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A European Network for the Investigation of Gender Incongruence: Endocrine Part

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ABSTRACT

Introduction: Cross-sex hormone therapy is an essential part of gender affirming treatment of transgender individuals. Studies systematically describing the physical and psychological effects of hormonal treatment of transgender persons are scarce.

Aim: The aim of the current protocol is to evaluate clinical and side-effects of cross-sex hormonal treatment in trans persons.

Methods: The European Network for the Investigation of Gender Incongruence (ENIGI) is a multicenter prospective study. Because of the relatively low prevalence of the condition and small number of specialized centers, international collaboration is warranted. Four European treatment centers, Ghent, Oslo, Florence, and Amsterdam, developed a common study and treatment protocol.

Main Outcome Measures: Outcome measures include hormonal and metabolic parameters, bone density, secondary sex and anthropometric characteristics, and physical and psychological well-being.

Results: Thus far, 333 trans women and 343 trans men have been included in the ENIGI Endocrine protocol. The study is still ongoing.

Conclusion: In recent years, the number of trans persons seeking gender affirming treatment has increased. However, well-designed prospective studies evaluating safety and effectiveness of current hormonal treatment protocols are lacking. Therefore we started the ENIGI collaboration. In this article we give a detailed description of the study protocol, objectives, and design of the ENIGI Endocrine protocol.

Key Words: Gender Dysphoria; Cross-Sex Hormonal Treatment; Prospective Cohort Study

INTRODUCTION

"Gender dysphoria" (GD) refers to the distress related to an incongruence between one's experienced gender and one's assigned gender that has been present for at least 6 months.[1] This condition has a great negative impact on physical, social, and psychological well-being and a large proportion of trans persons (but not all) desire gender affirming treatment.

For most trans individuals, hormonal treatment is an essential part of their sex change. Harry Benjamin (January 12, 1885–August 24, 1986) born in Germany, was an American endocrinologist and sexologist who was one of the pioneers treating patients with cross-sex hormones around the middle of the twentieth century. Although gender reassignment was still a matter of much debate, over half a century later hormone therapy and surgical reassignment are accepted as the mainstay treatment for gender dysphoria.[2]

Currently, endocrine treatment regimens are not standardized and include various forms, applications, and dosages of estrogens, progestins and (anti) androgens.[3] Thus far, no randomized intervention trials have been performed to determine the best treatment regimens. Furthermore, no large prospective studies have evaluated

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(side) effects of cross-sex hormone treatment (CHT) in a structured manner. The European Network for the Investigation of Gender Incongruence (ENIGI) collaboration creates a unique opportunity to perform the much-needed research in this field. The main objective of the study is to describe the clinical effects and side-effects of hormonal treatment in adults with GD. All participating centers will evaluate these clinical effects using the same standardized measurements and questionnaires.

Previously, Kreukels et al[4] presented the diagnostic protocol and psychological assessments used in the ENIGI Mental Health protocol. The close collaboration between both mental health and endocrine specialists offers a unique possibility for translational and interdisciplinary research. Furthermore, the international and multicenter approach of this study facilitates collaboration and exchange between different European expert centers. Another major advantage of this collaboration is that we can include larger number of patients in our study. This is of specific importance, because although patient numbers are increasing, GD is still a relatively rare condition.

AIMS

The aims of the endocrine part of the ENIGI collaboration is to evaluate the effects of CHT on hormonal and metabolic parameters, bone density, secondary sex and anthropometric characteristics, and physical and psychological well-being of trans persons. In this article, we present the study design and data collection procedures of the ENIGI Endocrine protocol.

SUBJECTS AND METHODS

Participating Centers

The ENIGI study is a multicenter prospective cohort study. So far, 4 West European gender identity clinics are participating in this collaboration. They include Ghent University Hospital, Belgium; VU University Medical Center in Amsterdam, the Netherlands; Rikshospitalet in Oslo, Norway; and University Hospital in Florence, Italy. These centers all use the ENIGI Mental Health protocol. The first trans persons were included in the ENIGI Endocrine protocol in 2010. Data presented in this article represent an update of our inclusions until March 12, 2015. At that time the first conference of the European Professional Association for Transgender Health (EPATH) was organized in Ghent, Belgium. Inclusion of trans persons is still ongoing in all participating centers.

The overall study protocol was approved by the Ethical Committee of Ghent University Hospital, Belgium. Every participating clinical center also obtained approval of their local ethical committees.

Study Participants

All patients underwent a standardized diagnostic procedure to confirm the diagnosis GD/gender incongruence and assess eligibility for treatment. The procedure of the ENIGI Mental Health protocol is described in Kreukels et al.[4] In short, the mean duration of the diagnostic phase varies between 6 and 12 months and consists of regular meetings with a psychologist and/or a psychiatrist. Once the diagnosis has been confirmed and there are no physical, psychological or social contraindications, hormonal treatment is started. In all centers, psychological counselling is offered during this phase, which is also called the "Social Transition Phase." During this 12- to 18-month period, persons experience life in the desired gender role on a daily basis. Once persons successfully go through this phase and there are no somatic contraindications, they are referred to the surgeon if they desire gender affirming surgery. All included subjects are above 16 years (Ghent and Oslo), above 17 years (Amsterdam), or above 18 years (Florence).

People are included in the ENIGI Endocrine protocol when they start medical treatment for GD/gender incongruence. Data on fulfillment of diagnostic criteria of current manuals for classification is collected in the ENIGI Mental Health protocol. Patients are eligible to participate if they have not used cross-sex hormones before and if they have sufficient knowledge of the native languages: Dutch or French for participants from Ghent, and Dutch, Italian, and Norwegian for patients from Amsterdam, Florence, and Oslo, respectively. At the start of CHT, patients receive oral and written information of the ENIGI endocrine protocol by their physician and written informed consent is obtained according to the institutional guidelines.

Treatment Protocol

Trans women

Cyproterone acetate in a once daily dose of 50 mg is started in combination with an estradiol agent. In Ghent, Amsterdam, and Florence, estradiol valerate is prescribed 2 mg twice daily, whereas in Oslo 4 mg is given in 1 single dose. Although not well established, the ENIGI medical team felt that risk of thrombosis from estrogens was greater with oral agents than with transdermal preparations, perhaps due to the "first pass effect" of the liver. Therefore the protocol calls for transdermal estrogens for patients older than 45 years of age. In Amsterdam and Ghent we prescribe estradiol patches in a dose of 100 mcg/24 hours. For some medical conditions, such as a history of thrombosis, treatment is started at a lower dose. In Florence patients can also choose from estradiol emidrate gel 1 mg twice daily.

Trans men

As not all testosterone treatment regimens are reimbursed in the different European countries, treatment protocols differ between the clinical centers. In Ghent and Oslo, testosterone undecanoate 1000 mg once per 12 weeks (Nebido) is prescribed. In the Netherlands, currently testosterone undecanoate injections are not covered by health insurance companies, so most patients choose between testosterone gel in a daily dose of 50 mg or testosterone esters 250 mg injections (Sustanon) every 2

weeks. If they prefer, testosterone undecanoate injections are prescribed.

In Florence, costs for hormonal treatment are covered by The National Health Service only in some regions. Trans men can choose between testosterone gel in a daily dose of 50 mg or different injectable formulations: testosterone undecanoate 1000 mg once per 12 weeks or testosterone enanthate 250 mg (Testo-enant©) injections every 2 weeks.

Data Collection

Demographic characteristics

At baseline (during the diagnostic phase) demographic characteristics are assessed. They include age, age of onset of GD, quality of life, education level (low: lower education and lower vocational; middle: secondary education, secondary vocational and high school; high: higher vocational, bachelor, master and PhD), living situation, employment (no employment, full-time, part-time, and education), and sexual orientation.

Clinical Measurements

At baseline and during each visit, body weight and height are measured in light indoor clothing without shoes. Additionally waist, hip, and chest circumference are measured. The chest circumference is measured at the level of the nipples and just below the breasts, so cup size can be inferred. Blood pressure is measured using an electronic blood pressure monitor. Grip strength at the dominant hand is measured using an adjustable hand-held standard grip device (JAMAR hand dynamometer, Sammons and Preston; Bolingbrook, IL, USA).[5,6]

The Ferriman-Gallwey score is used to evaluate and quantify body hair in trans men. Because of shaving and local manipulation the Ferriman-Gallwey score is not meaningful in trans women.[7] Acne is assessed using the Global Acne Grading Scale (GAGS).[8] At baseline a detailed medical history is taken to evaluate contraindications for hormonal treatment. At each visit current smoking status, alcohol use, medication use, and intercurrent diseases are assessed.

At all visits all clinical measures are filled out on a standardized form. After the visit this information is entered in our database manually. Measures that are used for clinical purpose are also documented in our medical records and include length, weight, blood pressure, current smoking status and alcohol use, intercurrent disease, and medication use.

Serum determinations

Venous blood samples are obtained between 8 AM and 10 AM after overnight fasting. Samples are analyzed at the local laboratory, as they are also used for clinical purposes. Serum measurements include haematocrit, alkaline phosphatase, gamma-glutamyltransferase, aspartate-aminotransferase, alanine-aminotransferase, creatinine, glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, 25-hydroxycholecalciferol, calcium, albumin, testosterone, estradiol, sex hormone-binding globulin, luteinizing hormone, prolactin, and prostate-specific antigen (in trans women aged 45 years and older).

Serum Bank

Next to the serum samples drawn for clinical evaluation additional samples are stored for future research purposes. These samples are collected at baseline, after 3 months and 1, 2 and 10 years of CHT. Next to this, at baseline, buffy coats are stored for future DNA research. In the near future we will start by extracting DNA from these samples. All samples are stored at −80°C at every local center.

Bone Measurements and Body Composition

Body fat and lean mass, bone mineral content, bone area, and areal bone mineral density at the lumbar spine and left proximal femur (total hip and femoral neck region) are measured using dual X-ray absorptiometry (DEXA). In Ghent additional bone measurements are available using peripheral quantitative computed tomography (pQCT).[9] Bone measurements are performed at baseline, and after 1, 2, and 10 years of hormonal treatment.

Questionnaires

At each visit questionnaires are handed out by our secretary staff or physicians. It takes approximately 15 to 20 minutes to fill out these forms. The questionnaires are processed using TeleForm automated data extraction (Hewlett-Packard Company; Palo Alto, CA, USA).

Physical activity: Habitual physical activity is measured using the Baecke Activity Questionnaire, which consists of 16 questions classified into 3 different dimensions: work, sport, and non-sports leisure activity. Each domain can receive a score from 1 to 5 points, thus allowing a total score from 3 (minimum activity) to 15 (maximum activity).[10]

Experienced vocal performance: The Trans Voice Questionnaire is a 30-item self-administered questionnaire that evaluates the psychosocial consequences of voice disorders and consists of 3 dimensions: functional, physical, and emotional impairment. A score of 0 is equivalent to no disability and a score of 120 is equivalent to maximum disability.[11]

Side-effects questionnaire: We constructed a questionnaire to evaluate side-effects of hormonal treatment such as psychovegetative symptoms, physical complaints, cognition, emotionality and sexuality, genital complaints, and pain.

The following 4 questionnaires are part of the Mental Health-Endocrinology protocol:

Positive Affect Negative Affect Scale (PANAS): The PANAS is a 20-item questionnaire that measures long-term changes in affect. Participants are asked to what extent they experience certain

feelings such as anxiety, happiness, or guilt on a 5-point scale from "very little" to "very much".[12]

Aggression proneness: The STAXI-2 State Anger scale is a 15-item questionnaire that evaluates aggression. Participants rank certain statements along a 4-point continuum from "not at all" to "very much." The questionnaire evaluates angry feelings on 3 subscales: feeling angry, feeling like expressing anger verbally, and feeling like expressing anger physically.[13]

Sexual desire: The Sexual Desire Inventory (SDI) is a self-administered 14-item questionnaire that aims to measure sexual desire. The SDI measures the individual's thoughts as well as actual experiences. Fourteen questions assess the strength, frequency, and importance of an individual's desire for sexual activity with others and by themselves. The score ranges from 0 (no sexual desire) to 112 (maximum desire).[14]

Sexual orientation questionnaire: This short questionnaire consists of 4 questions in the "background data interview" which is part of the ENIGI Mental Health protocol. These questions evaluate gender roles in sexual fantasy, and sexual orientation.

Psychological questionnaires: For a detailed description of the additional psychological questionnaires used in the ENIGI Mental Health protocol, see Kreukels et al.[4] These questionnaires are administered at baseline (during the diagnostic phase) and evaluate gender identity and dysphoria, body image, quality of life, psychological functioning, and psychiatric comorbidity.

Follow-up and data management

The short-term follow-up consists of ENIGI visits at baseline and after 3, 6, 9, 12, and 24 months of CHT. We also will perform mid-term follow-up after 10 years of hormonal treatment. In the future we will introduce long-term follow-up visits as well.

Currently, data management is performed by dedicated PhD students and laboratory technicians. We exchange databases at our twice-yearly research meetings. In the near future, data exchange and storage will be coordinated by a central ENIGI data manager. All ENIGI participants are given an ENIGI research number by which their data are anonymized.

New research proposals are discussed at our ENIGI meetings and all senior research partners have to agree with the proposal. Upon agreement the researcher gets access to the data needed for that specific research question. New ENIGI partners are welcome to join our initiative by contacting one of the senior investigators and filling out a questionnaire that evaluates quality of delivered health care and number of trans persons treated.

RESULTS

The baseline characteristics of our study population are presented in Table 1. So far 333 trans women and 343 trans men have been included in the ENIGI Endocrine protocol. The mean age at start of follow-up was 30 years and 24 years for trans women and trans men, respectively. Most trans women are treated with estradiol valerate tablets (n = 221), whereas fewer patients are treated with transdermal oestradiol patches (n = 109) or estradiol gel (n = 3). The most frequently prescribed hormonal treatment for trans men is testosterone undecanoate injections (n = 177). Ninety trans men are treated with testosterone injections every 2 weeks (Sustanon or Testo-enant) and 76 trans men with testosterone gel. We have complete data on age and treatment at start of follow-up. Missing data is below 7 percent for all other characteristics presented in Table 1.

Table 1. Characteristics of the study population and cross-sex hormonel treatment regimens

	Trans women	Trans men
Total number of trans persons (n)	333	343
Number of trans persons per center (n)		
VU University Medical Centre Amsterdam	178	185
Ghent University Hospital	130	69
Rikshospitalet University Hospital Oslo	13	81
University Hospital Florence	12	8
Cross-sex hormonal treatment at start of study (n)		
Estradiol valerate 4 mg/day + CPA	221	
Estradiol patches 100 mcg/24 hours + CPA	109	
Estradiol gel* + CPA	3	
Testosterone im 1000 mg once per 12 weeks		177
Testosterone im 250 mg once per 2 weeks		90
Testosterone gel 50 mg/day		76
Age, median (range) (y)	30 (16–65)	24 (16–51)
Systolic blood pressure (mm Hg)	127 (3.3)	122 (6.2)
Diastolic blood pressure (mm Hg)	79 (3.1)	75 (4.5)
Body weight (kg)	77 (2.8)	71 (2.5)
Length (m)	1.77 (0.02)	1.67 (0.02)
BMI (kg/m^2)	24.4 (0.8)	25.3 (0.7)
Current smoking (%)	32.7	32.1
Alcohol, median (25th/75th percentile) (units/wk)	0 (0–2)	0 (0–2)

CPA = cyproterone acetate; im = intramuscular.
Data are presented as means (SD) unless stated otherwise.
*Patients treated with estradiol emidrate gel 1 mg twice daily.

DISCUSSION

The ENIGI Endocrine protocol studies the effects and side-effects of CHT in transgender individuals. Data collection began in 2010 and at time of the first EPATH conference in

March 2015 in Ghent (Belgium), 333 trans women and 343 trans men were included from 4 different European expertise centers.

Recently, the first results of the ENIGI collaboration were published. Wierckx et al evaluated side-effects and safety of CHT in 53 trans men and 53 trans women. For this study patients from Ghent University Hospital and Rikshospitalet University Hospital Oslo were included. The authors concluded that the treatment modalities used were effective and carried a low risk for side-effects and adverse events at short-time follow-up.[15] Furthermore, Van Caenegem et al showed that although trans women had a lower bone mineral density and cortical bone size before the start of CHT, their skeletal status was well preserved during the first 2 years of their hormonal treatment, despite substantial muscle loss.[16] For this study 49 trans women were included from Ghent University Hospital. Additional analyses using the (combined) data of the different centers are ongoing.

To our knowledge, the ENIGI initiative is the first large prospective cohort study to evaluate clinical and side-effects of CHT. In line with the findings of Wierckx et al, previous observational studies showed that CHT of trans men yields a low risk of adverse events in the short and longer term.[17,18] However, observational studies with longer follow-up times show that the incidence of cardiovascular disease in trans women is higher compared with the control population, whereas this increase is not observed in trans men on testosterone treatment.[17] Limitations of these studies include the retrospective design (all studies), relative low number of study subjects,[19-21] and relatively short median duration of follow-up of most of these studies (between 4.4 and 9.0 years).[19-23] This is of importance, because the largest increase in incidence of lethal cardiovascular events in trans women seems to occur after 10 years of CHT. This was shown by a study by Asscheman et al on total and cause-specific mortality of trans persons receiving hormonal treatment in which 966 trans women and 365 trans men were included with a median duration of follow-up of 18.5 years.[24] However, in this study most lethal ischemic cardiac events observed were related to ethinyl estradiol use. This synthetic estrogen is not part of current CHT protocols since the observation of the increased risk of venous thrombosis related to this compound.[22] Well-designed prospective studies in large samples are needed to evaluate safety and effectiveness of current hormonal treatment of trans persons.

In ENIGI, all centers use the same study protocol. We use the same standardized questionnaires and use the same devices to measure clinical outcomes. In this way we will be able to include patients from participating centers in combined studies and compare results of the different centers easily. However, the hormonal treatment protocols differ somewhat between participating centers. The most important reason for these discrepancies is that not all treatment modalities are reimbursed in the different European countries. For instance, testosterone undecanoate injections are not covered in the Netherlands. Although these differences were not introduced intentionally, they may provide us with insights into possible different clinical and side-effects of different treatment options for trans persons. Although we are not performing a randomized controlled trial, important clinical lessons will be learnt.

Up till now we have not collected data on total number of eligible patients, and number and reasons of non-participation. However, in our experience the majority of patients were very motivated to participate in the study and wanted to contribute to improve transgender health care. At our latest research meeting we agreed to start collecting these data from June 2016 onward. Another important improvement is that in the near future data storage and exchange will be coordinated by a central ENIGI data manager. However, due to lack of funding we have not been able to launch a central ENIGI website yet which can be used for data management, exchange of research protocols, and application of new ENIGI partners. Hopefully, with enough resources we will be able to launch this website in the future.

A better understanding and knowledge of clinical and side-effects of CHT will be essential to optimize hormonal treatment of trans persons. Once a substantial database has been collected, variations in products and dosages may be introduced in the hormonal treatment protocol of trans persons.

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