Association of genetic markers within the KIT and KITLG genes with human male infertility

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BACKGROUND: There is much evidence involving the KIT tyrosine kinase receptor and its ligand KITLG in the survival and proliferation of germ cells. Animal models and functional studies in humans suggest that this signalling pathway plays a role in male infertility. METHODS: We studied three and two single-nucleotide polymorphisms (SNPs) (rs3819392, rs3134885, rs2237012, rs10506957 and rs995030) located within the genomic region of the KIT and KITLG genes, respectively. A total of 167 idiopathic infertile men (sperm counts <5 million spz/ml) and 465 unrelated healthy controls from the same geographical region were genotyped for these SNPs. RESULTS: We found a statistically significant association of the rs3819392 polymorphism, which is located within the KIT gene, with idiopathic male infertility. In addition, a deviation from the Hardy–Weinberg equilibrium (HWE) law was observed for rs10506957 polymorphism within the KITLG gene only in the infertile group. CONCLUSIONS: Our data indicate that the KIT/KITLG system may be involved in a low sperm count trait in humans.

Key words: association study/KIT/KITLG/male infertility/polymorphism

Introduction

Primary or secondary infertility affects roughly 1 of 10 couples worldwide (WHO, 2002). In 51.2% of infertile couples, a male factor is identified (Tournaye, 2002). Numerous male infertility aetiological factors have been described (De Kretser and Baker, 1999). However, a considerable percentage of infertile men are currently classified as idiopathic.

Intensive studies in animal models and humans, both in vitro and in vivo, have emphasized the role of a myriad of genetic factors in the reproductive function of men (Lissens, 1999; Matzuk and Lamb, 2002). Nevertheless, the implication of a vast majority of these factors in male infertility remains to be clarified.

One of the most studied molecular systems in male fertility, the KIT/KITLG system, is constituted by the KIT receptor [OMIM:164920] encoded by the v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog gene (KIT) and its ligand KITLG [OMIM:184745] encoded by the KIT ligand gene (KITLG). This system is involved in physiological processes such as haematopoiesis, melanogenesis or germ cell development (Prasanth et al., 2004).

The KIT receptor belongs to a subclass III of the tyrosine kinase receptor family, which also includes platelet-derived growth factor receptor alpha (PDGFRα) and beta (PDGFRβ), fms-related tyrosine kinase 3 (FLT3) and colony stimulating factor 1 receptor (CSF1R) (Prasanth et al., 2004). The only ligand for the KIT receptor is KITLG, also called stem cell factor (SCF) (Prasanth et al., 2004). Once non-covalent dimers of KITLG bind to KIT receptors, dimerization and activation of the kinase domain of the receptor take place. Key tyrosine residues within the receptor dimers become phosphorylated and act as docking sites for transducer molecules that activate different molecular pathways (reviewed in Ronnstrand, 2004). The activation of the KIT/KITLG system gives rise to different cellular responses including adherence, migration, survival, proliferation, differentiation, maturation or secretion (Prasanth et al., 2004).

Studies in different animal models have highlighted the role of the KIT/KITLG system in male fertility (Sette et al., 1997; Blume-Jensen et al., 2000; Guerif et al., 2002; Kissel et al., 2000; Ohta et al., 2000).

In the adult human testis, the KIT receptor is expressed in early spermatogenic cells, the acrosomal granules of round spermatids and the acrosome of testicular spermatozoa, whereas the expression of KITLG has been observed in human Sertoli and Leydig cells (Sandlow et al., 1996). Regarding human male infertility, the expression of KITLG in seminal plasma has been correlated with the sperm count because infertile

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men present lower levels of KITLG mRNA compared with normozoospermic individuals (Fujisawa et al., 1998). Moreover, it has been postulated that the KIT receptor possibly plays a role in the capacitation or acrosome reaction of human spermatozoa (Feng et al., 2004).

On the contrary, a study examining the Y721 residue of the human KIT receptor, analogous to mouse Kit gene Y719 residue known to be essential for male fertility (Blume-Jensen et al., 2000; Kissel et al., 2000), failed to identify any alteration of this residue amongst a group of 65 idiopathic azoospermic men (Grimaldi et al., 2002). No other structural genomic analysis has been performed to analyse the role of the KIT/KITLG system in human male infertility.

There are a large amount of data supporting the involvement of the KIT/KITLG system in the male reproductive function and the possible role of this system in male infertility both in animal models and in humans. However, to our knowledge, only one study looking for KIT gene structural genomic variants in human male infertility has been published (Grimaldi et al., 2002). We carried out a genetic association study to provide new clues regarding the role of the KIT/KITLG system in male infertility.

Materials and methods

Subjects

The centres of reference are Unidad de Reproduccion, Centro Gutenberg (Malaga); Servicio de Urologia, Hospital Universitario Virgen del Rocio (Sevilla); and Unidad de Andrologia, Hospital General Universitario de Alicante (Alicante).

A group of 167 infertile Caucasian men were recruited for this study. All these patients fulfilled the following inclusion criteria: primary infertility, the absence of any known cause of infertility, the absence of varicocele, the absence of radiotherapy or chemotherapy, azospermia or severe oligozoospermia (sperm counts <5 million spz/ml) and absence of Y-chromosome microdeletions according to Simoni et al. (2004).

To estimate population frequencies of the genetic markers analysed, 465 unselected race-matched healthy controls from the same geographical region were genotyped anonymously. By using general population individuals instead of normospermic fertile men as controls, we obtained a more conservative estimate [odds ratio (OR)] in different statistical analyses.

Written informed consent was obtained from all the patients and controls included in this study. The Institutional Review Boards of referral centres and Neocodex have approved this protocol. DNA banking and genetic tests included in this study have complied with the international regulations regarding the collection, treatment, storage and use of genetic data (International Bioethics Committee, UNESCO SHS-503/2001/CIB-8/3).

Selection of single-nucleotide polymorphisms

The single-nucleotide polymorphisms (SNPs) within the genomic region of human KIT and KITLG genes were selected attending to the data set (public release 19) of the International HapMap Project (http://www.hapmap.org). We only considered the data regarding the CEPH (Utah residents with ancestry from northern and western Europe) (CEU) analysis panel, which is presumably the HapMap group most similar to the participants in our study in terms of ethnical origin.

We used Haploview software version 3.2 (http://www.hapmap.org) to identify the haplotypic blocks within the KIT and KITLG genes proposed by the International HapMap Project for its own data set (public release 19). Haplotypic blocks are defined as contiguous segments of the genome that show limited haplotype diversity. Therefore, we tried to select markers not included in the same haplotypic blocks to avoid redundant information and with the aim of obtaining as much information as possible from the whole genomic sequence containing the KIT and KITLG genes.

Haploview software can use three different definitions for haplotypic block construction. We decided to use the definition proposed by Gabriel et al. (2002), which in our experience results in haplotypic block construction of low complexity.

According to Gabriel et al. (2002), the HapMap data set (public release 19) describes four haplotypic blocks (I–IV) within the KIT gene covering 70.36% of its genomic sequence. Haplotypic blocks III and IV are in incomplete linkage disequilibrium (LD) ($D^\prime = 0.90$). Therefore, we selected three SNPs (dbSNP IDs: rs3819392, rs3134885 and rs2237012) located within haplotypic blocks I, II and IV, respectively (Figure 1). For the KITLG gene, the HapMap data set (public release 19) establishes two haplotypic blocks (I–II) in near complete LD ($D^\prime = 0.98$) covering 96.38% of the KITLG genomic sequence. We selected an SNP marker for each KITLG haplotypic block (dbSNP IDs: rs995030 and rs10506957) (Figure 2).

Genotyping

Genomic DNA extraction from peripheral blood samples of cases and controls were carried out in a MagNa Pure LC instrument (Roche Diagnostics, Basel, Switzerland) using the MagNa Pure LC Isolation Kit Large Volume (Roche Diagnostics) in accordance with the manufacturer’s instructions. DNA aliquots at 10 ng/μl were prepared, and the rest of the stocks were cryopreserved at −20°C.

For each polymorphism, we performed PCR protocols in a final volume of 20 μl containing 20 ng of genomic DNA, 1.25 mM Cl2Mg, 62.5 μM deoxynucleotides triphosphates (dNTP), 50 pmol of each amplification primer and 2 IU Taq DNA polymerase (Roche Diagnostics). PCRs were carried out in a PTC-100® Peltier Thermal Cycler (MJ Research, Waltham, MA, USA). Amplification primers and PCR conditions are summarized in Table I.

We designed Pyrosequencing™ (Biotage AB, Uppsala, Sweden) genotyping protocols in accordance with the manufacturer’s instructions. Sequencing primers are summarized in Table 1. Representative plots for the three possible genotypes in each marker are included in Figures 1 and 2.

To test the accuracy of our genotyping protocols, we carried out genotyping quality controls. All of the markers included in this study were checked by bidirectional sequencing in a CEQ 8000 automatic sequencer (Beckman Coulter, Fullerton, CA, USA) using the amplification primers in, at least, five random samples to verify the genotypes obtained through Pyrosequencing™ technology (Biotage AB) (data not shown). In addition, genomic DNA from 10% of all peripheral blood samples included in this study was freshly extracted and genotyped. A minimum of 99% of genotype coincidence was demanded for all five markers.

Statistical analyses

For statistical analysis of genotype distribution, test for deviation of Hardy–Weinberg equilibrium (HWE) or two–point association studies, we employed tests adapted from Sasieni (1997). These calculations were performed on the online resource facility at the Institute for Human Genetics, Munich, Germany (http://ihg.gsdf.de). A $P$ value of <0.05 was considered statistically significant in each situation.
Haplotype analyses

We used Haploview software version 3.2 to test whether the selected markers are included or not in the same haplotypic block within the KIT and KITLG genes in our control and infertile groups. This software was also used to obtain the Lewontin’s standardized disequilibrium coefficient $D'$ (Lewontin and Kojima, 1960) to measure the LD between the selected markers.

Haplotype frequencies and haplotype–based association analysis were calculated using Thesias software available at http://genecanvas.ecgene.net.

Results

**KIT gene**

Allelic frequencies and genotype distributions of the three selected SNPs within the KIT gene in cases and controls are summarized in Table II. We did not observe departures from HWE for any of the markers in either cases or controls (Table II). Nevertheless, we did detect differences in the allelic frequencies of the rs3819392 marker between cases and controls (Table II). These differences are more prominent when we consider the G allele of rs3819392 marker as a risk factor in a recessive model (OR = 2.10, $P = 0.017$).

Results from Haploview software indicated that none of the three markers within the KIT gene were included in the same haplotypic block either in cases or in controls (data not shown). These results correspond with the haplotypic blocks proposed by HapMap (public release 19) for the CEU analysis panel. According to Haploview software results, none of the three markers are in complete linkage disequilibrium each other in either cases ($D' < 0.67$) or controls ($D' < 0.65$).

Five of the eight possible haplotypes resulting from the three markers combination presented a frequency >5% in both cases and controls. There was no difference in haplotypic frequencies between cases and controls (Table III).

**KITLG gene**

Allelic frequencies and genotype distributions of KITLG gene markers in cases and controls are summarized in Table IV. We observed from the HWE for the rs10506957 marker due to an excess of heterozygous infertile men. Low $P$ values were obtained for different association tests with this marker. However, neither departure from HWE nor statistical tests with low $P$ values were detected for the rs995030 marker (Table IV).

According to Gabriel et al. (2002), the two KITLG gene markers are not included in the same haplotypic block. In addition, these two markers are not in complete LD in cases ($D' = 0.75$) or controls ($D' = 0.86$), as also occurs in the CEU analysis panel from HapMap ($D' = 0.88$). No difference between

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Figure 1. (A) KIT gene genomic sequence size (kb). (B) Haplotypic blocks (I–IV) for the KIT gene included in the HapMap data set (public release 19). Linkage disequilibrium (LD) values ($D'$) between the haplotypic blocks are presented. (C) Schematic representation of the KIT gene. Vertical and horizontal lines represent exons and introns, respectively. (D) Representative pyrograms derived from the genotyping of three different individuals (homozygote for the wild-type allele and heterozygote and homozygote for the polymorphic allele) for the three polymorphism within the KIT gene. Arrows indicate the physical location of the three polymorphisms within the KIT gene.
haplotype frequencies between cases and controls was detected (Table V).

We also examined the allelic frequencies and genotype distributions of the five selected markers by comparing the azoospermic and the oligozoospermic men in the infertile group. No statistical significant differences were found between these two groups for any of the five markers analysed (data not shown).

To test the joint effects of the polymorphisms in the *KIT* and *KITLG* genes, we carried out a manual search for the five polymorphisms within the *KITLG* gene. Arrows indicate the physical location of the three polymorphisms within the *KITLG* gene.

**Table 1.** PCR amplification and sequencing primers: PCR amplification conditions

<table>
<thead>
<tr>
<th>Gene/polymorphism</th>
<th>Primers (5′→3′)</th>
<th>PCR conditions: ti(Ti)→td(Td)→ta(Ta)→te(Te)</th>
<th>XNC→tfe(Tfe)</th>
<th>PCR product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIT/rs3819392</td>
<td>Forward: GCCTAACAGGATGAACCTGC Reverse: B-GGCCCATTTAGAAAGCATGCT Sequencing: TTCTCATGACTCTGAAATC</td>
<td>300(95)→[15(95)→30(65)→15(72)]</td>
<td>X50→420(72)</td>
<td>250</td>
</tr>
<tr>
<td>KIT/rs3134885</td>
<td>Forward: B-GCTGCAATCTTATGACTGC Reverse: AGACCTTCCCCAAAGGGCAG Sequencing: TGGCCTCTTTGAAGAA</td>
<td>300(95)→[15(95)→30(65)→15(72)]</td>
<td>X50→420(72)</td>
<td>172</td>
</tr>
<tr>
<td>KIT/rs2237012</td>
<td>Forward: AGTGGAGAAACTAGGCACTG Reverse: B-CAAAAATCCACCTCCAGGAAT Sequencing: GAGGTGACACAGGACA</td>
<td>300(95)→[30(95)→15(60)→20(72)]</td>
<td>X50→420(72)</td>
<td>203</td>
</tr>
<tr>
<td>KITLG/rs10506957</td>
<td>Forward: B-TTTCTCAGTTTCCTCACAACAA Reverse: TGAACCATCTGTCACCTCC</td>
<td>300(95)→[30(95)→15(60)→30(72)]</td>
<td>X50→420(72)</td>
<td>214</td>
</tr>
<tr>
<td>KITLG/rs995030</td>
<td>Forward: AGAAGAACTTGCATGGAGCAGG Reverse: B-GTTACCCTCATTATCCAAGCAG Sequencing: CGTGTCTCACTGCA</td>
<td>300(95)→[30(95)→15(60)→30(72)]</td>
<td>X50→420(72)</td>
<td>238</td>
</tr>
</tbody>
</table>

a, annealing; B, biotin; d, denaturation; e, elongation; fe, final elongation; i, initial denaturation; NC, number of cycles; T, temperature (in Celsius degrees); t, time (in seconds).
Table II. Allelic frequencies and genotype distributions for the three markers within the KIT gene, single-marker statistical analyses

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>rs3819392</th>
<th>rs3134885</th>
<th>rs2237012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleotide change</td>
<td>G&gt;A</td>
<td>A&gt;C</td>
<td>A&gt;G</td>
</tr>
<tr>
<td>Genotyped (%)</td>
<td>98.4 (622/632)</td>
<td>98.4 (622/632)</td>
<td>98.7 (624/632)</td>
</tr>
<tr>
<td>Controls/cases</td>
<td>Controls</td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>Minor allele frequency (%)</td>
<td>A: 37.9</td>
<td>A: 30.1</td>
<td>C: 47.4</td>
</tr>
<tr>
<td></td>
<td>AA: 70</td>
<td>AA:15</td>
<td>CC: 101</td>
</tr>
<tr>
<td>Statistical studies, P value [OR (95% CI)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deviation from Hardy–Weinberg equilibrium in control individuals/infertile patients</td>
<td>0.39/0.98</td>
<td>0.77/0.69</td>
<td>0.79/0.98</td>
</tr>
<tr>
<td>Allele frequency difference test</td>
<td>0.01 [1.41 (1.08–1.86)]</td>
<td>0.75 [1.04 (0.81–1.34)]</td>
<td>0.96 [1.01 (0.66–1.51)]</td>
</tr>
<tr>
<td>Heterozygous test</td>
<td>0.14 [1.59 (0.85–2.95)]</td>
<td>0.94 [0.98 (0.62–1.55)]</td>
<td>0.88 [0.88 (0.16–4.76)]</td>
</tr>
<tr>
<td>Homozygous test</td>
<td>0.02 [2.10 (1.13–3.89)]</td>
<td>0.77 [1.08 (0.65–1.79)]</td>
<td>0.90 [0.90 (0.17–4.71)]</td>
</tr>
<tr>
<td>Allele positivity test</td>
<td>0.04 [1.82 (1.01–3.29)]</td>
<td>0.94 [1.02 (0.66–1.56)]</td>
<td>0.90 [0.90 (0.17–4.67)]</td>
</tr>
<tr>
<td>Armitage’s trend test</td>
<td>0.01 [1.43]</td>
<td>0.75 [1.04]</td>
<td>0.96 [1.00]</td>
</tr>
</tbody>
</table>

Odds ratios (ORs) are in brackets; 95% confidence intervals (95% CI) are in parentheses. P values below 0.05 are in boldface.

Table III. Haplotype construction using the three markers within the KIT gene: statistical analyses using Thesias software

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Allele rs3819392</th>
<th>Allele rs3134885</th>
<th>Allele rs2237012</th>
<th>Frequency in controls (%)</th>
<th>Frequency in cases (%)</th>
<th>Statistical analyses, P value [OR (95% CI)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>G</td>
<td>C</td>
<td>A</td>
<td>34.3</td>
<td>36.0</td>
<td>1b</td>
</tr>
<tr>
<td>2</td>
<td>G</td>
<td>C</td>
<td>G</td>
<td>6.0</td>
<td>5.6</td>
<td>0.74 [0.90 (0.46–1.76)]</td>
</tr>
<tr>
<td>3</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>18.7</td>
<td>25.7</td>
<td>0.14 [0.91 (0.91–1.93)]</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>6.4</td>
<td>4.2</td>
<td>0.22 [0.66 (0.34–1.28)]</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>29.0</td>
<td>22.7</td>
<td>0.13 [0.77 (0.54–1.08)]</td>
</tr>
</tbody>
</table>

Odds ratios (ORs) are in brackets; 95% confidence intervals (95% CI) are in parentheses.

Table IV. Allelic frequencies and genotype distributions for the two markers in the KITLG gene: single-marker statistical analyses

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>rs10506957</th>
<th>rs995030</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleotide change</td>
<td>T&gt;C</td>
<td>C&gt;T</td>
</tr>
<tr>
<td>Genotyped (%)</td>
<td>98.9 (625/632)</td>
<td>99.7 (630/632)</td>
</tr>
<tr>
<td>Controls/cases</td>
<td>Controls</td>
<td>Cases</td>
</tr>
<tr>
<td>Minor allele frequency (%)</td>
<td>C: 23.0</td>
<td>C: 21.2</td>
</tr>
<tr>
<td>Genotypic distribution</td>
<td>TT: 272</td>
<td>TT: 97</td>
</tr>
<tr>
<td></td>
<td>TC: 164</td>
<td>TC: 66</td>
</tr>
<tr>
<td></td>
<td>CC: 24</td>
<td>CC: 2</td>
</tr>
<tr>
<td>Statistical studies, P value [OR (95% CI)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deviation from Hardy–Weinberg equilibrium in control individuals/subfertile patients</td>
<td>0.91/0.01</td>
<td>0.26/0.30</td>
</tr>
<tr>
<td>Allele frequency difference test</td>
<td>0.50 [1.11 (0.82–1.51)]</td>
<td>0.87 [1.03 (0.76–1.40)]</td>
</tr>
<tr>
<td>Heterozygous test</td>
<td>0.02 [4.83 (1.11–21.01)]</td>
<td>0.17 [2.01 (0.73–5.49)]</td>
</tr>
<tr>
<td>Homozygous test</td>
<td>0.03 [4.28 (0.99–18.45)]</td>
<td>0.26 [1.75 (0.65–4.68)]</td>
</tr>
<tr>
<td>Allele positivity test</td>
<td>0.03 [4.49 (1.05–19.12)]</td>
<td>0.22 [1.83 (0.69–4.87)]</td>
</tr>
<tr>
<td>Armitage’s trend test</td>
<td>0.48 [1.32]</td>
<td>0.87 [1.11]</td>
</tr>
</tbody>
</table>

Odds ratios (OR) are in brackets; 95% confidence intervals (95% CI) are in parentheses. P values below 0.05 are in boldface.

Table V. Haplotypes construction using the two markers within KITLG gene: statistical analyses using Thesias software

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Allele rs10506957</th>
<th>Allele rs995030</th>
<th>Frequency in controls (%)</th>
<th>Frequency in cases (%)</th>
<th>Statistical analyses, P value [OR (95% CI)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T</td>
<td>C</td>
<td>73.8</td>
<td>74.0</td>
<td>1b</td>
</tr>
<tr>
<td>2</td>
<td>T</td>
<td>T</td>
<td>2.3</td>
<td>4.2</td>
<td>0.12 [1.79 (0.87–3.68)]</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>T</td>
<td>18.9</td>
<td>16.8</td>
<td>0.47 [0.88 (0.61–1.25)]</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>C</td>
<td>5.0</td>
<td>5.0</td>
<td>0.99 [1.00 (0.56–1.76)]</td>
</tr>
</tbody>
</table>

Odds ratios (OR) are in brackets; 95% confidence intervals (95% CI) are in parentheses.

*Haplotype used as reference.*
marker genotype combinations. All the statistically significant
genotype combinations that we observed included the
rs3819392 polymorphism, and none of them showed an OR
value above that observed for rs3819392 alone (data not
shown). These results indicate that the effect of these genotype
combinations was mainly due to the presence of the rs3819392
polymorphism of the KIT gene.

Discussion
The KIT/KITLG system is involved in human diseases. Hetero-
ygous loss-of-function mutations in the KIT gene are responsi-
ble for piebaldism [OMIM: 172800]. Furthermore, alterations
in the expression of the KIT receptor have been associated with
different tumours (Ronnstrand, 2004; Lennartsson et al., 2005).

In addition, this system seems to be altered in testicular
germ cell tumours (Looijenga et al., 2003; Kemmer et al., 2004;
Rapley et al., 2004; Nakai et al., 2005) demonstrating a role for
the KIT/KITLG system in the human testicular pathology.

A previous study published by one of us (M.D.F.) proposed
an experimental in vitro model for tumourigenic germ cell
transformation. In this model, the development of germ cell
tumours after estrogen exposure was mediated by KIT/KITLG
system and its downstream Akt/PTEN signalling pathway
(Moe-Behrens et al., 2003). This study stresses the possibility
that genetic factors could modulate the effects of environmen-
tal agents over the testicular function.

In this study, we have carried out the first analysis of genetic
polymorphisms within the human KIT and KITLG genes in
human male infertility. Our analyses revealed an association of
the KIT gene rs3819392 marker with a low sperm count trait in
our population. This finding suggests the possibility that the
rs3819392 marker could be in LD with a male infertility sus-
ceptibility factor located within the KIT gene or within another
nearby locus.

The SNP rs3819392 is included in the haplotypic block I of
the KIT gene, which comprises the promoter, the first exon and
part of the first intron of this gene (Figure 1). Therefore, muta-
tions or genetic variants within these genomic regions could be
relevant for the activity of the KIT gene.

Interestingly, PDGFRα belongs to the same family as the
KIT gene. Human PDGFRα gene is located ∼360 kb 5’
upstream from the KIT gene, and no other genes have been
identified between them.

Human Sertoli and Leydig cells are the only cell types
expressing PDGFRα protein in normal fetal and adult male
gonads (Basciani et al., 2002). The disruption of the Pdgfra
gene in mice impair the testis organogenesis and the Leydig
cell differentiation (Brennan et al., 2003). Moreover, an
aberrant transcript of the PDGFRα gene has been proposed as
a highly selective marker for the detection of early stages
of human testicular germ cell tumours (Mosselman et al.,
1996).

Taking into account these data, we think that it would be
interesting to analyse the joint effects of the KIT and PDGFRα
genes in male infertility and testicular carcinoma to identify
low penetrance alleles influencing the development or progres-
sion of these two pathologies.

Regarding the KITLG gene, we have detected a deviation of
the HWE law for the rs10506957 marker due to an excess of
heterozygous men only in the group of infertile patients. This
deviation is responsible for differences in genotypic distribu-
tions between controls and cases for the rs10506957 marker
(Table IV). Departures from the HWE law may be due to geno-
typing errors. However, we carried out strict genotyping qual-
ity controls during this study (see Materials and methods)
for all the markers analysed, which make it unlikely that genoty-
ping errors have been overlooked.

An additional cause of HWE departure is the genetic stratifi-
cation in the studied population. However, all the individuals
included in this study are Caucasians, as their parents and
grandparents are. In addition, all patients and controls came
from the same geographical region.

On the contrary, a previous study looking for large-scale
variations in the human genome detected a loss of a chromo-
somal region of ∼172 kb comprising the whole genomic
sequence of the KITLG gene (Iafrate et al., 2004). This
genomic rearrangement was found in heterozygosis in 1 of
39 unrelated healthy individuals (2.5%), and it is included in
the Database of Genomic Variants (http://projects.tcag.ca/
variation/).

Therefore, we cannot rule out the possibility that this or
other genomic rearrangements affecting part or all of the
KITLG gene nucleotide sequence could affect our genotyping
results in a fraction of our series. In this sense, we studied the
possibility of characterizing this rearrangement in our infertile
samples through alternative PCR-based technologies.

Additionally, it has been proposed that departures from
HWE law in cases may be indicative of genetic association
between the marker and the phenotype under study (Hoh et al.,
2001). Furthermore, the same phenomenon can be expected for
genetic markers under certain disease models (Wittke-Thompson
et al., 2005).

Furthermore, this is not the first HWE departure found in a
human male infertility association study for an SNP marker
within a candidate gene (Galan et al., 2005). We are currently
performing meta-analyses of male infertility genetic associa-
tion studies with the aim of testing whether HWE departure in
genetic markers is a common feature in male infertility studies
that have been published. We think that the low sperm count
trait is a very susceptible phenotype for transmission distortion
phenomena previously described in mice, flies and humans
(Zollner et al., 2004). Transmission distortion is a biological
process by which the transmission of chromosome to surviving
offspring is skewed away from Mendelian predictions. One
cause of transmission distortion that specifically affects the
male sex is called ‘gametic selection’, where sperm with a par-


ticular genotype manage to disrupt or otherwise outperform
their competitors (Zollner et al., 2004). This mechanism
should account for the HWE departure observed in genetic
markers associated with male infertility.

On the contrary, the infertile men included in this study did
not present varicocele; this has not been assessed in the control
group. We recognize that this issue could be considered as a
limitation of our study. In fact, we cannot rule out the effect of
any of the polymorphisms analysed in this study on varicocele.
In this sense, specific genetic association studies assessing the presence or absence of varicocele in both case and control groups would be necessary to establish the role of genetic factors in the development of varicocele.

We should not forget that male infertility is considered to be a complex trait influenced by both environmental and genetic factors. In this sense, we are planning to analyse the joint effect of the KIT/KITLG system with other previously published male infertility genetic susceptibility factors such as polymorphisms within the estrogen receptor alpha gene (Galan et al., 2005; Guarducci et al., 2006), the androgen receptor gene (Yong et al., 2003), the cAMP-responsive element modulator gene (Vouk et al., 2005) or the gr/gr deletion on the chromosome Y (Repping et al., 2003; Giachini et al., 2005). We are also interested in analysing the effects of other genes interacting with the KIT/KITLG system, specifically the PDGFRA gene and the PIK3/Akt/PTEN signalling pathway that has been demonstrated to mediate the activity of the KIT/KITLG system in infertility in animal models (Blume-Jensen et al., 2000; Kissel et al., 2000).

In conclusion, although we recognize that our findings do not present a relevant clinical significance, our results indicate that genetic variants within the genomic sequences of the KIT and KITLG genes could play a relevant role in human male infertility. Additional analyses are necessary to establish clearly the effects of the SNPs analysed in this study on the activity of the KIT/KITLG system in the male reproductive function.

Acknowledgements

The authors thank the patients and controls for their participation in this study. This work was supported by the European Commission (QLK4-CT-2002–02403) and the Ministerio de Ciencia y Tecnología (QLK4-CT-2002–02403), and the Ministerio de Ciencia y Tecnología. This work was supported by the European Commission (QLK4-CT-2002–02403) and the Ministerio de Ciencia y Tecnología. This work was supported by the European Commission (QLK4-CT-2002–02403) and the Ministerio de Ciencia y Tecnología.

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Submitted on April 1, 2006; resubmitted on May 16, 2006; accepted on June 30, 2006

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