Reproductive BioMedicine Online (2016) 32, 218–224

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Deletion of GOLGA2P3Y but not GOLGA2P2Y is a risk factor for oligozoospermia

Sanjukta Sen [a], Rupesh Agarwal [a], Prafulla Ambulkar [b], Indira Hinduja [c], Kusum Zaveri [c], Jyotsna Gokral [d], Asoke Pal [b], Deepak Modi [a,*]

[a] *Molecular and Cellular Biology Laboratory, National Institute for Research in Reproductive Health (ICMR), JM Street, Parel, Mumbai, 400 012, India;* [b] *Human Genetic Division, Department of Anatomy, Mahatma Gandhi Institute of Medical Sciences, Sevagram, 442102, Wardha, India;* [c] *Hinduja IVF Centre, PD Hinduja Hospital and Medical Research Center, Veer Savarkar Marg, Mahim, Mumbai, 400016, India;* [d] *Department of Reproductive Endocrinology and Infertility, National Institute for Research in Reproductive Health (ICMR), JM Street, Parel, Mumbai, 400012, India*

* Corresponding author. *E-mail address:* deepaknmodi@yahoo.com (D Modi).

Sanjukta Sen has completed her masters degree in biochemistry, and is presently pursuing a doctorate degree in applied biology. Her area of interest is genetics of male infertility, with a focus on Y chromosomal genes and male infertility. She is also interested in the reproductive outcome of patients with male infertility opting for ICSI for biological parenthood.

Abstract The AZFc locus on the human Y chromosome harbours several multicopy genes, some of which are required for spermatogenesis. It is believed that deletion of one or more copies of these genes is a cause of infertility in some men. *GOLGA2LY* is one of the genes in the AZFc locus and it exists in two copies, *GOLGA2P2Y* and *GOLGA2P3Y*. The involvement of *GOLGA2LY* gene copy deletions in male infertility, however, is unknown. This study aimed to investigate the association of deletions of *GOLGA2P2Y* and *GOLGA2P3Y* gene copies with male infertility and with sperm concentration and motility. The frequency of *GOLGA2P3Y* deletion was significantly higher in oligozoospermic men compared with normozoospermic men (7.7% versus 1.2%; $P = 0.0001$), whereas the frequency of *GOLGA2P2Y* deletion was comparable between oligozoospermic and normozoospermic men (10.3% versus 11.3%). The deletion of *GOLGA2P3Y* but not *GOLGA2P2Y* was significantly higher ($P = 0.03$) in men with gr/gr rearrangements, indicating that *GOLGA2P3Y* deletions increase the susceptibility of men with gr/gr rearrangements to oligozoospermia. Furthermore, men with *GOLGA2P3Y* deletion had reduced sperm concentration and motility compared with men without deletion or with deletion of *GOLGA2P2Y*. These findings indicate *GOLGA2P3Y* gene copy may be candidate AZFc gene for male infertility.

KEYWORDS: AZFc, GOLGA2LY, gr/gr rearrangements, oligozoospermia, sperm count, sperm motility

http://dx.doi.org/10.1016/j.rbmo.2015.11.001
1472-6483/

Introduction

Cytogenetic and molecular analyses of men with defective spermatogenesis have identified microdeletions in the q arm of the Y chromosome. Between 2 and 10% of men with oligozoospermia or azoospermia are estimated to harbour Yq microdeletion, and it is currently the leading genetic cause of male infertility (Abid et al., 2008a; Ambulkar et al., 2014; Krausz et al., 2014; Massart et al., 2012; Sen et al., 2013a). Yq microdeletions involve loss of one (or more) of the three AZF loci namely, AZFa, AZFb and AZFc. Among these three, the deletion of AZFc locus is most commonly observed (Ambulkar et al., 2014; Foresta et al., 2001; Massart et al., 2012; Sen et al., 2013a; Simoni et al., 2008).

The human AZFc is palindromic in nature, and has several repeat regions making it susceptible to self recombination during meiosis, which makes it most susceptible to deletions (Kuroda-Kawaguchi et al., 2001; Skaletsky et al., 2003). Withstanding this fact, along with complete AZFc deletions, genetic analysis of infertile men has led to identification of partial deletions within the AZFc locus. Referred to as the gr/gr rearrangements or AZFc subdeletions, these rearrangements remove 1.6–1.8 Mb, which is about 50% of the AZFc region (Ferlin et al., 2005; Repping et al., 2003; Stouffs et al., 2008). The gr/gr rearrangements are more frequently observed in azoospermic and oligozoospermic men compared with normozoospermic counterparts (Ferlin et al., 2005; Giachini et al., 2005; Lo Giacco et al., 2014; Sen et al., 2015; Stouffs et al., 2011). Unlike the Yq microdeletions, however, the gr/gr rearrangements are also observed in 2–5% of fertile and normozoospermic men (Lo Giacco et al., 2014; Sen et al., 2015; Stouffs et al., 2011; Tüttelmann et al., 2007). What makes some men harbouring gr/gr rearrangements susceptible to male infertility is presently unclear. Although ethnicity and genetic background of Y chromosome have been demonstrated to be a cause; variation in the genes deleted within the AZFc locus in individuals with gr/gr rearrangements has been suspected to be the causative factor (Lo Giacco et al., 2014; Sen et al., 2015).

The AZFc locus is structurally unique, and is composed of three palindromes housing 11 families of transcription units (Kuroda-Kawaguchi et al., 2001; Skaletsky et al., 2003). All the genes in AZFc locus exist in multiple copies and are expressed in the testis (Kuroda-Kawaguchi et al., 2001; Skaletsky et al., 2003; Tse et al., 2003). It is believed that gr/gr rearrangements remove copies of some key AZFc genes like Deleted in Azoospermia (*DAZ*) and Chromodomain on the Y chromosome,1 (CDY1) causing male infertility (Ferlin et al., 2005; Krausz et al., 2009; Sen et al., 2015; Stouffs et al., 2008). In addition to these genes, however, the gr/gr rearrangements also remove copies of three additional genes, namely Golgi autoantigen, golgin subfamily a, 2-like, Y linked (GOLGA2LY), Basic protein on Y chromosome, 2 (*BPY2*) and Testis specific transcript, Y linked 4 (*TTY4*) (Noordam et al., 2011; Stouffs et al., 2008). Whether the deletion of one or more copies of these genes may also be a cause of infertility is presently unclear. As not all men harbouring gr/gr rearrangements are infertile, it is possible that there might be heterogeneity in the deletion of these genes, which may be the determinant of the susceptibility of some men to infertility. Although variation in DAZ and CDY1 copy deletions in men with gr/gr rearrangements has been reported to increase the susceptibility of gr/gr rearrangements to male infertility (Choi et al., 2012; Krausz et al., 2009; Lu et al., 2009; Sen et al., 2015), the involvement of other genes is still unknown.

GOLGA2LY is a 4.8Kb gene present in two copies on the AZFc locus, referred to as *GOLGA2P2Y* and *GOLGA2P3Y* for the 5′ and the 3′ copy, respectively. The gr/gr deletions generally remove either the *GOLGA2P2Y* copy or *GOLGA2P3Y* copy depending on the site of recombination (Krausz et al., 2009; Noordam et al., 2011). Controversies exist on the association of *GOLGA2LY* copy deletions and male infertility. Noordam et al. (2011) showed that infertile males harbouring the AZFc subdeletion encompassing the GOLGA2LY had lower total motile sperm count compared with controls. Lu et al. (2014) failed to report any significant association of *GOLGA2LY* gene copy number variations with male infertility. Therefore, it is unclear if deletions of *GOLGA2P2Y/3Y* may be a causative factor for male infertility. As *GOLGA2LY* is transcribed in the testis (Kuroda-Kawaguchi et al., 2001), and is located within the region deleted in gr/gr rearrangements, we hypothesize that loss of *GOLGA2LY* gene copies may be also associated with male infertility.

In the present study, we aimed to determine the association of the deletions of the *GOLGA2LY* gene copies *GOLGA2P2Y* and *GOLGA2P3Y* with oligozoospermia. The effect of these gene copy deletions were also evaluated on sperm concentration and motility.

Materials and methods

Ethics statement

The study was conducted independently at the National Institute for Research in Reproductive Health, Mumbai, and Mahatma Gandhi Institute of Medical Sciences, Sevagram, India. The Mahatma Gandhi Institute of Medical Sciences Ethical Committee approved the study on 30 December 2006 and the Ethics Committee for Clinical Studies of National Institute for Research in Reproductive Health approved the study on 20 December 2005. Written informed consent was obtained from all study participants.

Study participants

The details of inclusion and exclusion criteria for the participants have been described previously (Ambulkar et al., 2014; Sen et al., 2013b). Participants were classified on the basis of sperm concentration, and were grouped into normozoospermic men (sperm concentration ≥15 million/ml) and oligozoospermic men (sperm concentration <15 million/ml) as per the World Health Organization 2010 guidelines (WHO, 2010). Participants with karyotypic abnormalities, obstructive azoospermia, hypogonadism, hypoandrogenism, chronic diseases, history of mumps, history of fever, varicocele and history of pelvic/spinal injuries were excluded. None of the patients reported heavy smoking, alcohol intake, or both. All the men were screened for Yq microdeletion