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Corresponding Author: Prof Armando Reyes-Engel, M.D., Ph.D.

Corresponding Author's Institution: University of Malaga

First Author: Miriam Cortés

Order of Authors: Miriam Cortés; Jose L Royo; Arturo Reyes-Palomares; Ana Lendínez; Maximiliano Ruiz; Armando Reyes-Engel

Abstract: To establish the association of multiple polymorphisms, traditionally related to neurotransmission, in a population grouped into normospermic controls (n=182) and idiopathic infertile men with asthenozoospermia (n=103), analyzed as a case-control study and as a quantitative association of each genotype, using the entire population, with seminal parameters. Ten neurotransmission-associated genes were mapped by SNP analysis performed by qPCR with TaqMan probes. HTR2A rs6313 GG subjects exhibited a higher risk of asthenozoospermia (P=0.038). MAOA rs3788862 G carriers also displayed an increased risk of asthenozoospermia (P=0.021). SLC18A1 rs1390938 G allele was also more frequent among such cases (0.75 vs. 0.87, P<0.001) and scored P=0.001 on the Armitage trend test, while SLC18A1 rs2270641 showed P=0.018 and P=0.013 on the Armitage trend test. Quantitative analysis revealed a correlation between MAOA rs3788862 and sperm motility (P=0.023) while SLC18A1 rs1390938 was correlated with sperm count and motility (P<0.001), respectively. The results show that the analyzed gene polymorphisms of HTR2A, MAOA and SLC18A1, which are mainly related to neurotransmission, are individually associated with asthenozoospermia through variation in sperm count and motility, without detectable allelic or genotype interaction between them.

Suggested Reviewers:

Opposed Reviewers:

**Cover Letter**

Department of Surgery, Biochemistry and Immunology
Faculty of Medicine
University of Malaga
29071 Malaga, Spain
Tel: +34 952 131 531
E-mail: engel@uma.es.es

**Reproductive Biomedicine Online**
Editors
Gedis Grudzinskas, London, UK
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**Reference:** Sperm count and motility are quantitatively affected by functional polymorphism of classically related to neurotransmitters mood regulators *HTR2A*, *MAOA* and *SLC18A*.

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Our research has the novelty that is the first time that a case-control study is clearly correlated and reinforced by a subsequent quantitative study. Overall, here we find that *MAOA* and *SCL18A* are, key genes in maintaining extra and intracellular levels of neurotransmitters, associated with asthenozoospermia. This association could be based on changes in the functionality and activity mediated by their receptors present in the sperm. We cannot rule out that other neurotransmitters play a role in intracellular metabolism in the sperm.

The material contained in the manuscript has not been published, has not been submitted, or is not being submitted elsewhere for publication. All the authors agreed to submit the manuscript to RBM Online.

Trusting you find the article of interest, we remain

Yours,

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# Title

Sperm count and motility are quantitatively affected by functional polymorphism of classically related to neurotransmitters mood regulators *HTR2A*, *MAOA* and *SLC18A*.

## Short title

*MAOA* and *SLC18A1* in asthenozoospermia

# Author

Miriam Cortés [a], Jose-Luis Royo [a], Arturo Reyes-Palomares [a, b], Ana M. Lendínez [a, c], Maximiliano Ruiz-Galdón [a, d], Armando Reyes-Engel [a].

Affiliations

a. Department of Biochemistry, Molecular Biology and Immunology, Faculty of Medicine, University of Malaga, 29071, Malaga, Spain.

b. Instituto de Fertilidad Clínica Rincón, IVF Laboratory and I+D+i, 29730, Malaga, Spain.

c. Biochemistry Service, Agencia Sanitaria Costa del Sol, Malaga, 29603, Marbella, Malaga, Spain.

d. Clinical Analysis Service, Virgen de la Victoria University Hospital, 29071, Malaga, Spain.

# Corresponding author

Armando Reyes-Engel

University of Malaga - Faculty of Medicine

Department of Biochemistry, Molecular Biology and Immunology

29071, Malaga, Spain

e-mail: engel@uma.es

Phone number: +34 952 131 531

# Permanent address

Same as corresponding author.

## Abstract

To establish the association of multiple polymorphisms, traditionally related to neurotransmission, in a population grouped into normospermic controls (n=182) and idiopathic infertile men with asthenozoospermia (n=103), analyzed as a case-control study and as a quantitative association of each genotype, using the entire population, with seminal parameters. Ten neurotransmission-associated genes were mapped by SNP analysis performed by qPCR with TaqMan probes. HTR2A rs6313 GG subjects exhibited a higher risk of asthenozoospermia ($P$=0.038). MAOA rs3788862 G carriers also displayed an increased risk of asthenozoospermia ($P$=0.021). SLC18A1 rs1390938 G allele was also more frequent among such cases (0.75 *vs*. 0.87, $P<0.001$) and scored $P$=0.001 on the Armitage trend test, while SLC18A1 rs2270641 showed $P$=0.018 and $P$=0.013 on the Armitage trend test. Quantitative analysis revealed a correlation between MAOA rs3788862 and sperm motility ($P$=0.023) while SLC18A1 rs1390938 was correlated with sperm count and motility ($P<0.001$), respectively. The results show that the analyzed gene polymorphisms of *HTR2A*, *MAOA* and *SLC18A1*, which are mainly related to neurotransmission, are individually associated with asthenozoospermia through variation in sperm count and motility, without detectable allelic or genotype interaction between them.



## Keywords

Asthenozoospermia; polymorphism; neurotransmission; male infertility.

## Introduction

Infertility affects around one in seven couples worldwide and male factor infertility accounts for around 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 (Organization, 2010). This decreased motility may be due to 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). However, many cases of AZS are of unknown etiology and are impossible to diagnose using routine medical tests (Ortega et al., 2011). Due to the high number of idiopathic AZS, many studies have evaluated the contribution of genetic risk factors analyzing functional single nucleotide polymorphisms (SNPs) in a wide variety of potential target genes (Aston and Carrell, 2009; Aston et al., 2010; Fruhmesser et al., 2013; Liu et al., 2016).

Spermatozoids and neurons share similar membrane characteristics and features. Moreover, many neuron receptors are also present in sperm cells, which have even been labelled “neurons with a tail” (Meizel, 2004). Sperm shares excitability functions with neurons but lacks the synaptic mechanism and exocytosis by acrosome reaction (Meizel and Son, 2005). The most common neurotransmitters are serotonin (5-hidroxitriptamine or 5-HT), dopamine (DA) and norepinephrine (NE), and their receptors and/or carriers 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 DA and 5-HT receptors (Urra et al., 2014), while 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 mobility.

One of the most widely studied molecules involved in neurotransmission is 5-HT. Receptors HTRAT1, HTRAT2 and HTR3 could influence sperm mobility in response to changes in extracellular 5-HT availability (Fujinoki, 2011). Moreover, these changes in sperm activity are unusual in that they exhibit an optimal concentration range of intra and extracellular 5-HT where sperm shows suitable 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 DA and its receptors and transporters, are present in sperm and have also been linked to mobility and 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 NE, 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 is treated with NE, capacitation is improved and this also increased fertilization rates during *in vitro* fertilization 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, it could suggest that neurotransmitters are involved in the development and maturation of sperm cells. In the literature, there are several studies showing associations of multiple SNPs with asthenozoospermia (Aston and Carrell, 2009; Aston et al., 2010). However, no new studies which have been performed were able to reproduce these results. Therefore, there is an urgent need for independent research groups to undertake observational association studies of SNPs in AZS. In this paper, we have studied the association between different functional SNPs in genes related to metabolism and neurotransmission in a group of normospermic and asthenozoospermic human males.

## Material and methods

### *Subjects*

The selected population sample involved 285 European men. The control group consisted of 182 normospermic subjects who had normal semen analysis values according to 2010 WHO criteria ($\geq 1.5\cdot 10^{7}\cdot$spermatozids$\cdot$mL$^{-1}$, (A+B+C) motility ≥40%, viability ≥58%, and typical morphology ≥4%). The asthenozoospermic group were drawn from studies of infertile couples at the Infertility Unit of the Gynecology Department of Children's Hospital Materno Infantil (Malaga, Spain) and consisted of 103 subjects with progressive motility A+B+C <40% (Supplementary Fig. 1). All asthenozoospermic patients were classified as idiopathic after a comprehensive andrological examination including medical history and physical examination, semen analysis, scrotal echography, hormonal analysis, karyotype and Y chromosome microdeletion screening. Patients who presented the following abnormalities: cryptorchidism, varicocele, previous testicular trauma, obstructive azoospermia, recurrent infections, iatrogenic infertility, pituitary hypogonadism, karyotype abnormalities, and Y chromosome microdeletions

including gr/gr deletions, were excluded. All participants signed an informed consent form and their data was stored by the interviewers. The study protocol was approved by the ethics committee of the University of Malaga in accordance with the Declaration of Helsinki.

*DNA extraction*

DNA was extracted from semen samples using the salting-out method proposed by Miller *et al.*, 1988 (Miller et al., 1988). Briefly, the cellular fraction was obtained by centrifugation, discarding the supernatant and re-suspending the pellet in PBS. After the final centrifugation, Lysis Buffer I (0.1 M Tris-HCl pH 8 + 0.1 M EDTA pH 8) and Lysis Buffer II (0.1 M Tris-HCl pH 8 + 0.1 M EDTA pH 8 + 1% SDS), proteinase K and 6 M NaCl were added for cell lysis. The samples were centrifuged and washed several times using an ice-cold 6 M NaCl buffer. The DNA was precipitated using 100% isopropanol, washed in ice-cold 70% ethanol and re-suspended in DNAse-free water. DNA purity was measured using the NanoDrop 1000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA), evaluating saline contamination (260/230 ratio, >2.00) and the presence of protein (260/280 ratio, >1.8). Subsequently the sample was adjusted to a DNA concentration of 50 $ng \cdot mL^{-1}$, aliquoted and stored at -80 ºC until genotyping.

*Genotyping*

SNPs analysis was performed by qPCR with TaqMan probes using a commercial microarray technique (OpenArray TaqMan® Genotyping System, Applied Biosystems). The obtained results were subsequently processed using TaqMan Genotyper® Software. The probes and primers were designed by Applied Biosystems based on the sequences adjacent to the SNPs of interest. Detailed information on each probe is available upon request. The genes and SNPs included in the study are listed in Table 1.

*Statistical analysis*

Hardy-Weinberg equilibrium (HWE) analysis for each polymorphism and case-control study was completed using the SNPStats on-line tool (http://bioinfo.iconcologia.net/snpstats/start.htm) and the Helmholtz Centre for Human Genetics web tool (https://ihg.gsf.de/ihg/snps.html). All polymorphisms included in downstream analysis showed call rates >0.95 and HWE *P*-values>0.05, except for polymorphisms mapping chromosome X (rs3813929, rs3788862, rs979605 and rs3027452). Quantitative analysis was performed using IBM SPSS v22.

# Results

Firstly, we performed a case-control association comparing genotype distributions between normospermic *vs.* asthenozoospermic groups. Of the 15 tested SNPs, four were mapped into

*HTR2A*, *MAOA* and *SLC18A1* genes, showing statistically significant association with AZS (Table 2). HTR2A rs6313 GG subjects exhibited a higher risk of AZS following a recessive model (OR=2.14, *P*=0.038). MAOA rs3788862 allele G frequency was significantly higher among cases of AZS (0.77 *vs*. 0.89, *P*=0.001), causing G-carriers to display an increased risk of AZS (OR=2.29, IC95 [1.114 - 4.705], *P*=0.021). Meanwhile, SLC18A1 rs1390938 G allele was also more frequent among AZS cases (0.75 *vs.* 0.87, *P*<0.001), whereas SLC18A1 rs2270641 showed an OR=1.849, IC95 [1.105 - 3.092], *P*=0.018. Armitage trend tests for SLC18A1 rs2270641 and rs1390938 were both positive (*P*=0.001 and 0.013, respectively), suggesting an additive effect.

To characterize the nature of these associations, we decided to perform a series of quantitative studies. An initial exploration of the sperm count and motility data revealed a logarithmic correlation, so we performed a logarithmic transformation and observed that the obtained data fitted into a normal distribution (Kolmogorov-Smirnov, *P*-value>0.05, Fig. 1).

Once we observed that sperm count and mobility followed a normalized distribution, we decided to approach the study in two complementary ways. On the one hand, we performed a quantitative study of allele and genotypic frequencies and their relation to the quantifiable values of sperm quality. On the other hand, the classic grouping cases and sticking to the ranges agreed diagnostic AZS according to the above spermatic values *versus* controls. Firstly, we analyzed the correlation between sperm count and motility. We found that both parameters were positively correlated (β=0.634, *P*<0.001). This analysis was performed using data from both groups of men (n=285). In addition, we observed that MAO rs3788862 was significantly associated with increased sperm quality (Spearman ρ=0.140, *P*=0.023), while SLC18A1 rs1390938 exhibited a significant correlation with sperm count and mobility (Spearman *P*-value<0.001, in both cases), as shown in Fig. 2. No significant correlation was observed for HTR2A polymorphisms.

As the initial data showed that sperm count and motility are dependent variables, we performed a regression analysis using one of the quality parameters as a covariate on each occasion. Our results showed that the effect for SLC18A1 rs1390938 was still significant for sperm count after co-varying for motility (β=0.204, Pearson's *P*-value=0.042). They showed that the effect was mainly related to count, but can also affect motility (trend *P*=0.08). MAOA rs3788862 lost its significance, suggesting that it affects sperm count and motility equally. Interaction analysis between both genotypes showed no epistasis, which suggested that their role could be independent. However, the loss of statistical power due to the reduction of the population grouping while at least two polymorphisms were analyzed for their interactions could compromise the robustness of these results.

## Discussion

The similarities between the neuronal neurotransmission system and sperm membrane architecture, as well as their potential implications for fertility, have been studied in several models. Many components assumed to be exclusive to neuronal functions are also found in sperm, including metabolites, receptors and transporters. In this study, functional polymorphisms were selected from candidate genes related to the neurotransmission system with the intention of assessing their implications for sperm quality and quantity. Nowadays, most of this data comes from studies in which patients suffered mood disorders and therefore also underwent pharmacological treatments whose targets are transporters (Cavariani et al., 2015), receptors or enzymes involved in the distribution of intracellular and/or extracellular serotonin (Jimenez-Trejo et al., 2007; Koyuncu et al., 2012; Tanrikut et al., 2010), dopamine (Cavariani et al., 2015; Ramirez et al., 2009; Urra et al., 2014) or noradrenaline (Bellentani et al., 2011; Borges et al., 2013). These studies show that changes in neurotransmitter levels produce significant variations in sperm quality. Similarly, the addition of a direct neurotransmitter blocking pathway produces clear effects on sperm activity, affecting both concentration and motility (Fujinoki, 2011; Way and Killian, 2006). Our results show a clear association between different SNPs mapping of *HTR2A*, *MAOA* and *SLC18A1* genes and AZS risk. Quantitative studies corroborate the nature of this association with sperm motility and/or sperm count for *MAOA* and *SLC18A1* gene. The associated SNPs should be correlated functionally with the distribution and maintenance of intracellular and extracellular neurotransmitter levels as occurs with the BDNF polymorphism (Czira et al., 2012). *MAOA* and *MAOB* are responsible for the degradation of biogenic amines, while *SCL18A1* handles the traffic between extra and intracellular compartments. MAOA associated allele G for SNP1 (rs3788862) has been reported as the maximum element related to post-surgery pain and outcomes derived from changes in amine levels (Kim et al., 2006). On the other hand, SLC18A rs1390938 is a missense variant whose G-allele (threonine) has been associated with decreased activity in neurotransmitter transport (Khalifa et al., 2012). Therefore, we could consider that a reduction in available neurotransmitter levels could affect receptor binding. Studies in which neurotransmitter transport is inhibited showed an increase in extracellular amines that, in the case of the synapse, exerts a more effective neurotransmission (German et al., 2015).

Nonetheless, the influence of extracellular neurotransmitter concentrations on sperm quality is completely unknown. The use of SSRIs has been observed to increase DNA fragmentation in the sperm (Koyuncu et al., 2012; Safarinejad, 2008). The information that is available about the effect of MAO inhibitors is very poor. However, a recent study with D-Deprenyl (dextro-N-propargyl-N-methylamphetamine), a MAOB specific inhibitor, reported an improvement in sperm capacitation. Although D-Deprenyl is supposed to be a MAOB selective inhibitor, at the

high reported doses it could also affect MAOA (Mihalik et al., 2015). In the early 1990s, a study focused on the effect of Iproniazid, a non-selective irreversible monoamine oxidase inhibitor, on MAO activity and suggested that infertile men presented increased MAO activity in seminal plasma (Roberge et al., 1984). In this context, another study linked increased 5-hydroxyindoleacetic acid with hypothetically higher 5-HT levels, due to increased MAO activity, with lower sperm parameters, including concentration and motility, and thus a lower fertility potential also (Ortiz et al., 2010). These observations from earlier independent studies support our results, in which we have clearly demonstrated the existence of a specifically associated polymorphism that can influence MAOA and its relationship with AZS.

On the other hand, sperm neurotransmitter levels have several functions which differ from those of the nervous system and which relate mainly to motility, such as the quick recruitment of sperm based on their number and output of the epididymis or increase in synthesis (Bellentani et al., 2011; Jimenez-Trejo et al., 2007). It is also known that if we add 5-HT or NE to an *in vitro* sperm culture, motility is increased significantly (Fujinoki, 2011; Jimenez-Trejo et al., 2012; Way and Killian, 2006). In contrast, inhibition of 5-HT transport via SSRIs produces sperm disablement not always related to motility (Koyuncu et al., 2012; Safarinejad, 2008; Tanrikut and Schlegel, 2007). Serotonin activity is mediated by different receptors present in the sperm (Jimenez-Trejo et al., 2012; Jimenez-Trejo et al., 2007). We can therefore deduce that neurotransmitters produce a change in sperm motility upon interaction with their ligands. However, our results show that the polymorphisms of one of the serotonin receptors (*HTR2A*) present only a weak association between the AZS condition and its polymorphism. The His452Tyr (G allele) variant of the serotonin 2A receptor has a reduced capacity to activate phospholipase C and D that could be related to an impaired response to 5-HT (Hazelwood and Sanders-Bush, 2004). These findings coincide functionally with the result obtained for the *MAOA* and *SLC18A1* gene variants. In this sense, it has been suggested that 5-HT could play a role in dynein phosphorylation and its implications for performance of energy metabolism in the sperm cell (Jimenez-Trejo et al., 2012). The main associated gene polymorphisms, *MAOA* and *SCL18A*, are related to the regulation of neurotransmitter concentration inside and outside of sperm. The functional effects of these variants may be due to the fact that increased MAOA activity associated with decreased transport would result in a lower availability of neurotransmitters. This lower ligand activity over the surface of the sperm or lower activity of the receptor (HT2A receptor) would explain the association with AZS. The case-control study and subsequent quantitative study leads us to propose this hypothesis. We cannot rule out the possibility that this effect would be more evident in larger populations. Overall, here we find that *MAOA* and *SCL18A* are key genes in maintaining extracellular and intracellular levels of neurotransmitters associated with asthenozoospermia. This association could be based on

changes in the activity mediated by their receptors present in the sperm, but we cannot rule out that some, such as 5-HT, play a role in intracellular metabolism in the sperm.

## Funding

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## Acknowledgements

The authors thank Traducciones HumanBioMed for help with English language editing.

## Figure captions

**Fig. 1.** Distribution of the variable sperm count after logarithmic transformation (A) and its correlation with sperm quality (B).

**Fig. 2.** Box plot illustrating the effect of rs1390938 on sperm count (A) and rs3788862 on sperm quality.

**Supplementary Fig. 1**. Distribution of sperm quality between cases and controls (A). Box plot showing the differences in total sperm count between controls and AZS cases.

# Tables

**Table 1**. Genes and SNPs included in the study.

| Gene | | Cytoband | SNP ID | Position (GRCh38.p7) | Change |
|---|---|---|---|---|---|
| COMT | Catechol-O-methyltransferase | 22q11.21 | rs4633 | 19962712 | C>T |
| | | | rs4680 | 19963748 | G>A |
| | | | rs4818 | 19963684 | C>G |
| | | | rs6269 | 19962429 | A>G |
| HTR1A | 5-hydroxytryptamine receptor 1A | 5q11.2-q13 | rs6295 | 63962738 | C>G |
| HTR2A | 5-hydroxytryptamine receptor 2A | 13q14-q21 | rs6313 | 46895805 | C>T |
| HTR2C | 5-hydroxytryptamine receptor 2C | Xq24 | rs3813929 | 114584047 | C>T |
| HTR3B | 5-hydroxytryptamine receptor 3B | 11q23.1 | rs1176744 | 113932306 | A>C |
| MAOA | Monoamine Oxidase A | Xp11.3 | rs3788862 | 43658116 | T>C |
| | | | rs979605 | 43742116 | T>C |
| MAOB | Monoamine Oxidase B | Xp11.23 | rs3027452 | 43798542 | G>A |

| SLC18A1 | Solute carrier family 18 vesicular monoamine 1 | 8p21.3 | rs1390938 | 20179202 | T>C |
|---|---|---|---|---|---|
| | | | rs2270641 | 20180955 | A>C |
| SLC18A2 | Solute carrier family 18 vesicular monoamine 2 | 10q25.3 | rs363371 | 117226885 | G>A |
| TPH1 | Tryptophan hydroxylase 1 | 11p15.3-p14 | rs1800532 | 18026269 | C>A |

**Table 2.** Case-control comparison between normospermic (n=183) and asthenozoospermic subjects (n=103).

| Gene | SNP | Genotype | Controls | Cases | Allelic | OR | *P*-value | Armitage test |
|---|---|---|---|---|---|---|---|---|
| | | AA | 36 | 11 | | 1 | - | |
| | | AG | 67 | 44 | | 2.149 | 0.057 | |
| *HTR2A* | rs6313 | GG | 49 | 32 | | 2.144 | 0.038[a] | 0.106 |
| | | | 0.46 | 0.38 | 0.097 | | | |
| | | C | 148 | 83 | | 1 | - | |
| HTR2C | rs3813929 | T | 27 | 13 | | 0.859 | 0.675 | 0.675 |
| | | | 0.85 | 0.86 | 0.556 | | | |
| | | AA | 72 | 41 | | 1 | - | |
| | | AC | 83 | 43 | | 0.910 | 0.727 | |
| HTR3B | rs1176744 | CC | 20 | 11 | | 0.921 | 0.748[a] | 0.825 |
| | | | 0.65 | 0.66 | 0.828 | | | |
| | | A | 40 | 11 | | 1 | - | |
| *MAOA* | rs3788862 | G | 135 | 85 | | 2.290 | 0.021 | 0.021 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0.77 | 0.89 | 0.001 | | | |
| | rs979605 | G | 135 | 77 | | 1 | - | |
| | | A | 36 | 14 | | 0.682 | 0.266 | 0.266 |
| | | | 0.79 | 0.85 | 0.115 | | | |
| MAOB | rs3027452 | G | 141 | 86 | | 1 | - | |
| | | A | 33 | 14 | | 0.696 | 0.293 | 0.293 |
| | | | 0.81 | 0.86 | 0.137 | | | |
| COMT | rs4633 | CC | 52 | 32 | | 1 | - | |
| | | TC | 87 | 39 | | 0.728 | 0.283 | |
| | | TT | 38 | 27 | | 0.858 | 0.669 | 0.760 |
| | | | 0.54 | 0.53 | 0.751 | | | |
| | rs4680 | GG | 52 | 29 | | 1 | - | |
| | | AG | 88 | 42 | | 0.856 | 0.601 | |
| | | AA | 37 | 26 | | 0.975 | 0.928 | 0.555 |
| | | | 0.54 | 0.52 | 0.545 | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | rs4818 | CC | 61 | 40 | | 1 | - | |
| | | GC | 85 | 36 | | 0.646 | 0.123 | |
| | | GG | 29 | 22 | | 0.776 | 0.327 | 0.992 |
| | | | 0.59 | 0.59 | 0.992 | | | |
| | rs6269 | AA | 56 | 39 | | 1 | - | |
| | | AG | 94 | 39 | | 0.596 | 0.065 | |
| | | GG | 30 | 22 | | 0.706 | 0.181 | 0.772 |
| | | | 0.57 | 0.58 | 0.769 | | | |
| *SLC18A1* | rs1390938 | AA | 9 | 4 | | 1 | - | |
| | | AG | 69 | 17 | | 0.554 | 0.365 | |
| | | GG | 99 | 78 | | 1.272 | 0.694 | 0.001 |
| | | | 0.25 | 0.13 | <0.001 | | | |
| | rs2270641 | TT | 83 | 32 | | 1 | - | |
| | | TG | 74 | 49 | | 1.717 | 0.050 | |
| | | GG | 20 | 18 | | 1.849 | 0.018 [a] | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0.68 | 0.57 | 0.011 | | | 0.013 |
| | | GG | 124 | 65 | | 1 | - | |
| | | GA | 48 | 31 | | 1.232 | 0.450 | |
| *SLC18A2* | rs363371 | AA | 4 | 4 | | 1.284 | 0.348 | |
| | | | 0.84 | 0.81 | 0.282 | | | 0.281 |
| | | GG | 80 | 42 | | 1 | - | |
| | | GT | 72 | 44 | | 1.164 | 0.573 | |
| TPH1 | rs1800532 | TT | 21 | 9 | | 1.086 | 0.749 | 0.941 |
| | | | 0.67 | 0.67 | 0.940 | | | |

(a) refers to a recessive model of inheritance (11+12 *vs.* 22) while (b) reflects a dominant model of inheritance (11 *vs.* 12+22) being 1 the reference and 2 the mutant allele.

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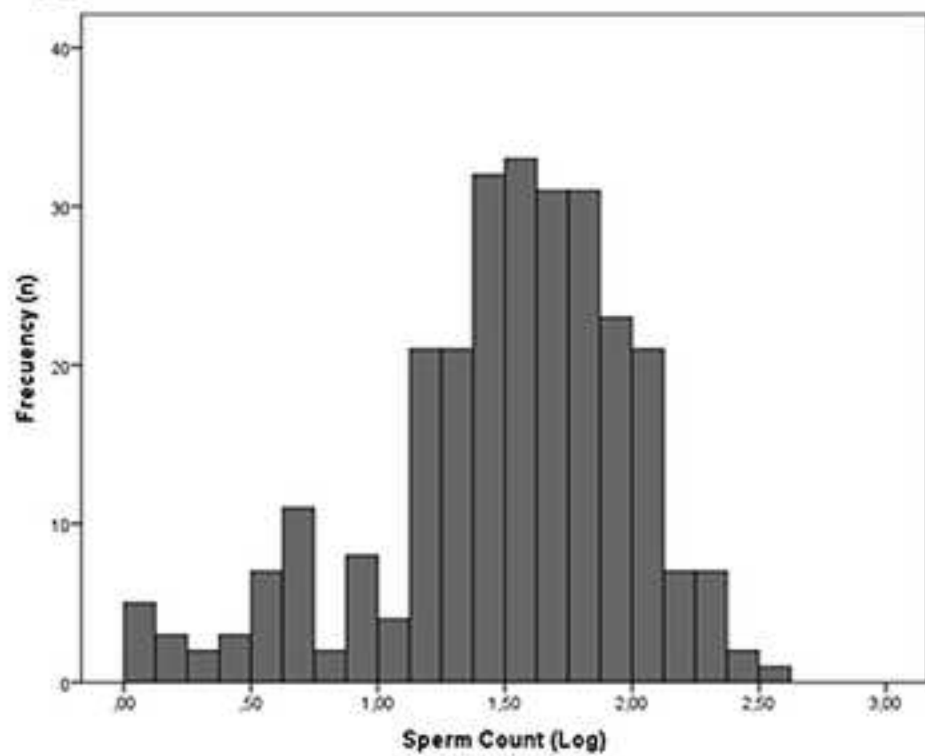
Figure 1

# Figure 1

## A



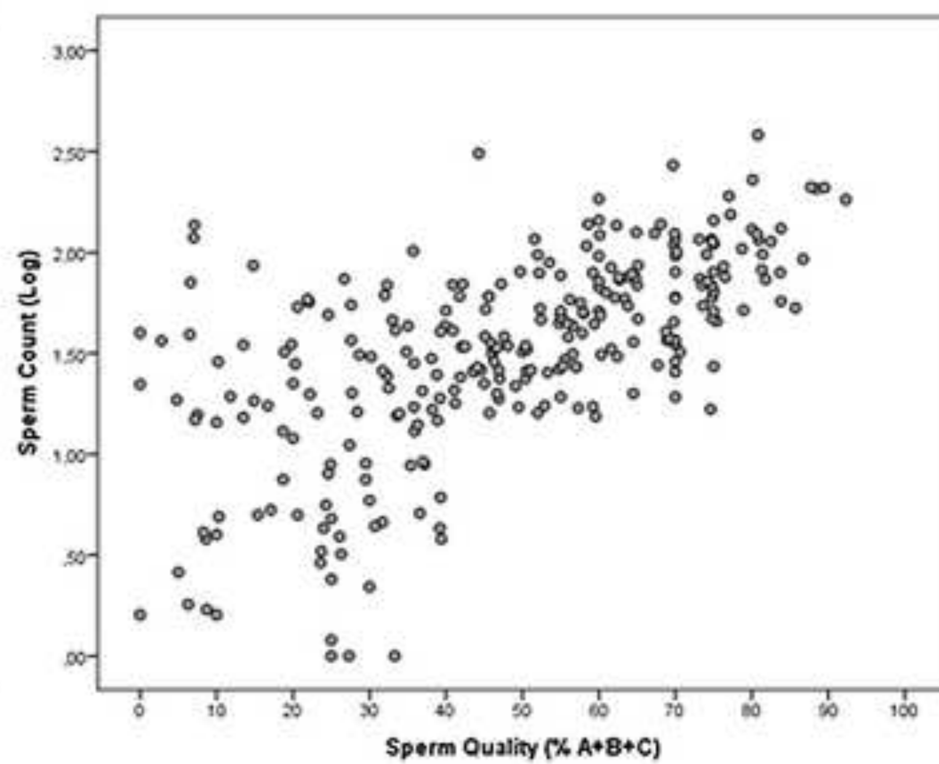
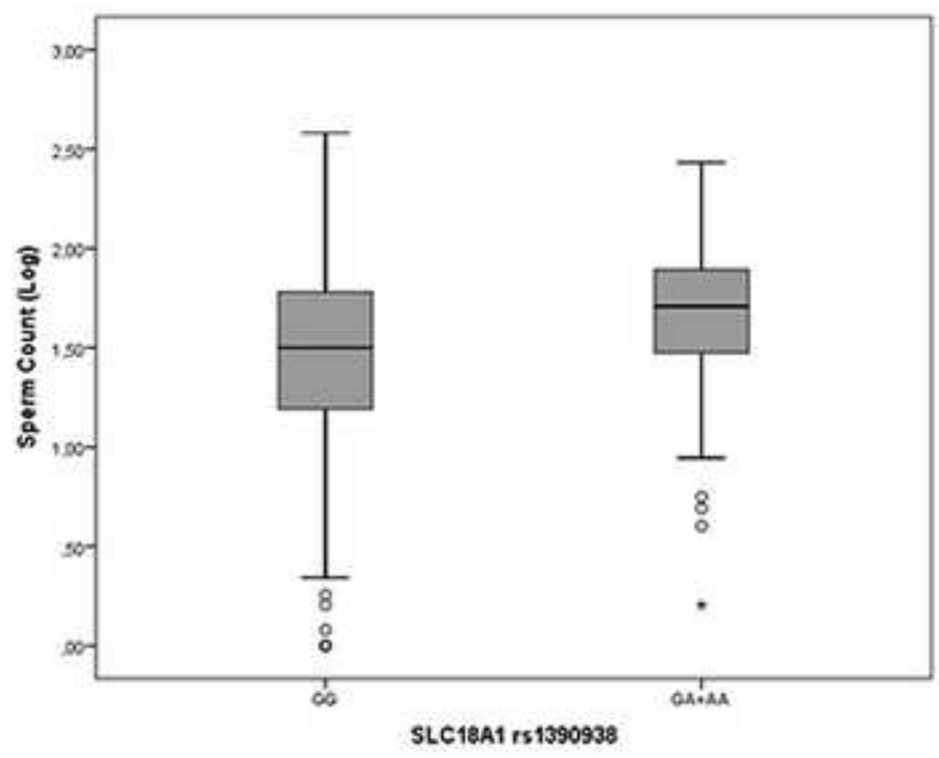



Figure 2

# Figure 2

**A**

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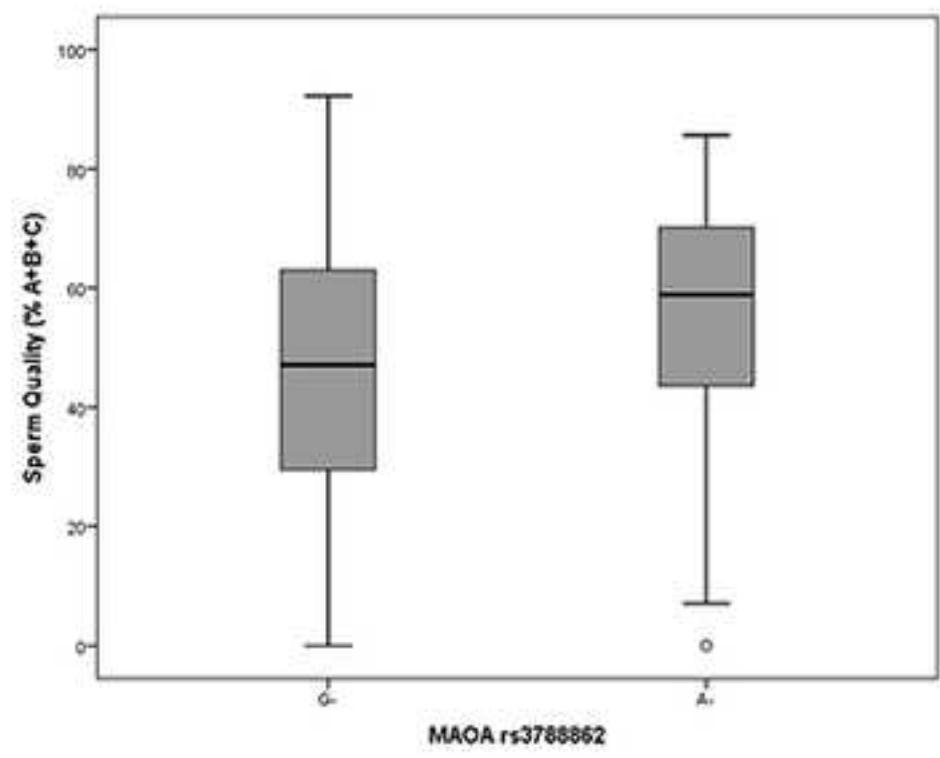

*Author photo

***Author Biography (50 words max.)**

**Armando Reyes-Engel; M.D. and Ph.D.**

Professor Armando Reyes-Engel is Doctor in Medicine and professor in Biochemistry and Molecular Biology and Clinical Genetics in the Faculty of Medicine at Malaga University, with a main focus on fertility issues.

***Key Message (50 words max.)**

## Key message

The genes *MAOA* and *SCL18A* related with the metabolism of neurotransmitters are key genes in maintaining extracellular and intracellular levels of neurotransmitters, which are associated with asthenozoospermia.

Servicio Andaluz de Salud
**CONSEJERÍA DE SALUD**

**La Subcomisión de Investigación Clínica del Hospital Universitario "Virgen de la Victoria", de Málaga, reunida el día 18 de noviembre de 2008, con la asistencia de sus miembros:**
D. Francisco Javier Estebaranz García, D. Alberto Delgado García, D. Ramón Porras Sánchez, Dª Isabel Lucena González, D. Javier Alzueta Rodríguez, D. Emilio Alba Conejo, D. Cristóbal Corral Leal, Dª Carmen Verge González, Dª Carolina Conejo Gómez, D. José M. Trigo Pérez, Dª Leonor Ruiz Sicilia, D. Fernando Cardona Díaz, Dª Blanca O'Donnell Cortes.

Ha evaluado la propuesta para que se lleve a cabo en el Centro el proyecto de investigación, "**Influencia del metabolismo del eje folatos-homocisteina en la fertilidad humana. Seguimiento de la selección genética inducida por folatos y relación del eje con las anomalías cromosómicas más comunes.**"

- El proyecto evaluado cumple con los requerimientos legales exigibles y el planteamiento metodológico es correcto.
- El protocolo establece claramente los objetivos.
- Los riesgos y molestias previsibles para el sujeto están definidos acotados y justificados, con la cobertura que procede.
- Existe consentimiento informado, la hoja de información para los sujetos es comprensible y completa y se contempla el procedimiento de garantía de confidencialidad e intimidad.
- Esta Comisión considera que, **D. Armando Reyes Engel,** de la Universidad de Málaga, **D. Maximiliano Ruiz Galdón**, del Hospital "Virgen de la Victoria" de Málaga, y su equipo, están capacitados para llevar a cabo este proyecto, que es de desarrollo factible en este Centro, aprobando en todos sus términos la realización del mismo.

En Málaga, a 24 de noviembre de 2008

EL SECRETARIO

Fdo.: Ramón Porras Sánchez

EL PRESIDENTE

P.A.

Fdo.: Fco. Javier Estebaranz García

Hospital Virgen de la Victoria
Campus Universitario Teatinos, s/n.
Apartado 13.007
Teléf. 951 032 000
29010 - Málaga

Cód. 000054

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