of Sex Development (DSD) are congenital conditions defined by atypical chromosomal, gonadal or anatomic sex [1]. The estimated incidence of genital ambiguity is 1 in 5000 to 1 in 4500 births [2,3,4]. The most recent classification is based on chromosomal setting: DSD related to sex chromosome abnormalities and DSD related to 46,XY or 46,XX karyotype [5]. 45,X/46,XY mosaicism, included in sex chromosome abnormalities DSD, have an overall incidence of about one in 10.000 [6].

45,X/46,XY mosaicism is characterized by heterogeneous clinical presentation [7-10]. Most prenatally diagnosed subjects (90–95%) present normal male phenotype at birth; abnormalities are only found in a small percentage (5–10%) [7,10,11,12]. Data derived from postnatally ascertained cases exaggerate the extent of genital and other abnormalities by over representing the phenotypically abnormal minority [7].

Possible genital presentations are: male or female regular, males with ambiguous features and hypovirilization (micropenis, lack of scrotal fusion, incomplete testicular descent, hypospadia) or females with ambiguous features and virilization (clitoromegaly, labio-scrotal fusion) [7-10]. Typical somatic signs of the Turner syndrome are observed in some cases (palpebral ptosis, epicanthus, webbed neck, short stature, shield chest, scoliosis) with possible prenatal and postnatal growth retardation [10,13]. Short stature may be the only somatic feature [10,13,14], which is usually related to the *SHOX* ("*Short Stature Homeobox*") gene haploinsufficiency in 45,X cell line [15,16]. Some development abnormalities can be associated to 45,X/46,XY mosaicism: heart malformations, kidney malformations, Meckel diverticulum, bilateral inguinal hernias [7,8]. As regards to gonads, functional testes can more often occur and streak or dysgenetic gonads are also possible, even in the presence of normal male external genitalia [7]. Gonads can be set at any point of the testicular descent path: streak gonads are found most frequently at intra-abdominal level, while regularly developed testes often reach inguinal-scrotal level [17]. There is a higher risk of neoplastic transformation in dysgenetic

gonads, which is not limited to subjects with abnormal genitalia [7]. The incidence of gonadoblastoma in 45,X/46,XY individuals is reported to be approximately 15% [11,18]. In rare cases ovary stromal-like tissue with scattered primordial follicles has been observed [12]. Müllerian structures are occasionally found, resulting from absent or inadequate production of anti-Müllerian hormone by Sertoli cells [12].

The treatment of individuals with DSD requires the attention of many professionals. Although the medical–surgical treatment is highly important, quality of life also largely depends on the psychosocial management [19]. There is little data concerning the clinical course of people with 45,X/46,XY mosaicism and, in particular, psychosocial development has not been adequately investigated [20,21].

This study focuses on methods that enable clinicians to investigate the alignment of physical characteristics, gender identity and quality of life in people with DSD. In particular, the study reports the application of these methods to a small cohort of people with 45,X/46,XY.

2. Materials and Methods

2.1. Aim

The aim of the study was to describe the physical phenotypes of a cohort of children and young adults with 45,X/46,XY mosaicism, psychosocial features focusing on gender identity in children and assessment of quality of life in young adults. A scoring system was proposed in order to make it easier to understand the process of gender identification and quality of life in DSD patients with specific physical phenotypes.

2.2. Participants

We analyzed the personal histories of ten subjects with 45,X/46,XY mosaicism followed up between 1985 and 2014 in the Department of Pediatric Endocrinology, Regina Margherita Children's Hospital, Turin: five children and five young adults, four reared as females and six as males. These subjects represent the entire consecutive series of patients with 45,X/46,XY mosaicism followed up in the hospital in the specific time period.

Table 1 summarizes clinical presentation, sex of rearing, gonadal features, genitourinary imaging and laboratory investigations in the first month of life in the ten subjects with 45,X/46,XY mosaicism. Reconstructive and gonadal surgeries are also presented.

2.3. Measures

Clinical presentation and history were assessed using specific measures, that are described below.

For the children, we analyzed gender identification by means of the Gender Identity Questionnaire for Children (GICQ), which was filled in by the parents [22].

For the young adults we used the World Health Organization Quality of life Assessment (WHOQOL-100) [23,24,25] in order to assess their quality of life.

2.3.1. Clinical presentation at birth and sex of rearing

Analysis of clinical presentation at birth was based on External Masculinization Score (EMS) [26]

(Table 2). The scores for each patient are shown in the second column of Table 1.

When selecting the sex of rearing, genital anatomy, surgical options, need for lifelong hormone replacement therapy, potential for fertility were taken into consideration. Genital anatomy was the most important aspect on which the decision was based. In the past, the presence of uterus was an indication for attributing female sex, while recently parental involvement in the decision has progressively grown, since consistent parental support is felt to be critical for the patient for assimilating sex assignment.

2.3.2. Perinatal investigations

Karyotype

Due to genital ambiguity at birth, karyotype was carried out on six subjects in order to determine the chromosomal sex; it was carried out prenatally for the remaining four by means of amniocentesis and was then confirmed at birth.

Genitourinary imaging

Genitourinary imaging was obtained by carrying out retrograde genitography and abdominopelvic ultrasound, in some cases completed by means of abdomen magnetic resonance. Retrograde genitography was performed for the anatomical outlining of the urogenital sinus and for localizing the position and entry of the urethra and vagina into the sinus. Abdominopelvic ultrasound and abdomen magnetic resonance were used for evaluating internal genitalia. In two subjects, signs of genital ambiguity has already been detected with prenatal ultrasound.

Hormone levels

The following hormones were investigated using standard methods in the first month of life (from 10^{th} to 30^{th} day of life):

- Follicle Stimulating Hormone (FSH);
- Luteinizing Hormone (LH);
- Testosterone.

Comorbidities

Complete clinical evaluation of each patient, in particular through renal ultrasound and echocardiography, was performed at birth in order to detect pathologies of any kind.

Table 1. Early clinical data, sex of rearing and surgical interventions in ten subjects with 45,X/46,XY mosaicism.

Subject	Clinical presentation/ Sex of rearing	Reconstructive surgery (age: years, months)	Gonadal features	Gonadal surgery (age: years, months)	Karyotype	Genitourinary imaging	Hormone levels (10th–30th day of life)	Comorbidities
1	EMS 4.5/12 Female	Clitoroplasty (1, 0/12) Vaginoplasty (1, 0/12)	Right gonad: *streak,* intra-abdominal Left gonad: dysgenetic, inguinal	Bilateral gonadectomy (0, 3/12)	45,X/46,XY prenatal (amniocentesis)	Uterus: present, hypoplastic Fallopian tubes: absent Urogenital sinus	FSH 3.6 mU/mL LH 2.4 mU/mL Testosterone 0.56 ng/mL	Horizontal nystagmus
2	EMS 0.5/12 Female	Vaginoplasty (0, 6/12) Clitoroplasty (0, 6/12–25, 0/12) Corrective surgery in vaginal introitus stenosis (25, 0/12)	Right gonad: *streak,* intra-abdominal Left gonad: dysgenetic, inguinal	Bilateral gonadectomy (0, 4/12)	45,X/46,XY postnatal	Uterus: present, regular structure Fallopian tubes: absent Urogenital sinus	FSH 3.5 mU/mL LH 2.1 mU/mL Testosterone 0.42 ng/mL	None
3	EMS 9.5/12 Female	Vaginoplasty (1, 0/12) Clitoroplasty (1, 0/12)	Right gonad: *streak,* inguinal Left gonad: dysgenetic, intrascrotal	Bilateral gonadectomy (1, 0/12)	45,X/46,XY prenatal (amniocentesis)	Uterus: present, regular structure Fallopian tubes: present on right side Female urethra: slightly longer, it flows in a small vestibule with a vagina of regular morphology	FSH 3.1 mU/mL LH 2.5 mU/mL Testosterone 0.3 ng/mL	None
4	EMS 4.5/12 Female	Vaginoplasty (0, 3/12) Clitoroplasty (0, 3/12)	Right gonad: dysgenetic, inguinal Left gonad: *streak,* intra-abdominal	Bilateral gonadectomy (0, 3/12)	45,X/46,XY prenatal (amniocentesis)	Suspected ambiguous genitalia at prenatal ultrasound Uterus: present, regular structure Fallopian tubes: absent Vagina with moderate uterine impression at cupola level Female urethra: straight, veru montanum absent, it flows in a large vestibule with a vagina of regular morphology	FSH 11.1 mU/mL LH 6.14 mU/mL Testosterone 1.12 ng/mL	None

5	EMS 5/12 Male	Urethroplasty (12, 0/12) Testicular prosthesis placement (18, 10/12)	Right gonad: *streak,* intra-abdominal Left gonad: dysgenetic, intra-abdominal	Bilateral gonadectomy (1, 6/12)	45,X/46,XYq- postnatal	Uterus: absent Fallopian tubes: absent Male urethra: hypospadic, veru montanum present	FSH 3.5 mU/mL LH 0.7 mU/mL Testosterone 0.8 ng/mL	None
6	EMS 6.5/12 Male	Chordee correction (recurvatio corrective surgery) (3, 0/12) Urethroplasty (4, 5/12)	Right gonad: regularly developed, intrascrotal Left gonad: dysgenetic, inguinal	Left gonadectomy (1, 9/12)	45,X/46,XY postnatal	Uterus: absent Fallopian tubes: absent Male urethra: hypospadic, veru montanum present	FSH 5.4 mU/mL LH 3.2 mU/mL Testosterone 1.2 ng/mL	None
7	EMS 8.5/12 Male	Chordee correction (recurvatio corrective surgery) (8, 0/12) Urethroplasty (8, 0/12)	Right gonad: hypoplastic, inguinal Left gonad: regularly developed, intrascrotal	Right orchidopexy (6, 0/12)	45,X/46,XY/47,XYY postnatal	Uterus: absent Fallopian tubes: absent Male urethra: hypospadic	FSH 4.5 mU/mL LH 2.3 mU/mL Testosterone 0.9 ng/mL	None
8	EMS 7.5/12 Male	Chordee correction (recurvatio corrective surgery) (1, 6/12) Urethroplasty (in two times) (2, 0/12–3, 6/12)	Right gonad: fusiform, inguinal Left gonad:dysgenetic, intra-abdominal	Right orchidopexy (0, 3/12) Left gonadectomy (0, 3/12)	45,X/46,X, i(Y)(p11) postnatal	Uterus: absent Fallopian tubes: absent Male urethra: hypospadic, veru montanum present	FSH 11.2mU/mL LH 5.1mU/mL Testosterone 1.5ng/mL	Common Atrioventricular Chamber
9	EMS 7.5/12 Male	Hysterectomy (1, 0/12) Vaginectomy (1, 0/12) Urethroplasty (in two times) (2, 4/12–3, 0/12)	Right gonad: regularly developed, inguinal Left gonad: *streak,* intra-abdominal	Right orchidopexy (0, 4/12) Left gonadectomy (0, 4/12)	45,X/46,XY postnatal	Suspected ambiguous genitalia at prenatal ultrasound Uterus: present, regular morphology Fallopian tubes: present on left side, hypoplastic Urogenital sinus in which a straight urethra flows, veru montanum absent	FSH 1.2 mU/mL LH 0.8 mU/mL Testosterone 0.3 ng/mL	None
10	EMS 12/12 Male	None	Right gonad: regularly developed, intrascrotal Left gonad: regularly developed, intrascrotal	None	45,X/46,XY prenatal (amniocentesis)	Uterus: absent Fallopian tubes: absent Male urethra	FSH 4.5 mU/mL LH 1.4 mU/mL Testosterone 1.3 ng/mL	None

Table 2. External Masculinization Score [26].

Feature	Score for Yes/No or condition
EMS	
Scrotal fusion	3/0
Micropenis	0/3
Urethral meatus	
- Normal	3
- Glandular	2
- Penile	1
- Perineal	0
Right and left gonad (score for each)	
- Scrotal	1.5
- Inguinal	1
- Abdominal	0.5
- Absent	0

2.3.3. Clinical course

The patients were evaluated and followed up between 1985 and 2014 in the Department of Pediatric Endocrinology, Regina Margherita Children's Hospital, Turin, Italy. The clinical course of each subject was monitored with annual outpatient checkups carried out by the pediatric endocrinologist as case manager. The clinical history was collected at each checkup, a general physical examination was performed and the following parameters were assessed: height, weight, growth rate, pubertal development according to Tanner staging [27].

In clinical histories, the attitudes and decisions concerning the following issues were analyzed:

- diagnosis and sex of rearing;
- reconstructive interventions;
- cancer risk management and preventive decisions in relation to gonadal function;
- growth pattern and human growth hormone therapy;
- pubertal development and sex hormone replacement therapy;
- gender identity in children and quality of life in young adults.

At the end of the follow up, in order to evaluate the alignment of phenotypic features to the attributed sex, we elaborated a clinical scoring system (rating from 1 = low alignment to 5 = high alignment) relative to: height, genital appearance, presence of gonads and pubertal development (Table 3).

The analysis of clinical histories highlighted a clinical outcome (height reached at the end of the follow up, results of reconstructive and gonadal surgeries, pubertal development) which the pediatric endocrinologist quantified for each subject by means of the clinical scoring system. The scores are shown in Table 4 in the "Total Clinical Scores" column; in the third, fourth, fifth and sixth columns the score for each specific feature is reported in brackets.

In order to evaluate gender identity and quality of life, the children's parents were given a questionnaire on gender identity while the young adults filled in a questionnaire on quality of life.

At the time of the interview, each subject was informed of the purpose of the study; then the patients and the children's parents released their informed consent. Parental consent was required for

those under 14 years of age, for subjects between 14 and 18 years of age the consent of patients and their parents was required, while subjects over 18 years of age signed their own informed consent.

Table 3. Clinical scoring system.

Feature	Score
Height	
< 3rd percentile	1
3rd–10th percentile	2
10th–25th percentile	3
25th–50th percentile	4
> 50th percentile	5
Male genitals	
hypospadias and recurvatio	1
hypospadias and surgically corrected recurvatio	2
surgically corrected hypospadias and recurvatio	3
surgically corrected hypospadias and no recurvatio	4
male normally shaped genitalia	5
Female genitals	
clitoral hypertrophy and urogenital sinus	1
partial reduction of clitoral hypertrophy and urogenital sinus	2
clitoroplasty and vaginoplasty with narrow orifice	3
clitoroplasty and vaginoplasty	4
female normally shaped genitalia	5
Gonads	
bilateral gonadectomy	1
unilateral gonadectomy of a streak gonad and contralateral dysgenetic gonad	2
bilateral dysgenetic gonads	3
unilateral gonadectomy of a streak gonad and contralateral regularly developed gonad	4
bilateral regularly developed gonads	5
Pubertal development score: according to Tanner staging [27].	

Table 4. Clinical data at the end of the follow up, clinical scores and GICQ/WHOQOL questionnaires' results in ten subjects with 45, X/46, XY mosaicism.

Subject	Age (years, month)	Height (clinical score)	Genitalia (clinical score)	Gonads (clinical score)	Pubertal development (clinical score)	Total Clinical Score	Gicq score	Whoqol-100 score		Comorbidities
								TOTAL SCORE	4 FACETS SCORE	
1	33, 0/12	144.1 cm << 3rd percentile (1)	Clitoroplasty and vaginoplasty results (4)	Bilateral gonadectomy results (1)	Adult (BV, PHV) with regular menses (5)	11/20 = 55%	–	–	–	Recurrent urinary tract infections; neurological disease (tremors, motor incoordination, nystagmus, cognitive impairment); erythematous desquamative chronic dermatitis
2	31, 0/12	153.5 cm 3rd–10th percentile (2)	Clitoroplasty, vaginoplasty and corrective surgery in vaginal introitus stenosis results (4)	Bilateral gonadectomy results (1)	Adult (BV, PHV) with regular menses (5)	12/20 = 60%	–	87.5%	Self-esteem: 87.5% Body image: 93.75% Interpersonal relationships: 93.75% Sexual activity: 93.75% Average: 92.2%	None
3	18, 0/12	158.5 cm 25th percentile (3)	Clitoroplasty results, presence of vaginal introitus stenosis (3)	Bilateral gonadectomy results (1)	Adult (BV, PHV) with regular menses (5)	12/20 = 60%	–	68.75%	Self-esteem: 62.5% Body image: 56.25% Interpersonal relationships: 68.75% Sexual activity: 0% Average: /	Overweight (BMI: 30 kg/mq), autoimmune thyroiditis
4	1, 0/12	72 cm > 50th percentile (5)	Clitoroplasty and vaginoplasty results (4)	Bilateral gonadectomy results (1)	Prepubertal (–)	10/15 = 66.7%	–	–	–	None
5	26, 11/12	166.2 cm > 10th percentile (3)	Urethroplasty results; intrascrotal testicular prosthesis (4)	Bilateral gonadectomy results (1)	Adult (PHV, GV) (5)	13/20 = 65%	–	–	–	Slight overweight (BMI: 26 kg/mq)

6	19, 0/12	152.8 cm << 3rd percentile (1)	Chordee correction and urethroplasty results (3)	Rright gonad: intrascrotal, 25 mL (periodic monitoring with scrotal ultrasound and markers assessment: βhCG and αFP); Left gonad: gonadectomy results (4)	Adult (PHV, GV) (5)	13/20 = 65%	–	43.75%	Self-esteem: 81.25% Body image: 31.25% Interpersonal relationships: 75% Sexual activity: 6.25% Average: 48.3%	Slight overweight (BMI: 27.3 kg/mq)
7	8, 0/12	125.8 cm 25th–50th percentile (4)	Chordee correction and urethroplasty results (3)	Right gonad: intrascrotal, 1 mL (periodic monitoring with scrotal ultrasound); Left gonad: intrascrotal, 2 mL (periodic monitoring with scrotal ultrasound) (5)	Prepubertal (–)	12/15 = 80%	4.80/5 = 96%	–	–	None
8	6, 3/12	104.6 cm < 3rd percentile (1)	Chordee correction and urethroplasty results (3)	Right gonad: intrascrotal, fusiform, 1 mL (periodic monitoring with scrotal ultrasound); Left gonadectomy results (2)	Prepubertal (–)	6/15 = 40%	4.90/5 = 98%	–	–	Mitral regurgitation of moderate degree, hemodynamically stable (result of intervention to the atrioventricular common chamber)
9	5, 2/12	95.5 cm < 3rd percentile (1)	Vaginectomy and urethroplasty results (3)	Right gonad: intrascrotal, 2 mL (periodic monitoring with scrotal ultrasound); Left gonadectomy results (4)	Prepubertal (–)	8/15 = 53.3%	4.80/5 = 96%	–	–	None
10	4, 10/12	110 cm 50th–75th percentile (5)	Normal conformed, no reconstructive surgery (5)	Intrascrotal, 2 mL (periodic monitoring with scrotal ultrasound) (5)	Prepubertal (–)	15/15 = 100%	4.36/5 = 87.2%	–	–	None

2.4. GIQC—"Gender Identity Questionnaire for Children"

The 16-item Gender Identity Questionnaire for Children was taken from a study originally carried out by P.H. Elizabeth and R. Green in 1984, in order to create a tool that assessed possible problems in gender identity development. In 2004 Johnson and colleagues presented the psychometric properties of this parent-report instrument after making a few changes. The final 14-item questionnaire has excellent psychometric properties, therefore Johnson and colleagues proposed it as a useful and affordable screening tool, characterized by quick and easy administration [22]. Each item is rated on a 5-point scale for frequency of occurrence, except for 3 items which also have a 'not applicable' option; the score ranges from 1 (more cross-gendered behavior) to 5 (more same-gendered behavior). A mean GIQC score was calculated (gender-referred group mean score: 2.83).

The Italian version of this questionnaire was developed in the Department of Psychology, at the University of Turin using the translation/back-translation method in order to assure equivalence in meaning. The pilot study in which the psychometric properties of the tool were explored [28] showed that this questionnaire can be used for measuring children's gender-related behavior in the Italian context. The preliminary statistical analysis indicated that excluding two of the items significantly increased the reliability of the questionnaire (Cronbach's Alpha 0.83). The mean score of the non-clinical Italian group was 4.31 which was similar to that of the original study carried out by Johnson and colleagues in which the control group showed a mean score of 4.20.

In line with the preliminary results of the pilot study on the Italian version of the GIQC, in our data analysis we calculated the score on the 12 items for evaluating our patients. We were able to use this tool thanks to the availability and cooperation of the research group from the Department of Psychology lead by the scientific coordinator Prof. Piera Brustia.

2.5. WHOQOL-100—World Health Organization Quality of Life assessment

The World Health Organization Quality of Life assessment (WHOQOL-100) filled in by the young adults was implemented by the World Health Organization in order to develop a quality of life assessment that would be applicable cross-culturally [23,24,25].

The WHOQOL is divided into six broad domains of quality of life. These are:

(a) physical domain (the individual's perception of his/her physical status);

(b) psychological domain (the individual's perception of his/her cognitive and affective status);

(c) level of independence;

(d) social relationships (the individual's perception of interpersonal relationships and social role);

(e) environment; and

(f) spirituality/religion/personal beliefs (the individual's perception of life meaning).

Within each domain there are several quality of life sub-domains (facets) that summarize the particular domain of quality of life.

This questionnaire provides a percentage global score concerning quality of life (cut off 50%: over 50% good quality of life, under 50% poor quality of life), which incorporates 24 specific facets belonging to the six domains of Quality of Life [23,24,25].

In our study, 4 of the 24 facets were assessed due to their important implication in quality of life and gender identity:

1. self-esteem,

2. body image,
3. interpersonal relationships,
4. sexual activity.

3. Results

3.1. Clinical course analysis

3.1.1. Surgeries and cancer risk management

The reconstructive and gonadal surgical operations performed on the ten patients and the ages at which the operations were carried out are reported in Table 1.

The four female subjects underwent early feminizing reconstructive surgery. Subject 2 underwent corrective surgery in order to repair vaginal introitus stenosis and review clitoroplasty at 25 years of age. There is generally need for further intervention of possible vaginal introitus stenosis, subsequent to early vaginoplasty in these subjects after pubertal age [1,29,30].

The four female subjects underwent early bilateral gonadectomy due to the presence of streak or dysgenetic gonads in order to prevent malignancy and psychological problems with the presence of testes in subjects reared as females. At histological examination gonadoblastoma was not detected in any of the cases.

In the six male subjects, reconstructive surgery had a greater variability in timing which was often performed later in life. Gonadectomy was carried out in the presence of streak or dysgenetic gonads: the decision of removing them depended on the absence of hormone production and of spermatogenesis process in the presence of significant cancer risk. At histological examination gonadoblastoma was not detected in any of the cases.

Gonadectomy was not performed on subjects with apparently regularly developed testes, because of their possible hormone production and consequent spontaneous pubertal development.

Orchidopexy to the scrotum was performed on cryptorchid subjects.

3.1.2. Growth and pubertal development: treatment with human Growth Hormone (hGH) and sex hormone replacement therapy

In females, subject 1 received hGH therapy for 7 years (from the age of 9 years and 3 months to the age of 16 years and 5 months) and she reached a final height below the 3rd percentile (Figure 1). The growth pattern of subject 2 was stable at the 3rd percentile up to 10 years of age, reaching final height between the 3rd and 10th percentile following a 6 year course of hGH therapy (from the age of 10 years and 1 month to the age of 16 years and 3 months) (Figure 1). Subject 3 presented an initially normal growth pattern that reached its peak at the 75th percentile at 7 years of age after which there was a reduced growth pattern: hGH therapy was carried out from the age of 14 years and 2 months to the age of 15 years and 8 months with the achievement of final height at the 25th percentile (Figure 1).

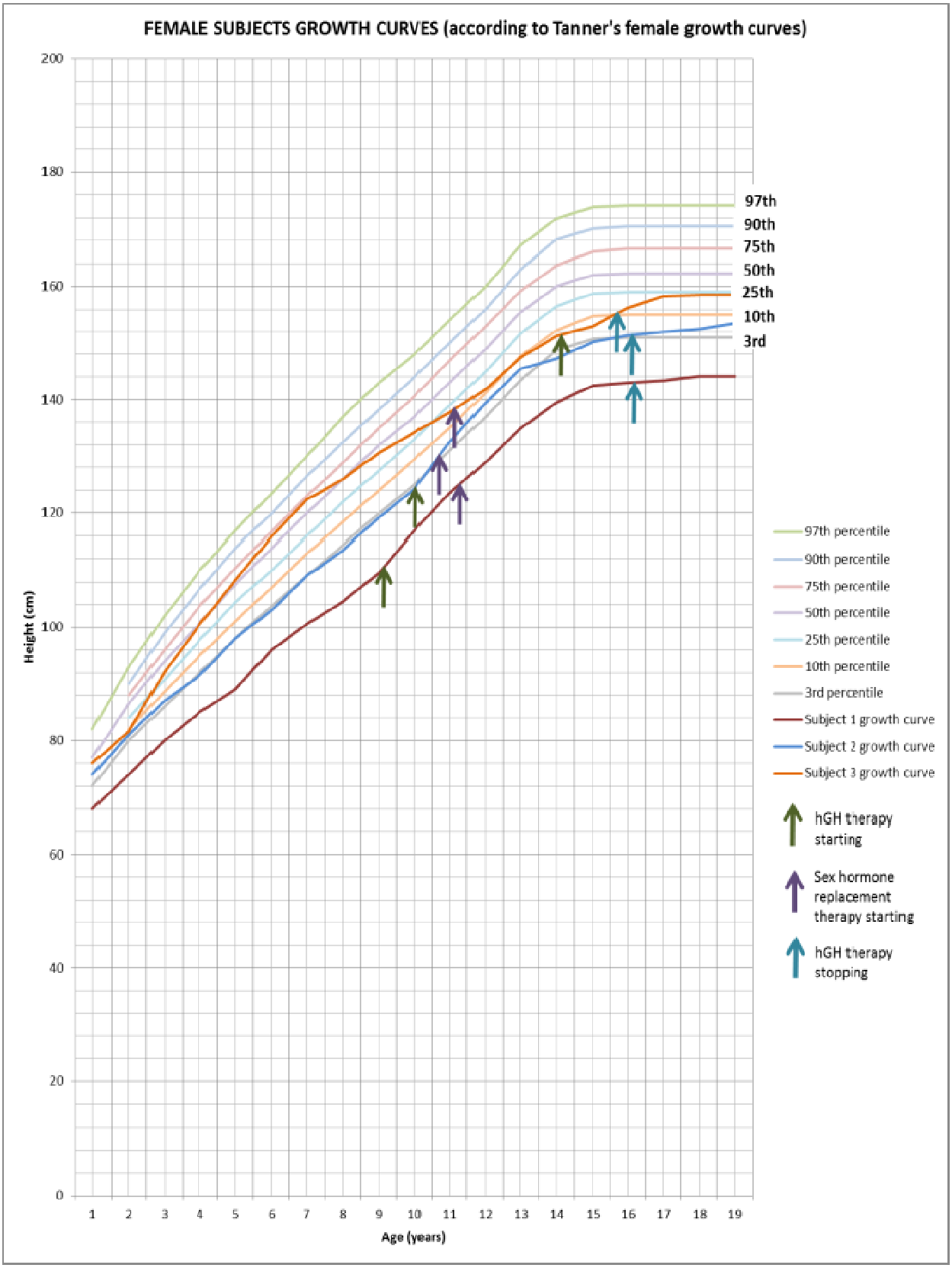

Figure 1. Female subjects growth according to Tanner [27].

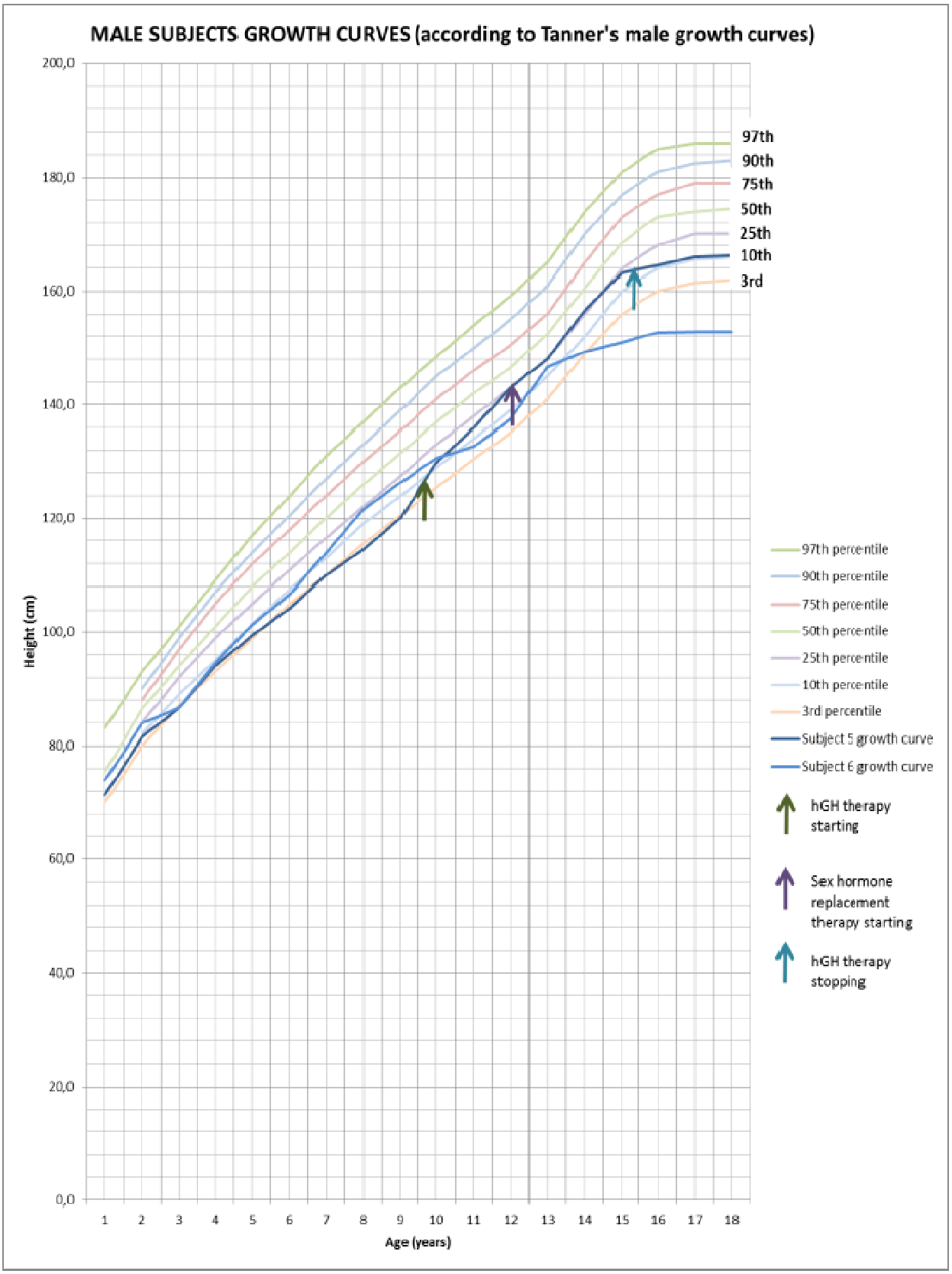

Figure 2. Male subjects growth according to Tanner [27].

The three young adults reared as females underwent sex hormone replacement therapy and reached complete adult development (according to Tanner staging) with regular menses (Figure 1).

Subject 4, a 12-month-old female, has not yet been submitted to any type of therapy.

In males, particular care was taken when considering hGH therapy due to the possible neoplastic risk in their potentially dysgenetic gonads submitted to such a proliferative stimulus. Following early bilateral gonadectomy, hGH therapy was administered to subject 5 from the age of 9 years and 8 months to the age of 15 years and 4 months: he reached final height above the 10th percentile. From the age of 12 years and 2 months he was administered testosterone (Figure 2). Therapy with human growth hormone was not administered to subject 6 because of noncompliance partially due to his concern about the possible neoplastic risk: his final height was below the 3rd percentile. His preserved gonad produced testosterone normally and spontaneous pubertal development occurred (Figure 2). The growth and gonadal features of the remaining four subjects (7-8-9-10) will be evaluated in the future as they were still in childhood at the end of the follow-up.

3.1.3. Clinical presentation at the end of the follow up

Table 4 summarizes the clinical presentation of the ten subjects at the end of the follow up in relation to the surgical operations and medical therapies carried out. Scores relating to the alignment of their phenotypic and functional features to the sex of rearing are presented and converted into percentages in order to carry out a more accurate comparison with the results of the questionnaires.

3.2. Gender identity and quality of life assessment

Table 4 shows the results of the gender identity questionnaire (GIQC) that was filled in by the parents of the four children (subjects 7-8-9-10) reared as males. The scores were high, indicating that the children developed and maintained a gender identity commensurate with the assigned sex.

It was not possible to investigated specific items of gender identity of subject 4 (the only child reared as female) since most of gender-related behaviors typically occur from 2–3 years of age, therefore her parents were not requested to fill in the questionnaire.

The WHOQOL-100 questionnaire was administered to the young adults in order to evaluate quality of life and the results are reported in Table 4.

The WHOQOL-100 questionnaire was not administered to subject 1 (reared as female) due to her cognitive impairment and to subject 5 (reared as male) because his family has opposed to prior attempts to interview him about his clinical condition.

3.3. Comparison of clinical scores to gender identity and quality of life scores

We compared clinical assessment with the results obtained from the questionnaires (GIQC in children and WHOQOL-100 in adults) as score percentages.

3.3.1. Children

It was not possible to evaluate the female children due to the age of the only child reared as female (subject 4).

For the male children we compared the clinical scores with the results of the questionnaire on gender identity (GIQC) (Table 4). Clinical scores in children included height, genital and gonadal features (total score = 15), as it was too early to assess pubertal development.

The male children developed gender behavior in line with the assigned sex. In subjects 7-8-9 GICQ score was higher than the clinical score; in subject 10 GICQ score was 87.2%, which was lower than the clinical score (100%).

3.3.2. Adults

The comparison of the young adults' clinical scores with the results of WHOQOL-100 questionnaire is reported in Table 4. The total scores of the 6 areas and of the 24 facets were evaluated. Among them, the following facets were subjected to further analysis (4-facets score):

1. self-esteem,
2. body image,
3. interpersonal relationships,
4. sexual activity.

For the only two female subjects the comparison between the clinical scores and questionnaire results showed that in subject 2 the total quality of life score and the 4-facets score were above 80%, thus exceeding the clinical score (60%); in subject 3 the total quality of life score was above 60% and similar to the clinical score (60%), while the 4-facets score was incomplete since she declined to answer the questions concerning her sexual activity (Table 4).

For the only young adult male who completed the questionnaire, the total quality of life score and the 4-facets score were below 50%, while the clinical score was 60% **(**Table 4**)**.

4. Discussion

Sex of rearing attribution in subjects with disorders of sex development depends on various issues: diagnosis, genital anatomy, surgical options, need for lifelong replacement therapy, potential for fertility, parental opinion and cultural practices [1,2]. The traditional approach which was mainly based on the physician's perspective and enabled him to attribute female sex on the basis of easier surgery [1,31] has progressively been overcome by the growing influence of parental opinion based mainly on personal and social experience. Early assignment of sex is necessarily grounded on the surrogate will of the parents [32]. Further information regarding the wellbeing of subjects with DSD from childhood to adult age is required in order to implement an appropriate strategy for assigning sex.

There is little or no available data on gender identity in DSD subjects: Meyer-Bahlberg found that, in genetic females with congenital adrenal hyperplasia, 5% expressed gender dysphoria, a much higher rate than that typically quoted and a few women transitioned from female to male gender [33]; Mazur affirmed that gender change from assigned-female to male is nil in complete androgen insensitivity syndrome [34]; Cohen-Kettenis and Reiner observed that gender change from female to male occurred in approximately 60% of XY DSD individuals who were assigned female as their sex of rearing at birth but with normal prenatal testosterone effects [35] and finally it was observed that gender change from female to male occasionally occurs in XY DSD individuals with low prenatal testosterone effects almost all of whom are assigned female at birth as their sex of rearing [36]. Only few studies regarding gender identity and psychosocial development in 45,X/46,XY mosaicism can be found in the literature [20,21].

Considering that active prenatal androgen effects appeared to dramatically increase the likelihood of recognition of male sexual identity independent of sex of rearing, we can assume that in our cohort there may have been partially deficient prenatal brain androgenisation [37].

It is essential to consider that patients with DSD are a heterogeneous group regarding gender identity and gender role outcome when planning treatment. In recent years there has been a shift toward multidisciplinary evaluation and treatment of DSD with the active involvement of the patient and family [38].

We propose methods for facilitating the association between physical findings and gender identification and quality of life in DSD studies. This study reports the use of these methods on a small cohort of subjects with 45,X/46,XY mosaicism divided into two age groups: children and young adults.

In children the limitations of our study were incomplete growth and pubertal development. In this age group strong identification with the assigned sex was observed, which was even stronger in those who had surgically reconstructed genitalia. Previous studies underline that gender identification in DSD appears to be less problematic during the early and middle stages of childhood than during adolescence and adulthood [39].

The Gender Identity Questionnaire for Children data show that the four-year-old male child with normal genital phenotype (subject 10) had a gender identity score of 87.2%, while the other children with genital anomalies had higher identity scores. In our opinion in subject 10, stereotypic male behavior, typical of mid childhood, was not so evident considering his age, while in the others, it was amplified to confirm the belonging to male gender. Nevertheless it is important to note that during childhood the correlation between gender identity and phenotypic appearance can be incomplete. Therefore it is essential to follow up the evolution of gender identity in these children especially at pubertal age, which is a critical phase for psychosexual development [40].

For the young adults quality of life was investigated by means of the WHOQOL questionnaire. There is a lack of consensus concerning conceptualization, operational definition and measurement of quality of life since it is actually an umbrella term covering various aspects such as functioning, health status, perceptions, life conditions, behavior, happiness, lifestyle and symptoms [41]. The WHOQOL questionnaire investigates quality of life from a broad perspective including: physical domain, psychological domain, level of independence, social relationships, environment and spirituality/religion/personal beliefs [23,24,25].

In the cases of the two young female adults (subject 2 and 3) the clinical scores were not balanced with quality of life scores: in fact they had similar clinical (60% in both) and different quality of life scores (87.5 % in subject 2 and 68.75% in subject 3). Additional variables regarding individual traits and social-familial context should be investigated in order to explain these differences. These variables are neither predictable nor easily editable [42]. Health related quality of life describes a person's satisfaction by integrating current life circumstances as well as the ability to cope and adjust to adversity: it is a complex construct that is strongly associated to family life, parents' responses and well-being, the quality of the social environment and other important factors [42]. Quality of life depends on subjective appraisal rather than objective measurements [41]. It is preferable to assess overall quality of life rather than health-related quality of life and it should be defined and measured in terms of life satisfaction [41].

For both sexes it was observed that some specific items reached insufficient scores, especially sexual activity and body image for the male (31.25%). This last observation is in line with a previous study which found that boys with DSD showed impairment concerning body image: they had a more

negative body image of the primary sexual characteristics compared with male controls [43]. It is important to remember regarding body image that the male subject had a reduced adult height suggesting that the possibility of growth hormone therapy should be carefully considered due to the strong effect of being short on body image perception.

Other studies investigated health-related quality of life in subjects with DSD. Kleinemeier E, Jurgensen M, Lux A, et al. observed that the psychological well-being of adolescents with DSD was not impaired, but outcomes related to adolescent developmental tasks such as sexual activity showed impaired participation and especially girls with DSD reported having fewer sexual activities than female controls [43]. For these subjects partnership and sexuality were identified as being difficult issues in life [44].

One of the females from our small cohort did not answer questions related to sexual activity and this could be due to difficulty in facing questions regarding sexual matters. Because of the elevated risk of sexuality-related problems, adequate and timely counseling regarding sexuality and relationships is of great importance: adolescents do not only need medical and sexual education, but they should also have the opportunity to discuss their concerns repeatedly with a mental health professional in private [45,46].

There is a report in literature claiming that adolescents who needed hormonal treatment to induce puberty have impaired well being compared to those who entered puberty spontaneously [43]. It is especially important to consider this in subjects reared as male since the risk of neoplastic transformation of the gonads should be weighted taking into account the possibility of a spontaneous pubertal development in presence of functional testicles.

5. Conclusion

Although the proposed scoring system should be tested on a larger control population, the comparison of clinical outcome with gender identity and quality of life provided us with important information for using a more complete approach for treatment, including psychosocial assessment.

The results should be carefully interpreted due to the limitations of the study. The findings are not easily generalizable since it was an observational study and lacks representativeness of the affected population.

Clinical and psychosocial assessment in people with DSD is mandatory in order to better direct care processes and adequate tools are required for a detailed analysis. In this sense the Clinical scoring system, the Gender Identity Questionnaire for Children and the World Health Organization Quality of Life assessment could prove to be important tools for investigating the alignment of physical characteristics and development with gender identity and quality of life.

Conflict of Interest

All authors declare no conflicts of interest in this paper.

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