Polymorphic CAG repeat length of androgen receptor gene and male-to-female gender incongruence: a systematic review and meta-analysis of case-control studies

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Running Title: Androgen receptor polymorphism and gender dysphoria

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ABSTRACT

Introduction. It has been hypothesized that male-to-female (MtF) gender incongruence could result from an antenatal impaired androgen activity on the developing brain. As the length of polymorphic CAG repeat sequences in the androgen receptor (AR) gene is inversely correlated with AR transcriptional activity, some studies explored a possible association between long CAG repeats and MtF gender incongruence. Yet results remain inconclusive.

Aim. To systematically evaluate whether a difference exists in the length of AR CAG repeat sequences between MtF individuals and men without gender incongruence.

Methods. A thorough search of Medline, SCOPUS, Cochrane library, Web of Science, and CINAHL databases was carried out to identify suitable case-control studies. Methodological quality of the included articles was assessed using the Newcastle-Ottawa Scale. In the absence of between-studies heterogeneity, as assessed by the Cochrane Q and I^2 tests, standardized mean differences (SMDs) in the length of AR CAG repeats were combined using a fixed effect model. Funnel plot and trim-and-fill analysis were used to assess publication bias.

Main Outcome Measures: The association of MtF gender incongruence with longer length of AR CAG repeat sequences was evaluated by calculating pooled SMD with 95% confidence interval (CI).

Results. Five studies included in the quantitative analysis collectively provided information on 795 MtF individuals and 1,355 control men. At the overall estimate, the MtF group exhibited a significantly longer length of AR CAG repeat sequences (pooled SMD 0.13, 95%CI: 0.04 to 0.22; P = 0.005; I^2 = 0%, $P_{\text{for heterogeneity}}$ = 0.51). Sensitivity analysis demonstrated the high stability of the result. Funnel plot revealed a possible publication bias and the trim-and-fill test detected two putative missing studies. Nevertheless, the significant association persisted even when pooled estimate was adjusted for publication bias.

Clinical Implications: These findings could suggest a contribution of a genetically-mediated impairment in androgen signaling in development of MtF gender incongruence.

Strength & Limitations: This is the first meta-analysis exploring the relationship between AR CAG repeat polymorphism and gender incongruence. However, interactions with other functional genetic variants were not explored and caution should be exercised when generalizing these results due to the possible variability in the distribution of CAG repeats among different populations and ethnic groups.

Conclusion: MtF population exhibits significantly longer polymorphic CAG repeat sequences in the AR gene. Further studies are warranted to elucidate whether, how and to what extent multiple functional variants in sex hormone signaling genes could be associated with gender incongruence/dysphoria.

Systematic review registration: PROSPERO ID: CRD42017070782**.**

INTRODUCTION

Gender dysphoria in the fifth version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5),[1] also referred to as gender incongruence in the 11th revision of the International Classification of Diseases (ICD-11),[2] was formerly known as transsexualism in ICD-10[3] and gender identity disorder in DSM-IV-TR.[4] This condition is characterized by a deep incongruence between experienced gender and biological sex,[3] which underpins a motivational thrust to social transition from male-to-female (MtF) or female-to-male (FtM), resulting, in many cases, also in a somatic transition through cross-sex hormone treatment and sex reassignment surgery.[1] Although reported prevalence may vary widely accordingly to different definitions, in a recent meta-analysis (Collin et al., 2016),[5] the overall prevalence estimates of transgender-specific diagnoses were 2.5 per 100,000 for FtM and 5.8 per 100,000 for MtF.

The etiology of gender incongruence is still largely unknown and under debate. While, on the one hand, it has been claimed that psychosocial factors, including dysfunctional family dynamics[6] and traumatic childhood experiences,[7] could play a key role, on the other hand, in a neurobiological perspective, some evidence point to a permanent structuring of gender identity starting during the fetal life.[8] Neuroanatomical *post mortem* studies focused on two sexually dimorphic brain structures, the central subdivision of the bed nucleus of the *stria terminalis* (BSTc) and the interstitial nucleus of the anterior hypothalamus-3 (INAH3), which in men are twice as large as in women and contain twice as many neurons, but in MtF individuals appear to be "female-like" both in volume and neuron density.[9-11] Also *in vivo* neuroimaging studies revealed specific regions in the brain of persons with gender incongruence resembling that of their experienced gender rather than that of their biological sex.[12-14] According to the classical organizational theory,[15] sexual dimorphic development of the brain would be an endocrine-driven process with testosterone representing the key determining factor: prenatal/neonatal exposure to testosterone promotes a "male-typical" brain development, whereas a brain "feminization" occurs in the relative absence of testosterone. This notion, that would lead to gender incongruence being considered as a reflection of an aberrant early organizational influence of testosterone on the developing brain,[16-18] has gained support from the study of specific congenital endocrine clinical models. Genetic males with complete androgen insensitivity syndrome (CAIS), due to mutations in the gene of androgen receptor (AR), develop as phenotypical women and experience a congruent female gender identity.[19,20] On the contrary, females with congenital adrenal hyperplasia, which induces an increase in adrenal androgens, can display male-typical behaviors, reduced satisfaction with the female sex of assignment and reduced heterosexual interest.[21,22]

On this basis, and following on from concordance rates among monozygotic twins,[23] pointing to a possible genetic contribution to gender incongruence development, some authors have investigated whether functional genetic variants influencing sex-hormone signaling could be

associated with gender identity disorders. One of the candidate polymorphisms involves the AR gene. In the exon 1, AR gene contains a polymorphic sequence of cytosine–adenine–guanine (CAG) repeats, encoding for a polyglutamine chain which affects the DNA transcriptional activity of AR. As the length of the polyglutamine chain correlates inversely with the receptor transcriptional activity[24-26] and men exhibiting exceptionally long CAG repeats experience clinical features of androgen insensitivity,[27,28] it has been hypothesized that such a polymorphism could be responsible for an "undermasculinization" of the brain. However, the association between long CAG repeat sequences in AR and MtF gender incongruence reported by some authors[29,30] has been denied by others.[31-33]

As small sample sizes in most studies might have contributed to inconclusive results, we carried out a systematic review with meta-analysis of the available evidence to comprehensively assess whether a statistically significant difference exists in the length of AR CAG repeat sequences between MtF people and healthy control men.

MATERIAL AND METHODS

The study was conducted according to the Cochrane Collaboration and to the statement of Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA).[34] It also complies with the guidelines from Meta-analyses Of Observational Studies in Epidemiology (MOOSE).[35] The PRISMA and MOOSE checklists have been presented as **Supplemental Tables 1** and **2**. The study is registered in the PROSPERO International Prospective Register of Systematic Reviews with the number CRD42017070782 (https://www.crd.york.ac.uk/PROSPERO/).

Systematic search strategy

Three independent authors (AB, FP and GS) performed an extensive search in MEDLINE (PubMed), SCOPUS,Cochrane Library, WEB OF SCIENCE, and CINAHL including the following

free and vocabulary terms: “gender identity”, “gender dysphoria”, “gender incongruence”, “GID”, “transsex*”, “transgender*”, “male-to-female”, “MtF”, “androgen receptor”, “testosterone receptor”, “androgen receptor repeat*”, “testosterone receptor repeat*”, “androgen receptor polymorphism*”, “testosterone receptor polymorphism*”, “CAG repeat*”, “androgen receptor polyglutamine”, and “testosterone receptor polyglutamine”, using the Boolean functions AND/OR. The search was restricted to English-language studies enrolling human participants. If it was not clear from the abstract whether the paper contained relevant data, the full text was retrieved. We scrutinized the reference lists of the identified articles to find additional pertinent studies.

Inclusion and exclusion criteria

The outcome of interest was a difference in the length of polymorphic AR CAG repeat sequences between MtF individuals and men without gender incongruence. Eligibility criteria for the study selection were: 1) observational case-control studies involving MtF people (cases) and a male healthy control group; 2) availability of mean length ± standard deviation (SD) of AR CAG repeat sequences in both groups. When the same population sample was used for multiple publications, the study with the largest number of cases was included. Two independent reviewers (AB and SD) evaluated for eligibility the full text of all selected studies and, where disagreement occurred, a third reviewer (FF) took a decision after open discussion.

Data extraction

Data were extracted from the selected papers by including the first author, publication year, geographic region and setting of enrollment, diagnostic tools (ICD-10, DSM-IV/DSM-IVTR or DSM-5) for definition of cases (MtF transsexualism, GID, or GD, respectively), the total number of cases and controls, the mean length ± SD of AR CAG repeat sequences in both groups. Additional information, when available, included ethnicity and mean age ± SD or age range of the participants.

When summary statistics were not fully reported, these were calculated whenever possible,[36] and, when data were missing or inconsistent, the authors of the original paper were contacted to obtain the necessary information.

Quality assessment

The quality of the included studies was assessed using the "star system" of the Newcastle-Ottawa Quality Assessment Scale (NOS).[37] The minimum score was 0 stars and the maximum that could be awarded was 9 stars. Studies getting scores ≥6 stars were regarded as good quality studies. Quality assessment was performed by two reviewers (AB and CC) and any disagreement was resolved by involving a third reviewer (SF) who re-evaluated the original study.

Statistical analysis

The association of MtF gender incongruence with longer length of AR CAG repeats was assessed by calculating a pooled standardized mean difference (SMD) with a 95% confidence interval (CI). In the absence of heterogeneity between the studies, data were combined using a fixed effect model. Cochrane Chi-square X^2 (Cochrane Q) statistic and I^2 test were performed to analyze heterogeneity between the results of different studies. An I^2 >50% and/or P ≤0.05 indicated substantial heterogeneity.[38] Sensitivity analysis was performed by sequential omission of individual studies to determine the contribution of each study to the pooled estimate, thus evaluating the stability of the result.

Publication bias was graphically explored through a funnel plot wherein a symmetric inverted funnel shape arises from a "well-behaved" data set, in which publication bias is unlikely.[39] To correct for publication bias, Duval and Tweedie's "trim-and-fill" analysis was carried out, as previously reported.[40-42] Briefly, in the presence of asymmetric funnel shape, this test detects

putative missing studies to rebalance the distribution and provides an adjusted pooled estimate taking the additional studies into account, thus correcting the analysis for publication bias.[43]

Data were analyzed using the package 'metafor' of R statistical software (version 3.0.3; The R Foundation for Statistical Computing, Vienna, Austria) and the Review Manager (RevMan) of the Cochrane Library (version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

RESULTS

Study selection

From electronic search we retrieved a total of 121 articles and two additional papers were obtained after manual search. After removal of duplicate, 103 studies were left, of which 91 were excluded as irrelevant based on title and abstract reading. Hence, as shown in **Figure 1**, a total of 12 articles were identified, of which 6 met the inclusion criteria: one study was included in the qualitative synthesis only[44] and 5 studies were also included in the meta-analysis.[29-33] Details of the articles included in the quantitative synthesis are reported in **Table 1**.

Quality of included studies

Quality rating of the meta-analyzed studies, based on the NOS score, is outlined in **Table 2**. Three articles were considered to be of good quality,[30,32,33] scoring 6; whereas two articles were assessed to be low/moderate.[29,31] In particular, in all studies, a bias in representativeness of cases could not be ruled out, since only individuals referred to gender identity hospital clinics were enrolled. Furthermore, a risk of selection bias occurred in control group, as, in most of the studies, controls were selected within a hospital setting and only in the study by Henningson et al,[31]

community controls were recruited. Finally, in the study by Hare et al[29] different populations from Australia and Los Angeles were compared.

Synthesis of results

The five studies including in the meta-analysis collectively gave information on 795 MtF individuals and 1,355 control men. Overall, as shown in **Figure 2**, the length of CAG repeat sequences of AR gene was significantly longer in the MtF group (pooled SMD 0.13, 95% CI: 0.04 to 0.22; P = 0.005; I^2 = 0%, $P_{\text{for heterogeneity}}$ = 0.51). Sensitivity analysis was performed to assess the contribution of individual studies to the overall estimate. As shown in **Figure 3**, similar pooled SMDs and 95% CIs were generated with the exclusion of each study, thus indicating the high degree of stability of the result.

Publication bias

As shown in **Figure 4**, the asymmetry of the funnel plot suggested a possible publication bias and, accordingly, the trim-and-fill analysis identified two putative missing studies on the right side of the distribution. Nevertheless, even after adjusting for publication bias, the pooled estimate indicated a significant association of MtF gender incongruence with longer length of CAG repeat sequences (pooled SMD 0.14, 95%CI: 0.06 to 0.23; P = 0.0008; I^2 = 4.1%, $P_{\text{for heterogeneity}}$ = 0.6).

DISCUSSION

To date, discordant results have been produced by the scarcely available comparative studies evaluating the difference in the length of AR CAG repeat sequences between MtF individuals and men without gender incongruence. While no association was found in three studies,[31-33] findings from the two largest series published so far[29,30] go in the opposite direction. In line with data from

the general population, where CAG repetitions in the AR gene averaged about 20,[45] in the carefully selected studies included in the present meta-analysis, the mean number of repeats in healthy control men ranged from 18.6 ± 2.5[31] to 21.9 ± 2.9.[33] Overall, when compared with control group, quantitative synthesis revealed significantly longer CAG repeat sequences in MtF individuals, with a pooled SMD of 0.13 (95% CI 0.04, 0.22).

It should be recognized that while the gender incongruence is a rare disorder, the gene polymorphism investigated in the present study is relatively common, suggesting that the here revealed association would not reflect a direct independent causal link. It is likely, indeed, that variants in different sex hormone signaling genes involved in brain sexual differentiation, together with other factors, could influence the development of gender identity/incongruence. Animal studies suggested a quite surprising crosstalk between testosterone and estrogens in exerting masculinizing/defeminizating effects on the developing male brain. In rodent models, indeed, such effects would be exerted by testosterone both directly[46] and following its conversion into estrogen by the brain aromatase (CYP19A1) with consequent activation of central estrogen receptor α (ERα) and β (ERβ).[15] In this scenario, not only the AR gene polymorphism, but also variants involving aromatase and ERs genes might represent reasonable candidates in searching for possible genetic factors underlying the gender incongruence. In particular, two short tandem repeat polymorphisms drew attention: the cytosine-adenine (CA) repeat in intron 5 of the ERβ gene and the thymine-thymine-thymine-thymine-adenine (TTTA) repeat in intron 4 of the CYP19A1 gene. It has been reported that the length of CA and TTTA is directly proportional to the activities of ERβ[47] and aromatase,[48,49] respectively. In a study by Fernández et al,[50] the number of ERβ CA repeats was significantly higher in FtM individuals than in healthy female controls, and subjects with the genotype homozygous for long alleles exhibited a two-fold higher likelihood of developing FtM gender incongruence. Interestingly, when analyzed by logistic regression models, interactions of (CAG)n-AR with (CA)n-ERβ[30,44] and with both (TTTA)n-CYP19A1 and (CA)n-ERβ[31] were

significantly overrepresented in MtF people when compared to male controls. Unfortunately, the dearth of studies refrained us from carrying out quantitative analyses of gene-gene interaction data.

Some limitations of this meta-analysis, other than the aforementioned lack of information about other functional genetic variants, have to be recognized. Firstly, the inclusion of a limited number of studies. This, however, resulted from a strict screening and selection of the literature. Although only five studies were included in the quantitative analysis, as a whole, taking into account the prevalence of gender incongruence, they provided information on a relatively large number of MtF individuals. In any case, in spite of the low number of studies, at the sensitivity analysis, similar pooled SMDs and 95% CIs were generated when the studies accounting for the largest proportion of the global study population and with the highest weight in contributing in the pooled estimate[29,30] were excluded, thus indicating the very high degree of stability of the result. Secondly, as most studies enrolled Caucasian populations (**Table 1**), caution should be exercised when generalizing these results due to the possible variability in the distribution of CAG repeats among different populations and ethnic groups. Finally, the funnel plot revealed a possible publication bias, suggesting that published studies might be a not fully representative sample of the available evidence. Nevertheless, corrected pooled SMD, taking into account two putative missing studies identified by the trim-and-fill analysis, demonstrated that publication bias did not substantially affect the overall estimate.

In conclusion, in the present meta-analysis, the first carried out so far on this topic, MtF people exhibited significantly longer polymorphic CAG repeat sequences in the AR gene. Further studies are warranted to elucidate whether, how and to what extent multiple functional variants in sex hormone signaling genes could interact with each other, and with other factors, in influencing the development of gender identity/incongruence in different human population and ethnic groups.

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FIGURE LEGENDS

Figure 1. Flow diagram showing an overview of the study selection process.

Figure 2. Forest plots depicting the standardized mean difference in the length of CAG repeats in androgen receptor gene between male-to-female (MtF) individuals and healthy control men. Diamond indicate the overall summary estimate and width of the diamond represents the 95% confidence interval (CI); boxes indicate the weight of individual studies in the pooled result. df, degrees of freedom; IV, inverse variance; SD, standard deviation.

Figure 3. Sensitivity analysis showing the influence of each individual study on the pooled standardized mean difference (SMD) with 95% confidence interval (CI) in the length of CAG repeats of androgen receptor gene between male-to-female (MtF) individuals and healthy control men.

Figure 4. Funnel plot for the analysis of standardized mean difference in the length of CAG repeats of androgen receptor gene between male-to-female (MtF) individuals and healthy control men. The trim-and-fill analysis identified two putative missing studies (white circle) on the left side of the distribution.