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Sperm count and motility are quantitatively affected by functional polymorphisms of *HTR2A*, *MAOA* and *SLC18A*

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KEY MESSAGE

The products of genes *MAOA* and *SCL18A*, both related to the metabolism of neurotransmitters, are key genes in maintaining extracellular and intracellular levels of neurotransmitters, and each has polymorphisms associated with asthenozoospermia.

A B S T R A C T

Spermatozoa and neurones share similar membrane characteristics and features. Associations of multiple polymorphisms traditionally related to neurotransmission were investigated. Infertile men were grouped into controls with normospermia (n = 182) and idiopathic infertile men with asthenozoospermia (n = 103), and analysed as a case-control study and as a quantitative association of each genotype. Ten neurotransmission-associated genetic variants were mapped by SNP analysis using quantitative polymerase chain reaction with TaqMan probes. Men with *HTR2A* rs6313 had a higher risk of asthenozoospermia (OR = 2.14; P = 0.04). *MAOA* rs3788862 G carriers displayed an increased risk of asthenozoospermia (OR = 2.29; P = 0.02). The *SLC18A1* rs1390938 G allele was more frequent among such cases (0.75 versus 0.87; $P < 0.01$ and $P < 0.01$ for Armitage trend test); for *SLC18A1* rs2270641 P = 0.02 (case-control frequency) and P = 0.01 (Armitage trend test). *MAOA* rs3788862 was correlated with sperm motility (Spearman ρ = 0.14; P = 0.02); *SLC18A1* rs1390938 was correlated with sperm count and motility (Spearman ρ = 0.20; $P < 0.01$). Gene polymorphisms of *HTR2A*, *MAOA* and *SLC18A1*, related to neurotransmission, are individually associated with asthenozoospermia through variation in sperm count and motility, without detectable allelic or genotype interaction.

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Introduction

Infertility affects around one in seven couples worldwide, and male-factor infertility alone accounts for around one-half of all cases (Alam, 2009). The most common causes of male infertility include endocrine dysfunction, varicocele, post-testicular obstruction, inadequate spermatogenesis and abnormal sperm morphology. Asthenozoospermia (AZS) is defined as a low proportion of progressive sperm motility (grade A + B + C of progressive motility <40%) according to 2010 World Health Organization criteria (2010). This decreased motility may be caused by various factors, such as lengthy sexual abstinence, unhealthy lifestyle, abnormal semen liquefaction, sperm dysfunction, partial obstruction of the seminal tract, varicocele, infection or known genetic causes (Gaur et al., 2007; Gdoura et al., 2007; Luconi et al., 2006; Marmar et al., 2007). Many cases of AZS, however, are of unknown cause, and are impossible to diagnose using routine medical tests (Ortega et al., 2011). Because of the high number of idiopathic AZS, many studies have evaluated the contribution of genetic risk factors analysing functional single nucleotide polymorphisms (SNP) in a wide variety of potential target genes (Aston and Carrell, 2009; Aston et al., 2010; Fruhmesser et al., 2013; Liu et al., 2016).

Spermatozoa and neurones share similar membrane characteristics and features. Moreover, many neurone receptors are also present in sperm cells, which have even been labelled 'neurons with a tail' (Meizel, 2004). Sperm share excitability functions with neurones but lack the synaptic mechanism (Meizel and Son, 2005). The most common neurotransmitters are serotonin (5-hydroxitriptamine [5-HT]), dopamine and norepinephrine, and their receptors, carriers, or both, have been widely studied in sperm functions. The classic neural receptors detected in sperm have been classified according to their functional and metabolic role. Acrosomal reactions have been linked to dopamine and 5-HT receptors (Urra et al., 2014), whereas purinergic (Gorodeski, 2015), nicotinic (Kumar and Meizel, 2005; Meizel and Son, 2005), angiotensin II (Gianzo et al., 2016), cannabinoid (Amoako et al., 2013), and olfactory receptors (Flegel et al., 2015) have been associated with motility.

One of the most widely studied molecules involved in neurotransmission is 5-HT. Receptors HTRAT1, HTRAT2 and HTR3 could influence sperm motility in response to changes in extracellular 5-HT availability (Fujinoki, 2011). Moreover, these changes in sperm activity are unusual because spermatozoa require an optimal concentration range of intra and extracellular 5-HT for appropriate motility (Jimenez-Trejo et al., 2012). This capability could influence interactions of oocyte and sperm in the uterine environment during fertilization, as it has been suggested that serotonin receptors can modulate sperm motility and the vaginal environment (Fujinoki et al., 2016). Multiple researchers have shown that selective serotonin reuptake inhibitors (SSRIs) have direct effects on sperm quality (Akasheh et al., 2014; Koyuncu et al., 2012).

Other neurotransmitters, such as dopamine, and its receptors and transporters, are present in sperm and have also been linked to motility and the acrosome reaction (Jimenez-Trejo et al., 2012; Urra et al., 2014). It has also been observed that male rats treated with sibutramine, a non-selective inhibitor of 5-HT and norepinephrine, causes an acceleration of sperm transit time leading to a lower reserve in the epididymis (Bellentani et al., 2011; Borges et al., 2013). On the other hand, when sperm are treated with norepinephrine, capacitation is improved and is also associated with increased fertilization rates during IVF of bovine oocytes (Way and Killian, 2006).

Expressions of genes involved in synthesis, i.e. *TPH*, degradation, i.e. *MAOA* and *MAOB*, and distribution, i.e. *SLC16A1* and *SLC18A1*, of neurotransmitters have been found in sperm (Jimenez-Trejo et al., 2007). Although the role and functional activities of these molecules in sperm is uncertain, neurotransmitters are bioactive substances directly participating in neural transmission or regulation. Spermatozoa and neurones share similar features and some bioactive substances. These can act as transmitters, are commonly known as neurotransmitters, or both, and have biological roles in the reproductive system; however, this does not mean that spermatozoa should be directly termed 'neurotransmitters' in reproduction. It could, for example, suggest that neurotransmitters are involved in the development and maturation of sperm cells. Several studies have established associations between multiple SNP and asthenozoospermia (Aston and Carrell, 2009; Aston et al., 2010). No new studies, however, have reproduce these results. Therefore, observational association studies of SNP in AZS conducted by independent research groups urgently need to be undertaken. In this study, the association between different functional SNP in genes related to metabolism and neurotransmission in a group of men with normospermia and asthenozoospermia were studied.

Materials and methods

Participants

The selected population sample were recruited between 2013 and 2016, and involved 285 European men. Both the control groups and the groups with asthenozoospermia come from patients with infertility (more than 1 year of frequent unprotected intercourse where pregnancy is not achieved) at the Infertility Unit of the Gynecology Department of Children's Hospital Materno Infantil, Malaga, Spain. The semen sample was collected according to WHO 2010 protocol (World Health Organization, Department of Reproductive Health and Research, 2010). Patients obtained seman samples through masturbation without using a condom after 2–7 days of sexual abstinence. The sample corresponded to the total of the ejaculate. Samples were collected 1 h before delivery to the laboratory, i.e., between 8 and 9 am, transported between 20–37°C. Semen analysis was carried out by a single observer in two consecutive samples (after a 2-week interval). The Sperm-Class Analyzer® computer-assisted system (Microoptica Automatic Diagnostic System, Barcelona, Spain) was used to characterize and classify morphometric motility.

The participants were classified as normospermic or asthenospermic according to 2010 World Health Organization criteria. The resulting 182 men with normospermia ($\geq 1.5 \cdot 10^7 \cdot$ Spermatozoa $\cdot mL^{-1}$, (A + B + C) motility ≥40%, viability ≥58%, and typical morphology ≥4%) constituted the control group. The group with asthenozoospermia consisted of 103 men with progressive motility A + B + C less than 40% (**Supplementary Figure S1**). Cases also showed a significantly lower ($P < 0.001$) sperm count (mean ± SD 23.088 ± 24.247 million per ml) than controls (69.774 ± 55.620 million per ml). Neither cases nor controls were given any treatment that could alter the study of neurotransmitter, exposure to a chemical related to semen quality such as anti-depressants, calcium channel blockers, alpha-adrenergic blockers, anti-epilepsy, anti-retrovirals, and miscellaneous medications, including some antibiotics, such as tetracycline, chemotherapeutic agents specifically alkylating agents, such as cyclophosphamide, and anabolic steroids. Men with a body mass

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