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ARTICLE

Association of *CLOCK* gene variants with semen quality in idiopathic infertile Han-Chinese males

Jie Zhang [a,1], Xinliang Ding [b,1], Yingchun Li [c], Yankai Xia [d], Jihua Nie [a], Cao Yi [a], Xinru Wang [d], Jian Tong [a,*]

[a] *School of Public Health, Medical College of Soochow University, Suzhou 215123, China;* [b] *Department of Public Health, Wuxi Center for Disease Prevention and Control, Wuxi 214023, China;* [c] *Department of Physiology, Nanjing Medical University, Nanjing 210029, China;* [d] *Key Laboratory of Reproductive Medicine, Institute of Toxicology, Nanjing Medical University, Nanjing 210096, China*

[*] Corresponding author. *E-mail address:* tongjian@suda.edu.cn (J Tong). [1] These authors contributed equally to this work.

Dr. Jian Tong is a professor of the Soochow University in China and a principle investigator of several national and international research grants, including the International Atomic Energy Agency (IAEA), the China National Science Foundation and the National Key Program Foundation. The research fields are mainly concerned with radiation medicine, toxicology and public health.

Abstract Recent experimental animal studies suggested that the circadian locomotor output cycles kaput protein gene (*CLOCK*) may play an important role in male reproduction. So far, such data for humans are not available. This study used single-nucleotide polymorphisms (SNP) to examine the association between *CLOCK* and semen quality in a human population with idiopathic infertility. Three-variant genotyping of *CLOCK* and semen analysis were performed in 478 men with idiopathic infertility by SNP genotyping assays and computer-aided sperm analysis. Subjects carrying a C allele at rs3749474 (CC and TC) presented significantly lower semen volume ($P = <0.001$ and 0.001, respectively) compared with the TT genotype. Subjects carrying the rs3749474 CC genotype had significantly lower sperm number per ejaculate ($P = 0.026$) and sperm motility ($P = 0.021$) than TT genotype carriers. rs1801260 TC genotype carriers had significantly lower sperm motility compared with the TT genotype ($P = 0.028$). For the rs3817444 genotypes, CA and AA genotype carriers presented significantly lower semen volume compared with the CC genotype ($P = 0.022$ and 0.001, respectively). The findings suggest, as far as is known for the first time, an association between *CLOCK* genetic variants and altered semen quality in a human population with idiopathic infertility.

KEYWORDS: *CLOCK* gene, genetic variants, idiopathic infertility, semen quality

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Introduction

Circadian rhythms are the approximate 24-hour oscillations in behavioural or physiological processes that allow organisms to anticipate routine environmental changes and prepare for the appropriate alignment in order to adapt (Kovanen et al., 2010). The principal circadian rhythm in human is a fundamental aspect of physiology. A wide range of biological processes are influenced by the circadian clock, including body temperature, energy metabolism, hormone secretion and sleep–wake cycles (Kondratov et al., 2007). Recently, there has been growing interest to investigate the impact of circadian rhythm disruption on human health.

CLOCK, the gene for the circadian locomotor output cycles kaput protein, is located at q12 region of chromosome 4 and has a basic helix–loop–helix domain for binding DNA (Tsuzaki et al., 2010). *CLOCK* functions as an important positive enhancer of the circadian suprachiasmatic nucleus system (Garaulet et al., 2010). Genetic variations in *CLOCK* have been shown to be associated with sleep, mood, metabolic disorders such as metabolic syndrome (Takahashi et al., 2008) and obesity (Scott et al., 2008), small dense low-density lipoprotein (Tsuzaki et al., 2010), altered food consumption (Garaulet et al., 2010), diurnal preference (Katzenberg et al., 1998; Mishima et al., 2005), delayed sleep phase syndrome (Iwase et al., 2002) and psychiatric phenotypes (Benedetti et al., 2007, 2008; Serretti et al., 2003; Xu et al., 2010).

Recent studies revealed that *CLOCK* was expressed in the testis (Alvarez et al., 2003; Morse et al., 2003). *Drosophila* males with mutated *CLOCK* released less spermatozoa than their wild types, thereby reducing reproductive fitness (Beaver et al., 2002). *Mus musculus* with *CLOCK* mutations were reportedly subfertile (Chappell et al., 2003) and had higher pregnancy failure rates (Miller et al., 2004). These studies suggested that *CLOCK* may play an important role in male reproduction.

Single-nucleotide polymorphisms (SNP), as the most abundant class of inheritable human gene mutations, have attracted people's attentions in recent years. For *CLOCK*, an association study between genetic variants and the level of social activity has been performed in a Finland population, and the results suggested that *CLOCK* genetic variants have an association with social activity (Kovanen et al., 2010). However, so far, there are no reports in human population concerning the association between *CLOCK* genetic variants and male infertility. This study was aimed to examine association between three SNP of *CLOCK* and semen quality in 478 males with idiopathic infertility, which is a predominant disorder of male reproduction in the Chinese population (Guzick et al., 2001).

Materials and methods

Ethics statement

This study was approved by the ethics review board of Nanjing Medical University. From March 2005 to March 2007, 478 men with definite idiopathic infertility were consecutively recruited from the Affiliated Hospitals of Nanjing Medical University (NJMU Infertile Study). This study was done under full compliance with all government policies and the Helsinki Declaration. All the samples, including human blood and semen, were obtained legally from the affiliated hospitals of NJMU: (i) First Affiliated Hospital of Nanjing Medical University and (ii) Nanjing Maternal and Child Health Hospital Affiliated to Nanjing Medical University. They were strictly used for the research and not used for any commercial or inadequate activities. The protocol and consent form were approved by the Institutional Review Board of NJMU, China (FWA00001501, 25 March 2008). The participants were fully informed of the study: a written and signed agreement was obtained from each participant.

Subjects and semen analysis

All subjects were unable to conceive for at least 12 months. A complete historical and physical examination was performed. Those with a history of orchitis, cryptorchidism, obstruction, congenital bilateral absence of vas deferens, cytogenetic abnormalities and Y chromosome microdeletions were excluded from the study (Ji et al., 2008; Osborne et al., 2007). After completing a questionnaire, each subject donated 5 ml of blood for genomic DNA extraction and an ejaculate for semen analysis which was performed by the computer-assisted semen analysis system (WLJY-9000; Weili New Century Science and Tech Dev, Beijing, China) according to World Health Organization guidelines (World Health Organization, 1999). The association between *CLOCK* genetic variants and four parameters of semen quality were determined: (i) semen volume (ml); (ii) sperm concentration (10^6/ml); (iii) sperm number per ejaculate (10^6/ejaculate) which represented changes in sperm number; and (iv) sperm motility (%) which provided an estimation on the fertilizing ability of the spermatozoa both *in vivo* and *in vitro* (Elzanaty et al., 2005; Hirano et al., 2001; Larsen et al., 2000). All data were subjected to statistical analysis and the results presented were the means of at least two analyses.

SNP selection and genotyping

For *CLOCK*, there is no genetic variant data in the Han Chinese population, and the HapMap database is the only one that can be used to make the SNP selection. However, previous studies on other gene polymorphisms in the Han Chinese population, compared with populations from different regions in China, indicated that, overall, the genetic variant distributions are similar between the northern and southern Han (Ding et al., 2010a,b; Zhang et al., 2008). The SNP were therefore selected using genotype data obtained from unrelated Han Chinese in Beijing individuals in HapMap (HapMap Data Rel 24/phase II November 2008, on NCBI B36 assembly, dbSNP b126; http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap24_B36/). This study selected SNP that had a minor allele frequency >0.05 in Han Chinese in Beijing within the 114,338 bp of the human *CLOCK* gene, which was pinpointed to chromosome 4 56298660–56412997; 103 SNP were captured in this region. Using Haploview 4.0 software (Barrett et al., 2005; Verhaegh et al., 2008), a linkage disequilibrium plot of this region was made based on the R^2 values, which indicated the ability of a certain SNP to predict another SNP